

Serum calprotectin: a review of its usefulness and validity in paediatric rheumatic diseases

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ABSTRACT

In most childhood rheumatic diseases, specific diagnostic markers are not yet available. Therefore, a major emphasis in medical research today is directed to the discovery of new inflammation molecules, like calprotectin. Calprotectin (MRP8/MRP14) is a complex of calcium- and zinc-binding proteins that belong to the S100 protein family. This protein is directly released by leukocytes during the interaction with inflammatory activated endothelium at the site of inflammation. Increased plasma calprotectin levels have been found in inflammatory chronic diseases such as rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), inflammatory bowel diseases (IBD), multiple sclerosis, cystic fibrosis and systemic lupus erythematosus (SLE). In these diseases, serum calprotectin has been shown to correlate with disease activity and laboratory variables of inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). This review outlines the validity and the possible applications of calprotectin as a new inflammation marker in paediatric rheumatic diseases.

Introduction

In recent years, much progress has been made in understanding the pathogenesis and the treatment of many childhood rheumatic diseases. There remain, however, some unsolved problems such as the lack of specific diagnostic markers or the absence of criteria for evaluating the minimal residual disease.

To date, commonly used parameters, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), present some limits because they are markers for systemic inflammation and therefore cannot detect local inflammation nor can they predict the further

course of the disease. Hence, the need to look for more specific markers of inflammation that lead to the identification of new molecules such as calprotectin.

Calprotectin is a granulocyte and monocyte complex of calcium- and zinc-binding proteins that belong to the S100 protein family and is released during cell activation and turnover.

It is also referred as S100A8/A9, MRP8/MRP14 (myeloid-related-protein 8/14), calgranulin A/B, L1 protein and cystic fibrosis antigen (1-5) and is composed by two subunits of 8 kDa and 14 kDa, respectively MRP8 and MRP14. Both MRP8 and MRP14 form a stable non-covalent associated heterodimer, which is essential for the tetramerisation in calprotectin. The association into MRP8/MRP14 heterotetramer is a Ca²⁺-depending process (6-9).

Calprotectin represents 40% of neutrophil cytosolic proteins and 5% of monocytes, suggesting an important role in the activity of these cells; it is released during the interaction of leucocytes with inflammatory activated endothelium at the sites of inflammation (10, 11).

A large number of functions have been proposed for calprotectin: among intracellular functions, the calcium-dependent interaction of MRP8/MRP14 complex with cytoskeletal components, in particular with microtubules, vimentin, keratin and actin filament, is critical for phagocyte transendothelial migration (12-16).

When secreted to extracellular environments, calprotectin explains many activities, one of which is an antimicrobial activity (17-19), probably linked to its zinc molecules.

Another important extracellular function is the endothelial activation: calprotectin leads to loss of barrier function, apoptosis of endothelial cells, up-regulation of thrombogenic factors and

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an increase of junctional permeability (20, 21). Moreover, it promotes chemotaxis, upregulates the integrin receptors CD11b/CD18 on neutrophils facilitating its adhesion to fibronectin (22-25). Increased plasma calprotectin levels have been found in inflammatory chronic diseases such as rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), inflammatory bowel diseases (IBD), multiple sclerosis, cystic fibrosis and systemic lupus erythematosus (SLE) (26-31). Significant higher values have been reported in systemic onset JIA with 10–15 fold increase compared to patients with other inflammatory diseases such as IBD, SLE and RA (32). In these diseases, serum calprotectin levels have been shown to correlate with disease activity and laboratory variables of inflammation such as CRP and ESR. In addition, calprotectin results to have a stronger correlation with clinical disease assessments than ESR and CRP (29). Thus, calprotectin appears to be a considerably more sensitive marker of local inflammation even in the absence of signs or symptoms of disease, detecting a status of clinical but not immunological remission (33) and, therefore, can be a useful tool to predict a disease flare (34).

Fecal calprotectin (FC) concentrations have been shown to be a helpful non-invasive tool in the diagnosis of paediatric bowel inflammation in combination with other clinical and laboratory indices, to select children who should undergo gastrointestinal endoscopy. In clinical practice, FC is a validated test used in cases of suspected IBD for supporting diagnosis and, in cases of already known IBD, to confirm a relapse. However, its specificity seems to be low, so a negative test does not exclude this diagnosis (35-37). Serum calprotectin is elevated in children with IBD, possibly indicating clinical disease activity in these patients; however, its role has not yet been established in this disease (38).

In this review we will discuss the role of serum calprotectin in inflammatory rheumatic disease of children and its possible applications in clinical practice. We will use indifferently the terms calprotectin and MRP8/MRP14.

Calprotectin in juvenile idiopathic arthritis

JIA is the most common chronic rheumatic disease in children. It comprises a group of chronic arthritis of unknown origin that begin before the age of 16 years and persist for more than 6 weeks (39). The International League of Association for Rheumatology (ILAR) has provided the most recent classification, identifying seven subtypes (oligoarticular, rheumatoid-factor [RF]-positive polyarthritis, RF-negative polyarthritis, systemic-onset JIA, psoriatic arthritis, enthesitis-related arthritis, undifferentiated arthritis), many of which seem to represent different diseases characterised by distinct methods of presentation, clinical features, and, in some cases, genetic backgrounds (39).

Serum calprotectin:

correlation with JIA disease activity

In 1991, Berntzen *et al.* (29) studied for the first time MRP8/MRP14 serum concentration in 127 children affected by JIA. There were 79 children with pauciarticular onset, 33 with polyarticular onset and 15 with systemic onset-JIA. The results showed that serum calprotectin presented a stronger correlation with clinical disease activity than commonly used laboratory variables such as ESR and CRP. The authors suggested that serum MRP8/MRP14 concentration could be a marker of inflammation and a useful supplement in the evaluation of disease activity in children with JIA (29).

In another study Frosh *et al.* analysed serum and synovial MRP8/MRP14 concentration in 35 patients with pauciarticular-onset JIA (40). MRP8/MRP14 was found to be up to 5 times higher in serum samples of patients with active disease compared with those in remission. Besides, in each patient they reported significantly higher levels of MRP8/MRP14 in the synovial fluid (SF) than in the serum with differences that amounted to 50-fold increase in synovial fluid. A good correlation between SF and serum calprotectin values was demonstrated in all patients ($p < 0.01$). These data indicate that this protein is probably released at the sites of inflammation by monocytes and neutrophils.

Furthermore, after intra-articular therapy with triamcinolone, serum MRP8/MRP14 decreased significantly in therapy responders, whereas no differences were found in non-responders.

In this study the authors also confirmed a good correlation between MRP8/MRP14 serum levels and clinical markers of the disease; however, calprotectin demonstrated to be more sensitive than ESR and CRP in evaluating local inflammation.

The authors concluded that CRP and ESR reflect systemic involvement lacking the required sensitivity and specificity, while MRP8/MRP14 is directly produced in the sites of inflammation and this might explain its better sensitivity (40).

Serum calprotectin has been also found by other authors to be a marker of disease activity in patients who receive an autologous cell transplantation (ASCT) for refractory JIA (41). The occurrence of macrophage activation syndrome (MAS) in some of these patients is not preceded by significant changes in calprotectin concentration; so it was concluded that calprotectin is not useful for an early diagnosis of MAS (41).

Calprotectin in systemic-onset JIA

Two studies were conducted to analyse the expression of MRP8/MRP14 in patients with systemic-onset JIA. In the first one, Frosh *et al.* analysed MRP8/MRP14 concentration in the serum and in bioptic specimens from cutaneous rash during the initial phase of twenty children with systemic-onset JIA (32). In the skin specimens, infiltration of leukocytes expressing MRP8/MRP14 were found, while keratinocytes showed *de novo* synthesis of this protein indicating the activation of epithelial cells during the acute phase of the disease. Parallel to the impressive epidermal expression, serum of children affected showed extremely high concentrations of MRP8/MRP14. The epidermis, an organ of remarkable size, is likely to be responsible for the extraordinary high serum levels of MRP8/MRP14 found. Besides, serum calprotectin in systemic-onset JIA patients was 120-fold higher than in healthy controls, 12-fold higher than in children with active oligoarthritis

and 12-fold higher than in patients with various bacterial infections. It is also showed a close correlation with clinical and laboratory parameters of disease activity like fever, CRP and ESR (32). Longitudinal follow-up of 10 patients with systemic-onset JIA after the onset of an immune suppressive therapy with methotrexate (MTX) revealed a significant decrease of calprotectin, reaching normal values within 4 months in patients in remission. The authors concluded that in children with systemic-onset JIA, serum calprotectin could be a reliable tool for monitoring disease activity and to evaluate the response to MTX (32).

In a second study, Frosh *et al.* analysed the validity of serum calprotectin as a diagnostic challenge in children with fever of unknown origin (42). The aim of the study was to investigate if calprotectin might have a role in the differential diagnosis of JIA and other diseases with similar manifestations in an early phase. Serum MRP8/MRP14 was significantly more elevated in children affected by systemic-onset JIA compared to healthy controls and patients with systemic infections, acute lymphoblastic and myeloblastic leukaemia and neonatal onset multisystemic inflammatory disease (NOMID).

Classic inflammation markers such as CRP and ESR did not distinguish JIA and severe infectious disease, while MRP8/MRP14 was able to differentiate systemic-onset JIA from infections with a specificity of 95%. The authors concluded that calprotectin is a useful serum marker for an early diagnosis of systemic-onset JIA, in the presence of fever of unknown origin. Moreover, given the finding that it is also a strong inducer of IL-1 β expression on phagocytes, they suggested the possibility to use calprotectin as a therapeutic target in systemic-onset JIA (42).

Calprotectin as a marker of JIA relapse

An unsolved problem in JIA children in remission is the risk of relapse after therapy interruption. As of now, clinical or standard laboratory parameters like ESR and CRP do not identify patients with a high risk of relapse. Therefore, calprotectin has also been studied as a

predictive biomarker for disease flare in patients with clinically inactive JIA.

In a prospective study, Schulze zur Wiesch *et al.* compared serum calprotectin in two groups of JIA patients with inactive disease: the first group included patients who developed a relapse and the second included patients in stable remission for at least 12 months (34).

In patients who developed a relapse, MRP8/MRP14 was significantly higher than in those in stable remission for at least one year, whereas no differences were found between relapser and non-relapser for CRP and ESR (34).

The authors concluded that a subclinical disease activity may be present even months before clinical evidence of the disease and MRP8/MRP14 is elevated in these children. As a consequence, in clinical practice, calprotectin could be a useful marker to help modify therapy before a clinically apparent relapse (34). Gerst *et al.* included a larger population of 188 children with all forms of JIA (33). They analysed MRP8/MRP14 as well as S100A12, another protein belonging to SA100 family, and high sensitivity (hs) CRP as predictive biomarkers of relapse within a time of six months. S100A12 and MRP8/MRP14 were significantly higher in the serum of patients who subsequently developed flares compared to patients in remission, while no significant difference was found in hs CRP levels.

The authors suggested that MRP8/MRP14, as well as S100A12, give information about the status of innate immunity and are able to identify patients with a status of clinical but non-immunological remission (33).

A further study evaluated MRP8/MRP14 as a predictive marker of clinical relapse in systemic-onset JIA. Data confirmed the results of previous studies, demonstrating the significant increase of MRP8/MRP14 in serum of relapsers up to 6 months before flare, compared to non-relapsers (43).

Calprotectin: a guide for a correct Methotrexate (MTX) therapy

In two studies, calprotectin has been investigated as a predictive marker of stable remission after MTX withdrawal (44, 45).

Patients in stable remission, for at least one year after MTX discontinuation, had significantly lower calprotectin serum levels compared to patients who presented a relapse within one year after MTX withdrawal, whereas no difference was found for common markers of inflammation like ESR and CRP (44).

According to these authors, calprotectin may be a guide for the therapeutic decision about MTX-treatment in children with JIA (44).

Foell *et al.* confirmed these results and showed the accuracy of the biomarker calprotectin in predicting a relapse within 3 months from the MTX interruption (45).

More recently, Moncrieffe *et al.* demonstrated that high levels of baseline serum MRP8/MRP14 identify a subgroup of patients whose arthritis will improve on MTX. Indeed, all patients with a baseline MRP8/MRP14 serum concentration >3500ng/ml achieved ACR50 or better response in this study cohort (46). In conclusion, MRP8/MRP14 may be helpful in predicting relapse after MTX withdrawal and MTX response in children with JIA (44-46).

Serum calprotectin in vasculitis

Kawasaki disease

Kawasaki disease (KD) is an acute systemic vasculitis that primarily affects infants and young children (47, 48). KD is a self-limited illness that preferentially affects coronary arteries and, without an appropriate therapy, leads to the development of coronary aneurysms or dilatation in 15–25% of patients (49, 50). Intravenous infusion of high dose immunoglobulins (IVIG) has been shown to be effective in reducing systemic inflammation and coronary damage, but their mechanism of action is unclear (51, 52). Abe *et al.* studied MRP8/MRP14 plasma levels and its gene expression on blood mononuclear cells (PBMC) of 46 patients with KD (53). In this study, calprotectin plasma levels were found elevated in acute KD patients compared to febrile controls and were rapidly down-regulated after IVIG therapy. Persistent elevation of plasma MRP8/MRP14 after IVIG infusion was associated to a higher risk of developing coronary aneurysms in these patients (53).

It has been also demonstrated that calprotectin induces a thrombogenic and inflammatory response in human microvascular endothelial cells *in vitro* (54), whereby Hirono *et al.* hypothesised that it could directly induce an inflammatory and thrombogenic vascular response also *in vivo* in acute vasculitis syndromes such as KD (55).

Hirono *et al.* investigated patients with acute KD who had a different response to IVIG therapy with the aim to verify if MRP8/MRP14 could be a marker of disease activity and coronary arterial lesions (CALs) development (55). The results showed elevated serum calprotectin in all patients with acute KD exceeding those of healthy patients; after IVIG treatment, it significantly decreased in the group of responders within 24 hours and reached normal values within 4 weeks. In contrast, in non-responder patients, MRP8/MRP14 levels increased after the initial treatment and tended to subside after the second infusion. No difference in serum MRP8/MRP14 was observed between responders and non-responders before the IVIG therapy. The authors concluded that calprotectin has no predictive value of IVIG response, however, it is a more reliable marker than CRP that correlates with disease activity in individual patients (55). Moreover, the authors confirmed previous data showing that calprotectin could identify patients at risk for developing CALs.

Calprotectin in Henoch Schönlein Purpura

Henoch Schönlein Purpura (HSP) is an IgA-mediated immunocomplex vasculitis that predominantly affects children. The organs mainly involved are skin, joints, gastrointestinal tract and kidneys. Prognosis is most determined by the severity of kidney involvement. It has been shown that renal insufficiency, proteinuria degree and hypertension are predictors of severity for HSP (56, 57). To date, few useful serum markers for HSP disease activity have been identified (58). Kawasaki *et al.* studied children affected by HSP-nephritis (HSPN) to evaluate whether MRP8/MRP14 could be a reliable serum marker of renal injury (59).

The authors analysed clinical manifestations, laboratory findings, serum E-selectin and MRP8/MRP14, and histological and immunohistochemical findings at renal biopsy (59).

Patients were divided into two groups according to their MRP8/MRP14 serum values at the moment of renal biopsy: group 1 consisted of patients with less than median serum calprotectin values, group 2 included patients with calprotectin values greater than median.

Comparing clinical and laboratory findings obtained from the two groups, the authors observed that both urinary protein excretion and E-selectin levels in the patients of group 2 were significantly higher than in patients of group 1. Serum MRP8/MRP14 concentration was found to be strongly associated with serum E-selectin, a marker of endothelial injury. Comparing the histological data, the second group of patients had a more severe renal disease than the first group. The authors concluded that MRP8/MRP14 complex levels might be associated with the severity of renal injury and endothelial cell dysfunction in HSPN, probably because MRP8/MRP14 induces a thrombogenic and inflammatory response via human microvascular endothelial cells (59).

Calprotectin in autoinflammatory diseases

Cryopyrin-associated periodic syndromes (CAPS)

The term CAPS comprises a group of rare autoinflammatory diseases, which include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurological, cutaneous and articular syndrome (CINCA).

Wittkowski *et al.* monitored a total of 39 patients with CAPS treated with anti-IL-1 therapy. The authors demonstrated a link between MRP8/MRP14 and IL-1 β and hypothesised that MRP8/MRP14 could be a surrogate marker for IL-1 β driven inflammation (60), a sensible marker for monitoring disease activity, status of inflammation and response to IL-1 blockade in patients with CAPS (60).

Similar results were found in a group of Armenian patients with familial Medi-

terranean fever (FMF) before and after colchicine treatment (61).

Conclusion

In many paediatric rheumatic diseases, serum calprotectin has proved to be a valid index of inflammation which correlates with disease activity.

In JIA, calprotectin has demonstrated a higher sensitivity compared to routine inflammation markers (CRP and ESR) in identifying subclinical disease and can be considered a useful tool to guide therapeutic intervention. This higher sensitivity has been attributed to the fact that calprotectin is directly produced in the sites of inflammation by infiltrating myeloid cells. However, in a diagnostic setting its specificity is low, with the exception of fever of unknown origin, where it reaches a specificity of 95% in detecting systemic-onset JIA.

Serum calprotectin may also identify patients with JIA at risk of relapse after therapy interruption even if clinical signs of disease activity are absent.

Persistent elevation of serum calprotectin levels is also associated with higher risk of developing CALs in Kawasaki disease and kidney damage in HSP.

It is possible that, in the near future, serum calprotectin will become a routine examination test that can be used to monitor the disease progression and the treatment efficacy in paediatric rheumatic diseases. Further investigations are required to evaluate its diagnostic, prognostic and therapeutic potential.

Key messages

- In paediatric rheumatic diseases, specific diagnostic markers are not yet available so medical research is directed to the discovery of new inflammation molecules like calprotectin.
- In juvenile idiopathic arthritis serum calprotectin demonstrated to be a valid index of inflammation which correlates with disease activity.
- Calprotectin showed higher sensitivity in identifying subclinical disease compared to routine inflammation markers as C-reactive protein and erythrocyte sedimentation rate and it can be considered a useful tool to guide therapeutic intervention.

References

- SCHÄFER BW, HEINZMANN CW: The S100 family of EF-hand calcium-binding proteins: functions and pathology. *Trends Biochem Sci* 1996; 21: 134-40.
- ODINK K, CERLETTI N, BRUGGEN J *et al.*: Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* 1987; 330: 80-2.
- WILKINSON MM, BUSUTTI A, HAYWARD C, BROCK DJ, DORIN JR, VAN HEYNINGEN V: Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. *J Cell Sci* 1988; 91: 221-30.
- SCHÄFER BW, WICKY R, ENGELKAMP MK, MATTEI MG, HEIZMANN CW: Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: rationale for a new nomenclature of the S100 calcium-binding protein family. *Genomics* 1995; 25: 638-43.
- VOGL T, GHARIBYAN AL, MOROZOVA-ROCHE LA: Proinflammatory S100A8 and S100A9 Proteins: Self-assembly into Multifunctional Native and Amyloid Complexes. *Int J Mol Sci* 2012; 13: 2893-917.
- ISHIKAWA K, NAKAGAWA A, TANAKA I, SUZUKI M, NISHIHARA J: The structure of human MRP8, a member of the S100 calcium-binding protein family, by MAD phasing at 1.9 Å resolution. *Acta Crystallogr D Biol Crystallogr* 2000; 56: 559-66.
- ITOU H, YAO M, FUJITA I *et al.*: The crystal structure of human MRP14 (S100A9), a Ca²⁺-dependent regulator protein in inflammatory process. *J Mol Biol* 2002; 316: 265-76.
- VOGL T, LEUKERT N, BARCZYK K, STRUPAT K, ROTH J: Biophysical characterization of S100A8 and S100A9 in the absence and presence of bivalent cations. *Biochim Biophys Acta* 2006; 1763: 1298-306.
- KORNDÖRFER IP, BRUECKNER F, SKERRA A: The crystal structure of the human (S100A8/S100A9)₂ heterotetramer, calprotectin, illustrates how conformational changes of interacting α -helices can determine specific association of two EF-hand proteins. *J Mol Biol* 2007; 370: 887-98.
- ROTH J, VOGL T, SORG C, SUNDERKÖTTER C: Phagocyte-specific S100 proteins: a novel group of proinflammatory molecules. *Trends Immunol* 2003; 24: 155-8.
- YUI S, NAKATANI Y, MIKAMI M: Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. *Biol Pharm Bull* 2003; 26: 753-60.
- VOGL T, LUDWIG S, GOEBELER M *et al.*: MRP8 and MRP14 control microtubule reorganization during transendothelial migration of phagocytes. *Blood* 2004; 104: 4264-8.
- LOMINADZE G, RANE MJ, MERCHANT M, CAI J, WARD RA, MCLEISH KR: Myeloid-related protein-14 is a p38 MAPK substrate in human neutrophils. *J Immunol* 2005; 174: 7257-67.
- ROTH J, BURWINKEL F, VAN DEN BOS C, GOEBELER M, VOLLMER E, SORG C: MRP8 and MRP14, S-100-like proteins associated with myeloid differentiation, are translocated to plasma membrane and intermediate filaments in a calcium-dependent manner. *Blood* 1993; 82: 1875-83.
- RAMMES A, ROTH J, GOEBELER M, KLEMP T, HARTMANN M, SORG C: Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J Biol Chem* 1997; 272: 9496-502.
- GOEBELER M, ROTH J, VAN DEN BOS C, ADER G, SORG C: Increase of calcium levels in epithelial cells induces translocation of calcium-binding proteins migration inhibitory factor-related protein 8 (MRP8) and MRP14 to keratin intermediate filaments. *Biochem J* 1995; 309: 419-24.
- NIKI I, YOKOKURA H, SUDO T, KATO M, HIDAKA H: Ca²⁺ signaling and intracellular Ca²⁺ binding proteins. *J Biochem* 1996; 120: 685-98.
- STEINBAKK M, NAESS-ANDRESEN CF, LINGGAAS E, DALE I, BRANDTZAEG P, FAGERHOL MK: Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. *Lancet* 1990; 336:763-5.
- RUBARTELLI A, COZZOLINO F, TALIO M, SITIA R: A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. *EMBO J* 1990; 9: 1503-10.
- VIEMANN D, STREY A, JANNING A *et al.*: Myeloid-related proteins 8 and 14 induce a specific inflammatory response in human microvascular endothelial cells. *Blood* 2005; 105: 2955-62.
- VIEMANN D, BARCZYK T, VOGL T: MRP8/MRP14 impairs endothelial integrity and induces a caspase-dependent and -independent cell death program. *Blood* 2007; 109: 2453-60.
- RYCKMAN C, MCCOLL SR, VANDAL K *et al.*: Role of S100A8 and S100A9 in neutrophil recruitment in response to monosodium urate monohydrate crystals in the air-pouch model of acute gouty arthritis. *Arthritis Rheum* 2003; 48: 2310-20.
- NEWTON RA, HOGG N: The human S100 protein MRP-14 is a novel activator of the beta 2 integrin Mac-1 on neutrophils. *J Immunol* 1998; 160: 1427-35.
- ANCERIZ N, VANDAL K, TESSIER PA: S100A9 mediates neutrophil adhesion to fibronectin through activation of beta2 integrins. *Biochem Biophys Res Commun* 2007; 354: 84-9.
- EUE I, PIETZ B, STORCK J, KLEMP T, SORG C: Transendothelial migration of 27E10+ human monocytes. *Int Immunol* 2000; 12: 1593-604.
- BERNTZEN HB, MUNTKE E, FAGERHOL MK: The major leukocyte protein L1 as an indicator of inflammatory joint disease. *Scand J Rheumatol Suppl* 1988; 76: 251-6.
- BERNTZEN HB, MUNTKE E, FAGERHOL MK: A longitudinal study of the leukocyte protein L1 as an indicator of disease activity in patients with Rheumatoid Arthritis. *J Rheumatol* 1989; 16: 1416-20.
- BRUN JG, HAGA HJ, BØE E: Calprotectin in patients with Rheumatoid Arthritis; relation to clinical and laboratory variables of disease activity. *J Rheumatol* 1992; 19: 859-62.
- BERNTZEN HB, FAGERHOL MK, OSTENSEN M, MOWINCKEL P, HØYERAAAL HM: The L1 protein as a new indicator of inflammatory activity in patients with juvenile rheumatoid arthritis. *J Rheumatol* 1991; 18: 133-8.
- HAGA HJ, BRUN JG, BERNTZEN HB, CERVERA R, KHAMASHTA M, HUGHES GR: Calprotectin in patients with systemic lupus erythematosus: relation to clinical and laboratory parameters of disease activity. *Lupus* 1993; 2:47-50.
- RYCKMAN C, VANDAL K, ROULEAU P, TALBOT M, TESSIER PA: Proinflammatory activities of S100: Proteins S100A8, S100A9 and S100A8/S100A9 induced neutrophil chemotaxis and adhesion. *J Immunol* 2003; 170: 3233-42.
- FROSCHE M, VOGL T, SEELIGER S *et al.*: Expression of Myeloid-related proteins 8 and 14 in systemic-onset juvenile rheumatoid arthritis. *Arthritis Rheum* 2003; 48: 2622-6.
- GERSS J, ROTH J, HOLZINGER D *et al.*: Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. *Ann Rheum Dis* 2012; 71: 1991-7.
- SCHULZE ZUR WIESCH A, FOELL D, FROSCHE M, VOGL T, SORG C, ROTH J: Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2004; 22: 368-73.
- KOSTAKIS ID, CHOLIDOU KG, VAIPOPOULOS AG, VLACHOS IS, PERREA D, VAOS G: Fecal calprotectin in pediatric inflammatory bowel disease: a Systematic review. *Dig Dis Sci* 2013; 58: 309-19.
- FAGERBERG UL, LÖÖF L, MYRDAL U, HANSSON LO, FINKEL Y: Colorectal inflammation is well predicted by fecal calprotectin in children with gastrointestinal symptoms. *J Pediatr Gastroenterol Nutr* 2005; 40: 450-5.
- HENDERSON P, ANDERSON NH, WILSON DC: The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2014; 109: 637-45.
- LEACH ST, YANG Z, MESSINA I *et al.*: Serum and mucosal S100 proteins, calprotectin (S100A8/A9) and S100A12, are elevated at diagnosis in children with inflammatory bowel disease. *Scand J Gastroenterol* 2007; 42: 1321-31.
- RAVELLI A, MARTINI A: Juvenile idiopathic arthritis. *Lancet* 2007; 369: 767-78.
- FROSCHE M, STREY A, VOGL T *et al.*: Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. *Arthritis Rheum* 2000; 43: 628-37.
- WULFFRAAT NM, HAAS PJ, FROSCHE M *et al.*: Myeloid related protein 8 and 14 secretion reflects phagocyte activation and correlates with disease activity in juvenile idiopathic arthritis treated with autologous stem cell transplantation. *Ann Rheum Dis* 2003; 62: 236-41.
- FROSCHE M, AHLMANN M, VOGL T *et al.*: The Myeloid-related proteins 8 and 14 complex, a new ligand of toll-like receptor 4, and interleukin-1 b form a positive feedback

- mechanism in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum* 2009; 60: 883-91.
43. HOLZINGER D, FROSCH M, KASTRUP A *et al.*: The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. *Ann Rheum Dis* 2012; 71: 974-80.
 44. FOELL D, FROSCH M, SCHULZE ZUR WIESCH A, VOGL T, SORG C, ROTH J: Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? *Ann Rheum Dis* 2004; 63: 206-8.
 45. FOELL D, WULFFRAAT N, WEDDERBURN LR *et al.*: Methotrexate withdrawal at 6 vs 12 months in Juvenile Idiopathic Arthritis in remission. *JAMA* 2010; 303: 1266-73.
 46. MONCRIEFFE H, URSU S, HOLZINGER D *et al.*: A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein. *Rheumatology (Oxford)* 2013; 52: 1467-76.
 47. KAWASAKI T: Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* 1967; 16: 178-222.
 48. MELISH ME, HICKS RM, LARSON EJ: Mucocutaneous lymph node syndrome in the United States. *Am J Dis Child* 1976; 130: 599-607.
 49. YANAGISAWA M, KOBAYASHI N, MATSUYA S: Myocardial infarction due to coronary thromboarteritis, following acute febrile mucocutaneous lymph node syndrome (MCLS) in an infant. *Pediatrics* 1974; 54: 277-80.
 50. KATO H, KOIKE S, YAMAMOTO M, ITO Y, YANO E: Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. *J Pediatr* 1975; 86: 892-8.
 51. FURUSHO K, KAMIYA T, NAKANO H *et al.*: High dose intravenous globulin for Kawasaki disease. *Lancet* 1984; 2: 1055-8.
 52. NEWBURGER JW, TAKAHASHI M, BEISER AS *et al.*: A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med* 1991; 324: 1633-9.
 53. ABE J, JIBIKI T, NOMA S, NAKAJIMA T, SAITO H, TERAJ M: Gene expression profiling of the effect of high-dose intravenous Ig in patients with Kawasaki disease. *J Immunol* 2005; 174: 5837-45.
 54. VIEMANN D, STREY A, JANNING A *et al.*: Myeloid-related protein 8 and 14 induce a specific inflammatory response in human microvascular endothelial cells. *Blood* 2005; 105: 2955-62.
 55. HIRONO K, FOELL D, XING Y *et al.*: Expression of myeloid-related protein-8 and -14 in patients with acute Kawasaki disease. *J Am Coll Cardiol* 2006; 48: 1257-64.
 56. BUNCHMAN TE, MAUER SM, SIBLEY RK, VERNIER RL: Anaphylactoid purpura: characteristics of 16 patients who progressed to renal failure. *Pediatr Nephrol* 1988; 2: 393-7.
 57. COUNAHAN R, WINTERBORN MH, WHITE RH *et al.*: Prognosis of Henoch-Schönlein nephritis in children. *Br Med J* 1977; 2: 11-4.
 58. KAKU Y, NOHARA K, HONDA S: Renal involvement in Henoch-Schönlein purpura: a multivariate analysis of prognostic factors. *Kidney Int* 1998; 53: 1755-9.
 59. KAWASAKI Y, OHARA S, ABE Y *et al.*: The role of serum myeloid-related protein 8/14 complex in Henoch-Schönlein purpura nephritis. *Pediatr Nephrol* 2012; 27: 65-71.
 60. WITTKOWSKI H, KUEMMERLE-DESCHNER JB, AUSTERMAN J *et al.*: MRP8 and MRP14, phagocyte-specific danger signals, are sensitive biomarkers of disease activity in cryopyrin-associated periodic syndromes. *Ann Rheum Dis* 2011; 70: 2075-8.
 61. DZHNDOIAN ZT: Serum level of myeloid related protein MRP8/14 calprotectin in Armenian patients with Familial Mediterranean Fever. *Ter Arkh* 2012; 84: 45-8.