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Serum Calprotectin in active Systemic Lupus Erythematosus

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Abstract:

Systemic Lupus Erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by the production of autoantibodies, immune complex formation, and widespread inflammation. Monitoring disease activity remains a clinical challenge due to its heterogeneous presentation and fluctuating course. Traditional biomarkers such as anti-dsDNA antibodies, complement levels (C3, C4), and ESR/CRP have limited sensitivity and specificity in all patients. This has led to increasing interest in novel inflammatory markers such as serum calprotectin. Calprotectin is a calcium- and zinc-binding protein complex released predominantly by neutrophils during inflammation. It plays a critical role in the innate immune response and serves as a sensitive biomarker of inflammatory activity.

Keywords: Calprotectin, Inflammation, Biomarker, Inflammatory Bowel Disease, ELISA, Disease Activity, Neutrophils.

Introduction:

Calprotectin is a heterodimeric complex composed of the calcium-binding proteins S100A8 and S100A9, predominantly expressed in neutrophils and monocytes. It plays a crucial role in innate immunity through its antimicrobial properties and regulation of inflammatory processes via metal ion sequestration and Toll-like receptor 4 (TLR4) activation **(1).** Upon cellular activation or death, calprotectin is released into extracellular fluids, where it acts as a damage-associated molecular pattern (DAMP), amplifying inflammatory responses.

In clinical settings, fecal calprotectin has gained prominence as a reliable non-invasive biomarker for detecting gastrointestinal inflammation, particularly in distinguishing inflammatory bowel disease (IBD) from functional disorders such as irritable bowel syndrome (IBS) **(2).** Elevated levels of serum calprotectin have also been reported in systemic inflammatory conditions including rheumatoid arthritis, systemic lupus erythematosus, sepsis, and COVID-19, indicating its broader role beyond gastrointestinal pathology **(3).**

The stability of calprotectin in feces and serum, along with its strong correlation with disease activity and progression, renders it a valuable tool not only for diagnosis but also for monitoring treatment response. Despite its increasing use, the specificity of calprotectin in different clinical scenarios, its cutoff values, and its relationship with other inflammatory markers remain areas of ongoing investigation.

This study aims to explore the diagnostic and prognostic value of calprotectin levels in patients with [insert disease], and to assess its correlation with conventional inflammatory markers and clinical outcomes.

Calprotectin (CLP) is a soluble protein secreted by activated monocytes and neutrophils into the circulation it is involved in inflammatory processes and/or inhibition of microbial growth. CLP comprises a heterodimer belonging to calcium-binding protein of the S100 family and it is composed of two proteins named S100A8 and S100A9. In humans, these proteins are formed by two α-helix motifs that allow Ca^{2+} binding and other divalent metal ions such as Zn^{2+}. After binding of the ion binding, the complex S100A8/S100A9 can form the heterodimer or heterotetramer that are considered essential to intracellular and extracellular biological function **(Figure) (4)**.

It has been observed that S100A8 and S100A9 can circulate as separated molecules, but the heterodimer is the most stable form, and it plays a key role in the protein's biological interaction. CLP can be assessed in faecal or serum samples. It has been described that CLP is mainly involved in inflammatory diseases. Faecal CLP (fCLP) is specific for gastrointestinal diseases while serum CLP (sCLP) is more specific in autoimmune diseases (AID) **(5)**.

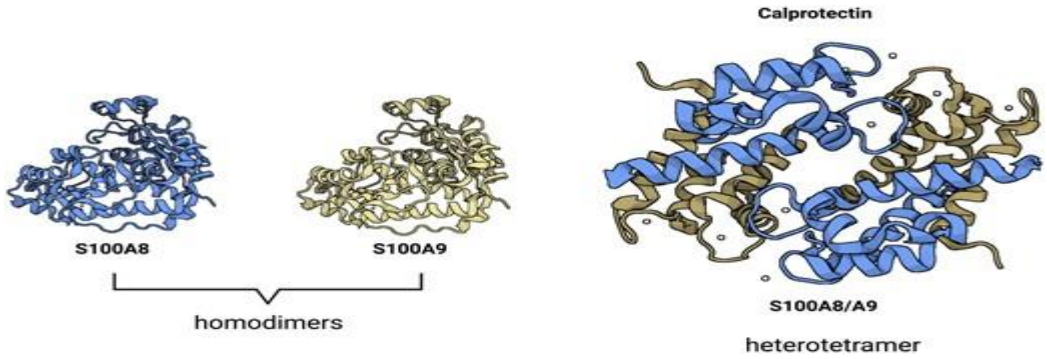

Figure 1: Structure of calprotectin (S100A8 and S100A9 proteins). S100A8 homodimer; individual subunits are shown in blue; S100A9 homodimer; subunits are shown in light brown; S100A8/A9 heterotetramer. Bound Ca^{2+} ions are shown by white spheres **(6)**

The serum level of CLP is usually reported below 1 µg/ml in healthy subjects but during inflammation the level may increase by 100 times. The faecal level of CLP (normal value up to 30 mg/l) provides a sensitivity and a specificity of 100% and 97%, respectively, in discriminating between active Crohn's disease (CD) and irritable bowel syndrome (IBS) **(6)**.

Intra and extracellular function of calprotectin

CLP is involved in both intracellular and extracellular functions. In fact, the S100A8/S100A9 complex regulates intracellular pathways of immune cells and modulates inflammatory response. It allows leucocyte recruitment through leucocyte chemotaxis and tissue infiltration. The extracellular function is mediated by binding to receptors for advanced glycation end-products (RAGE) and Toll-like receptor 4 (TLR4) which is the main CLP receptor. The bond between the heterodimer and TLR4 triggers signal transduction cascade that involves nuclear factor-κB and MyD88, which translocate into the nucleus and promote the expression of pro-inflammatory cytokine genes such as *TNF-α*, *IL6*, *IL8*, *IL23*, etc. **(Figure).** Moreover, extracellular CLP complexes contribute to chelation of different transition metal ions which are important for bacteria.

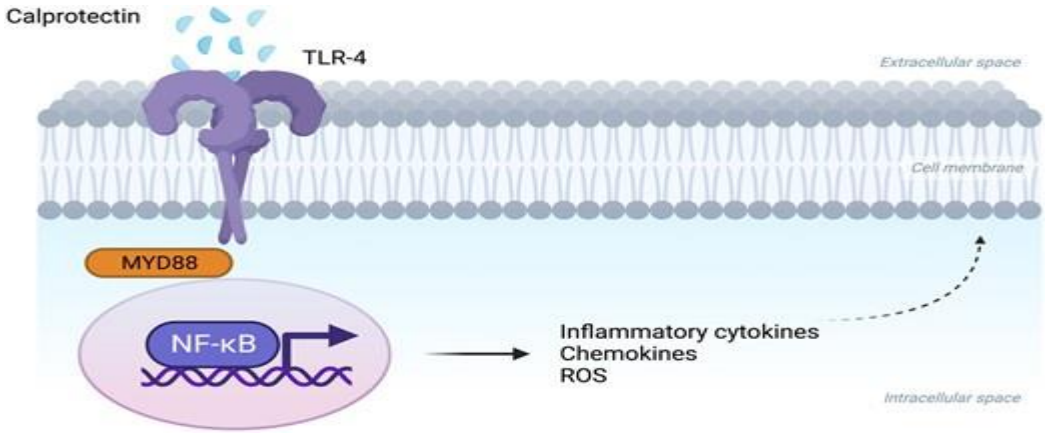

Figure 2: Extra and intracellular biological function of calprotectin. The extracellular function is mediated by Toll-like receptor 4 (TLR-4) which is the main calprotectin receptor. The bond between the heterodimer and TLR-4 triggers signal transduction cascade that involves nuclear factor-κB (NF- κB), which translocate into the nucleus

and promote the expression of pro-inflammatory cytokines chemokines, and reactive oxygen species (ROS) that drive inflammation **(6)**

Serum calprotectin

Serum calprotectin (sCLP) levels could be a good alternative to acute-phase proteins as a biomarker in autoimmune diseases (AID) such as ESR, CRP, etc. The onset and development of AID are the consequence of interactions between genetic and environmental factors, which result in dysregulation of the immune system, characterized by the occurrence of autoantibodies and autoreactive T cells. Based on this, circulating autoantibodies represent useful biomarkers of AID. These autoantibodies provide crucial diagnostic and prognostic information for the management of AID **(7)**.

In particular settings, autoantibodies are not necessarily specific for AID. In fact, it has been observed that certain autoantibodies can also appear in the blood of healthy individuals or in some particular physio-pathological situations, such as infections, the preclinical phase of AID or the administration of drugs **(8)**. It is noteworthy that the association between autoantibodies and risk of AID has attracted considerable attention. Numerous studies have shown that the presence of autoantibodies precedes the clinical onset of AID, and therefore could be a possible tool for AID screening or early diagnosis **(9)**.

AID include a large spectrum of clinically distinct entities that share a common aetiology: a misguided, self-directed immune response. sCLP levels might be a good alternative to acute-phase protein as a biomarker in AID. In fact, high levels of sCLP are detected in both systemic AID such as RA, SLE, SSc, myasthenia gravis (MG) and vasculitis, and in organ-specific AID such as Hashimoto's disease. Furthermore, sCLP levels are increased also in haemolytic AID such as autoimmune haemolytic anaemia **(Figure) (10)**.

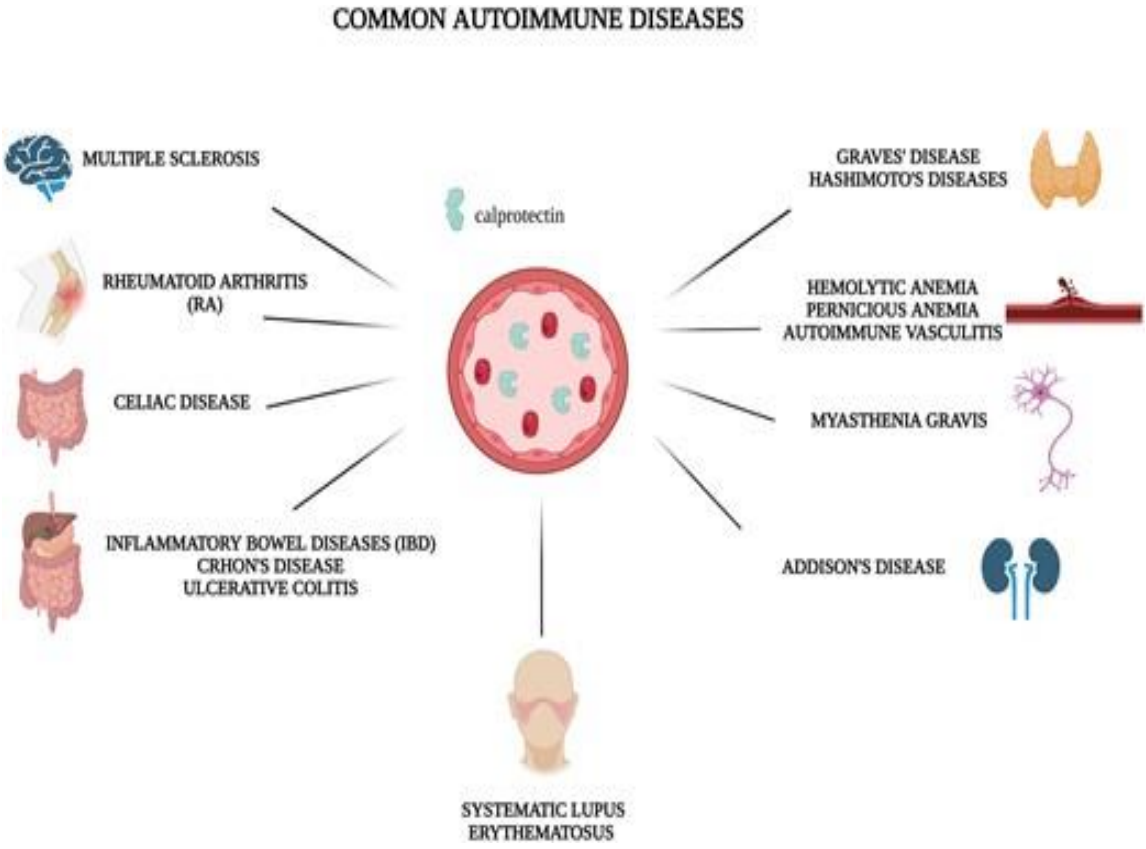

Figure 3: Serum calprotectinin common autoimmune diseases. Serum calprotectin levels could be a good alternative to acute-phase protein as a biomarker in the main autoimmune diseases. In fact, high levels of serum calprotectin are detected both in systemic autoimmune diseases such as RA, SLE, SS, SSc, myasthenia gravis and

vasculitis, and in organ-specific autoimmune diseases as Hashimoto's disease, coeliac disease, IBD and Addison's disease. Furthermore, serum calprotectin levels are increased also in haemolytic autoimmune diseases as autoimmune haemolytic anaemia **(6)**

Role of serum calprotectin on the adaptive immune system

sCLP is an important inflammatory biomarker and plays a key role on the adaptive immune response. CLP contributes to the induction of $CD8^+$ T cells during the activation process by antigen-presenting cells. This protein is a costimulatory enhancer together with CD40/CD40 ligand signalling and leads to the loss of tolerance of T cells. In a murine model of autoimmunity, the absence of S100A8 and S100A9 resulted in reduced IL-17 production by autoreactive $CD8^+$ T cells and in lower autoantibody production . Given its low molecular weight (36.5 kDa), CLP may diffuse from inflamed tissues to the blood circulation **(Figure) (6)**.

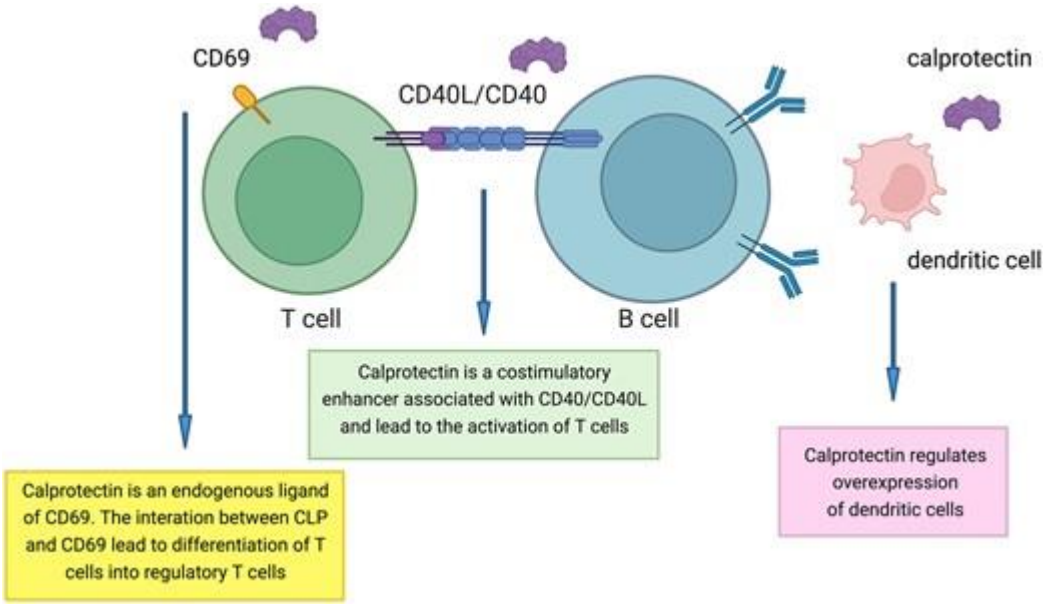

Figure 4: Role of serum calprotectin in autoimmune diseases. Calprotectin (CLP) may represent a connection between inflammation and the adaptive immune response. CLP contributes to the activation of $CD8^+$ T cells during the process of activation by antigen-presenting cells via dendritic cell overexpression. CLP is an enhancer of co-stimulation of the CD40/CD40L signal leading to $CD8^+$ T cell activation. Furthermore, CLP is an endogenous ligand of CD69. The interaction between CLP and CD69 leads to the differentiation of T cells into regulatory T cells **(6)**

Application of serum calprotectin

- **Rheumatoid arthritis**

In the literature, for patients affected by RA, a cut-off above 0.9 μg/ml was proposed to distinguish RA from non-inflammatory arthritis. In fact, sCLP levels are elevated in RA, but not in OA. RA is the most common chronic inflammatory joint disease, characterized by the presence of RF and ACPA. Both ACPA and RF together with inflammatory markers such as ESR and CRP have been included in the current 2010 ACR/EULAR classification criteria for RA **(11)**.

In a study, the authors highlighted the importance of sCLP in patients with RA who are receiving tocilizumab therapy to evaluate the inflammatory activity of the disease **(12)**.

- **Systemic lupus erythematosus**

Circulating CLP also increases in SLE patients. SLE is a systemic AID characterized by the loss of self-tolerance and the production of anti-nuclear components autoantibodies. This condition causes systemic organ inflammations, including joints, kidneys and skin. However, the diagnosis of SLE is complex given that the

pathophysiology is as yet unclear. Many studies have shown that serum CLP is high in patients with SLE compared with healthy controls **(13)**.

- **Vasculitis**

Other studies have investigated the role of CLP in vasculitis. Cutaneous and other vasculitides are specific inflammations of the blood vessel wall that can take place in any organ system of the body. Autoimmune vasculitis is a different group of diseases that cause damage to arteries, arterioles and capillaries. Vasculitis leads to destruction of the vascular wall resulting in haemorrhage and tissue ischaemia **(14)**.

ANCA-associated vasculitis (AAV) is an AID characterized by serum-positive ANCA, better known as MPO- or PR3-AAV, and the rapidly progressive glomerulonephritis which shows pauci-immune complex deposition in pathogenic biopsy. It has been observed in several studies that high levels of CLP are correlated with high levels of MPO and PR3. The sCLP of active MPO-AAV significantly increased (compared with inactive AAV and healthy controls) and were correlated with the severity of the disease **(15)**.

- **Systemic sclerosis**

Systemic sclerosis (SSc) is an AID characterized by fibrosis of skin and internal organs, vasculopathy, and dysregulation of immune system. A diagnostically important feature of immunological abnormalities in SSc is the presence of circulating ANA, which can be detected in 90–95% of patients. These include antibodies against topoisomerase (anti-TOPO I), kinetochore proteins (ACA), RNA polymerase enzyme (anti-RNAP III), ribonuclear proteins (anti-U11/U12 RNP, anti-U1 RNP, anti-U3 RNP) and nucleolar antigens (anti-Th/To, anti-NOR 90, anti-Ku, antiRuvBL1/2, anti-PM/Scl). SSc is an heterogeneous disease which leads to unclear diagnosis, and it is hard to predict its progression. For these reasons, it is necessary to study new biomarkers for diagnosis, progression and drug response **(16)**.

- **Sjögren's syndrome**

In many studies it has been shown that a high level of CLP is associated with mortality in SSc patients **(17)**. Sjögren's syndrome (SS) is a multifactorial systemic AID characterized by a wide spectrum of different clinical manifestations. The main symptoms are dry mouth and eyes. Conjunctivitis and periodontal disease, enlargement of the salivary glands, especially the parotid glands, dry cough, dry skin and dry genital organs. Other manifestations include arthralgias, paresthesias, asthenia and cutaneous vasculitis. High levels of sCLP were associated with positive anti-SSA e anti-Ro60 with higher incidence of carotid atherosclerosis **(18)**.

Table 1: Correlation between serum calprotectin and autoantibodies in different autoimmune diseases (6)

Disease	Association with specific disease features	Prognosis
RA	High levels are associated with positive RF and ACPA	High levels are predictive of disease relapse
SLE	High levels associated with positive Ab-anti-dsDNA	High levels are predictive of structural damage (skin damage, glomerulonephritis)
SS	High levels associated with positive anti-SSA and anti-Ro60	High levels associated with higher incidence of carotid atherosclerosis
SSc	High levels are associated with positive Ab-anti-hystone, anti-U1RNP anti-Th/To, anti-NOR 90, anti-Ku, antiRuvBL1/2 and anti-PM/Scl70	High levels are predictive of reduced survival
Vasculitis	High levels of CLP are correlated with high levels of MPO and PR3	High levels are predictive of disease relapse, associated with proliferative glomerulonephritis

CLP: calprotectin; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis; SS: Sjögren's syndrome.

Serum calprotectin in active SLE

CLP is released by phagocytes at the site of inflammation. After being released from neutrophils and monocytes, it modulates the inflammatory response. The extracellular CLP induces inflammation by binding to receptors for advanced glycation end-products (RAGE) and pattern recognition Toll-like receptors 4 (TLR4) located on the surface of leukocytes and endothelial cells. This causes the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α). Recent studies indicate that calprotectin may be a useful biomarker in monitoring the activity of autoimmune diseases, including SLE **(19)**.

Serum CLP levels are associated with certain inflammatory diseases such as SLE and inflammatory bowel disease. Patients with SLE demonstrate significantly higher serum calprotectin level as compared to healthy people. Calprotectin correlates with known markers of SLE exacerbation. Consistent with previous studies, serum calprotectin level was revealed to have potential as a rapid diagnostic biomarker of disease activity in patients with SLE **(20)**.

Serum CLP plays an important role in the regulation of adaptive immune responses **(21)**.

Elevated serum calprotectin in active SLE may reflect increased inflammatory activity by activating neutrophils and monocytes. Furthermore, this is because SLE is characterized by inflammation and immune system activation, which can lead to the release of calprotectin into the bloodstream so its elevated levels in serum can indicate heightened inflammatory activity, which is a hallmark of active SLE **(20)**.

In a study, it was found that CLP has high sensitivity (89.83%) and specificity (53.85%) in differentiating between SLE patients and healthy volunteers **(20)**.

Tyden et al demonstrated that the presence of anti-dsDNA antibodies was associated with increased calprotectin concentration, hypothesizing that the autoantibodies that attack dead cell elements contribute to elevated serum calprotectin levels by increasing phagocytosis and PMN activation, which is commonly observed in SLE **(22)**.

Furthermore, levels of serum calprotectin are associated with disease activity, high level of dsDNA antibodies and other SLE markers **(13)**.

Serum CLP promotes the induction of CD8-T cells that have pre-programmed cytotoxic functions. Further, serum calprotectin regulates the overexpression of dendritic cells, acts as an endogenous ligand of CD69, and is a costimulatory raiser of CD40/CD40L, increasing the activation of T-cells **(23)**. Serum CLP directly bound white cells such as macrophages, granulocytes, and monocytes and promoted the production of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, correlating with inflammation **(24)**.

In patients with SLE arthritis, Serum CLP levels were higher than in patients without arthritis **(21)**. Serum CLP is more specific in autoimmune diseases compared to Urinary calprotectin **(21)**.

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