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Review Article

Faecal Calprotectin: A reliable biomarker in Inflammatory bowel disease

Dr. Ripon Barua[1], Dr. Jogendra Nath Sarker[2]

[1]Lecturer of Microbiology, Sir Salimullah Medical College, Mitford, Dhaka. [2]Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University.

Abstract:

Calprotectin a calcium and zinc binding protein, is a breakdown product of WBC which can be detected and quantified in both stool and plasma and is markedly elevated in infectious and inflammatory conditions, including inflammatory bowel disease (IBD). Reference ranges for faecal calprotectin (FC) have been established in healthy adults and children and elevated concentrations of FC have been demonstrated in numerous studies of patients with IBD. The overall sensitivity and specificity for FC in IBD found to be as high as 78-100% and 76-100% respectively. The FC correlates well with histological inflammation as detected by colonoscopy with biopsies and has been shown successfully to predict relapses in patients with IBD. The FC has been shown to consistently differentiate IBD from irritable bowel syndrome (IBS) because it has excellent negative predictive value in ruling out IBD in undiagnosed, symptomatic patients. The FC may be also useful in determining whether clinical symptoms in patients with known IBD are caused by disease flares or non inflammatory complications/underlying IBS and in providing objective evidence of response to treatment. Although more studies are needed to define fully the role of FC, convincing studies and growing clinical experience point to an expanded role in the diagnosis and management of IBD.

Key words: Inflammatory bowel disease, Inflammation, Calprotectin

Introduction:

Inflammatory bowel disease (IBD) is a chronic condition characterized by recurrent episodes of inflammation in the gastrointestinal tract and includes Crohn's disease (CD) and ulcerative colitis (UC).[1] Serological and hematological parameters are used widely to assess intestinal inflammation in IBD but these systemic markers have low sensitivity and specificity for intestinal inflammation and correlate poorly with symptoms and clinical disease activity indexes.[2] Imaging studies such as CT and MRI scans, barium enemas can be useful in localizing intestinal inflammation but these studies often are expensive, have suboptimal sensitivity and/or specificity, and may be invasive or expose the patient to ionizing radiation. Endoscopy with biopsies is considered the gold standard for diagnosis of IBD and is also a tool for estimation of disease activity and efficacy of therapy.[3] But it is unsuitable for frequent use because it is an invasive and expensive procedure and often pose risk of various complications.[2,3] Non invasive, simple, sensitive, specific and harmless markers of intestinal inflammation have been looked for in recent years.[2] Calprotectin, a break down product of WBC was identified as a good marker to assess intestinal inflammation in IBD.[3,4] Faecal calprotectin (FC) is supposed to be a reliable biomarker that correlates more closely to histological than to macroscopic intestinal inflammation.[3]

Calprotectin and Inflammatory bowel disease:

Molecular structure and nomenclature of calprotectin:

The existence of the protein now known as calprotectin was suspected in the late 1970s. At that time, Fagerhol and co-workers searched for a marker of leukocyte turnover and in 1980 they published their discovery of a protein abundant in the cytoplasm of neutrophils. Provisionally they named it L1 or leukocyte derived L1 protein.[5] This protein was later shown to be a calcium-binding heterocomplex with a total molecular mass of 36.5 KDa and consisting of one light chain ($L1_L$) and two heavy chain ($L1_H$).[6,7] The name calprotectin was proposed when the protein was found to have antimicrobial properties and thereby a putative protective function.[8]

There are different nomenclatures of this protein given by different research groups during their study. The light chain of this protein was shown to be identical with the cystic fibrosis associated antigen (CFAg) described for the first time in 1973 when an abnormal protein band was found by isoelectric focusing of serum from patients with cystic fibrosis.[9] Other groups have used additional names for the light and heavy chains as Calgranulin A and B or myeloid-related protein 8 and 14 (MRP-8/14).[10,11] In recent time the name S100A8/S100A9 is frequently used for the heterocomplex to demonstrate that the protein belongs to the calcium-binding S100 family. The nomenclature of the S100 proteins was established according to the organization of the S100 genes. The complex form of this protein seems to be a prerequisite for biological functions. Diverse oligomeric structures of the protein have been found and the functional properties may vary among different types of complex formations. Recently a $(S100A8/S100A9)_2$ - tetramer formation was demonstrated in the presence of zinc and calcium.[12]

Origin and distribution of calprotectin:

The gene for calprotectin and other proteins from the S-100 protein family have been mapped to chromosome 1q21.[13] Calprotectin is found primarily within cells derived from the myelomonocytic cell lineage, e.g. predominantly in neutrophils, monocytes and macrophages but not in resting B or T lymphocytes. It is present both in the cytoplasm and on the plasma membrane in neutrophils and monocytes. Blood monocyte and tissue macrophages in acute inflammation are positive in calprotectin expression, whereas resident macrophages and macrophages present in chronic inflammation are negative.[14] Calprotectin is also detected in some cell types in addition to the cells in myeloid cell lineages.As an example, it was detected in keratinocytes in inflammatory dermatoses and squamous cell carcinoma, in a subset of micriglia in brain tissue with Alzheimer's disease and occasionally in endothelial cells, kidney tubules etc.[15,16]

In the neutrophils, calprotectin constitutes 5% of the total proteins and approximately 60% of the cytosolic proteins. Each neutrophil cell contains 5 to 25 picogram calprotectin per cell. In the monocytes, calprotectin accounts for approximately 1.6% of the total protein content.[17] Several research groups have stated the possibility of an extracellular secretion of calprotectin during neutrophil activation or during cell death and also following endothelial adhesion of monocytes. As a result it can be detected and quantified in fluids where inflammation is occurring for example serum, synovial fluid, cerebrospinal fluid, oral fluids, urine and faeces.[18] Elevated calprotectin concentrations have been found in recruitment of inflammatory cells because of an ongoing infection, inflammation or malignant disorder.[19,20]

Biological functions of calprotectin:

The biological properties of calprotectin are not fully known. Existing data show that calprotectin participates in the regulaton of inflammatory process in several ways. Following are some of the postulated biological activities of calprotectin: Immunoregulatory function, inhibition of immunoglobulin synthesis, antimicrobial activity, fungiostatic activity, chemotactic factor, intracellular signal transduction, apoptosis-inducing activity, growth inhibitory effect, cytotoxic effects on various tumor cell lines.[8,21,22]

Calprotectin is known to be both a calcium and zinc binding protein. By binding to zinc, it can reduce the local concentration of zinc. Thereby it deprives the microorganisms of zinc and also inhibits many zinc dependent enzymes. Zinc chelation that is mediated by histidine-rich regions of calprotectin represents an

important antimicrobial mechanism in host defense.[23,24] Calprotectin concentrations of 50-250 µg/ml were found to inhibit growth of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, lower concentrations (4-32 µg/ml) are sufficient to inhibit growth of *Candida albicans*. Cells expressing calprotectin are able to resist invasion by *Listeria monocytogenes* and *Salmonella enterica serovar Typhimurium*.[22]

It is likely that calprotectin represents a defense mechanism by protecting neutrophils and other calprotectin-expressing cells against microbes that invade the host's cell cytoplasm. Matrix metalloproteinases (MMPs), a family of zinc dependent enzymes, are important in many normal biological processes including angiogenesis and wound healing but also pathological processes such as inflammation, cancer and tissue destruction. Consequently by inhibiting these enzymes calprotectin is capable of regulating many important processes in the body.[8,22,25] Additionally, calprotectin seems to have growth inhibitory and cell death inducing effects on various cell types e.g. normal fibroblasts and different tumor cell lines. These properties suggest a regulatory role of calprotectin in inflammatory processes through the effect on the survival and/or growth state of fibroblasts and other cells involved in inflammation. The apoptosis inducing activity of calprotectin seems partly to be zinc dependent as well. It has been suggested that excessive concentrations of calprotectin for a long period might be cytotoxic and cause a local delay in tissue repair with subsequent tissue damage in chronic inflammation.[16]

Faecal calprotectin:

Calprotectin is quickly released into the gut at the onset of inflammation from the infiltrated inflammatory cells. Calcium-calprotectin complex to be resistant to both heat and proteolytic enzymes in the gut lumen and remains remarkably stable within faeces at room temperature for at least 7 days, making it possible for the patient to take the sample at home and send it to the laboratory by ordinary mail.[26] For this reason it is superior to relatively less stable faecal markers like neutrophil elastase, tumor necrosis factor-α (TNF-α), eosinophilic cationic protein (ECP) .[26,27]

Faecal calprotectin (FC) concentration is about 6 times that of normal plasma. Markedly elevated levels of calprotectin have been detected in the faeces of patients with infectious and inflammatory bowel conditions like active IBD, intestinal neoplasm, intestinal polyp, intestinal tuberculosis, NSAID induced enteropathy, infective gastroenteritis etc.[28] Young infants and children up to the age of 2 years have raised FC level and also following gastrointestinal surgery for IBD and other causes up to 2 months.[29] The raised FC level appears to be most apparent in the first week after birth which may be part of the body's physiological defence mecanism against yeasts and fungi, allowing early development of gut homeostasis.[30] Normal faecal calprotectin level is seen in healthy persons, patients with irritable bowel syndrome (IBS) and IBD in remission / mucosal healing.[2,18]

Roseth *et al.* first described the original enzyme-linked immunosorbent assay (ELISA) method for isolating and quantifying calprotectin in stool in 1992.[26] Polyclonal rabbit anti calprotectin antibodies were used in the ELISA and the results were provided in milligram calprotectin per litre of faecal homogenate. Since then, an improved, commercially available, validated ELISA has been developed in which smaller stool samples (100 mg) are extracted with 5 ml buffer in a closed tube. This assay is standardized well with ^{111}Indium-labeled neutrophilic granulocytes assay which is considered as gold standard for assessing disease activity of IBD. The newer assay measures calprotectin concentration in micrograms per gram rather than in milligrams per liter as in the original assay.[2,3]

Normal level/cut-off value for faecal calprotectin:

Studies show a variation of FC between 50-100 µg/g has been observed among normal healthy population.[31] Some studies show 50 µg/g of FC to be significant for IBD patients,[27,32] while it is 100 µg/g for others.[4]

The uses of faecal calprotectin in IBD:

I. Diagnostic exclusion of active IBD:

The FC is a measure of local gut inflammation rather than systemic inflammation which is not always apparent in IBD. It is not surprising therefore, that calprotectin has been demonstrated as more effective in identifying disease activity than other inflammatory markers such

as serum measurements of C-reactive protein, leucocyte count or erythrocyte sedimentation rate.[33] It has been demonstrated that FC level is significantly higher in patients with active IBD than in healthy controls and those with IBS. In this setting the overall sensitivity and specificity for FC may be as high as 78–100% and 76–100%, respectively.[30] Good sensitivity and specificity of FC detection in IBD, healthy persons and in IBS has been reported in several studies.[3,34] Among the Italian population, Carroccio *et al.* 2003 reported 100% sensitivity and 95% specificity for FC detection in IBD cases.[35] while in Egypt, Saadany *et al.* 2008 reported 100% sensitivity and 92% specificity.[4] Therefore measurement of FC by the physicians has changed the approach for management of IBD patients. A recent meta-analysis from Netherland reported that, there has been a significant reduction of colonoscopy by 67% in case of adult and 35% in case of children because of introducing FC as screening marker for IBD.[36] A normal FC level in a patient with active symptoms is therefore very reassuring and may be enough, in the correct context where the clinical risk of malignancy is low, to avoid further colonic investigation such as radiology or colonoscopy and allow a positive diagnosis of functional diarrhea or IBS at first clinic visit.[30]

II. Longitudinal monitoring of IBD:

Although crohn's disease activity index (CDAI) and ulcerative colitis activity index (UCAI) may give an indirect suggestion of disease activity, they are not direct measures of mucosal inflammation and may be raised by non-inflammatory symptoms. The FC may challenge the supremacy of the CDAI and UCAI by providing a real time indication of disease activity that can be measured longitudinally and that correlates well with clinical, endoscopic and histological activity in IBD. Normalization of calprotectin is a surrogate marker of endoscopic mucosal healing in patients being treated for active IBD.[37] Mucosal healing may be the optimal outcome of therapy in IBD, as it is associated with more sustained clinical remission and a reduced need for surgery.[38] A raised or normal biomarker result in a patient with IBD can therefore be a very useful decision making aid when investigating changing symptoms that may be due to an inflammatory flare of the disease, co-existing IBS, non inflammatory complications (eg, adhesions), or post operative changes such as bile acid malabsorption or short bowel syndrome.[39]

Faecal biomarker may also be of clinical use to monitor Crohn's disease following intestinal resection. In this setting inflammatory relapse is common, but can be difficult to identify clinically as symptoms may be due to IBS, bile salt malabsorption or altered gut anatomy, often necessitating radiological or endoscopic investigation. A UK study has shown that, in the normal postoperative period FC normalizes within 2 months. Therefore a single biomarker measurement in patients with symptoms after this time point may aid decision making about further investigation for surgical complications or disease recurrence if the biomarker is positive, or a trial of conservative treatment such as loperamide or cholestyramine if the biomarker is within the normal range.[39]

III. Predicting the clinical course of IBD:

An important question is whether single or sequential measurement of faecal biomarkers can predict the future course of IBD? If so, their use in clinical practice is potentially vast, allowing early counseling regarding the need for institution of immunosuppressive or biological drugs, or surgery. Equally biomarker measurement may allow discontinuation of unnecessary drugs in those at high likelihood of staying in remission long term.[40,41]

Plasma calprotectin:

The half life of plasma calprotectin is calculated to be 5 hours. Elevated plasma calprotectin concentrations found in patients with various inflammatory or malignant disorders and seem to reflect in increased leucocyte turnover or possibly the release of calprotectin at activation or cell death of these cells. In patients with severe bacterial infections the plasma calprotectin levels can rise upto 40 to 130 times the normal, while viral infections show normal or only slightly elevated calprotectin levels. At least in bacterial conditions, the plasma calprotectin concentrations tend to remain elevated for 2 to 3 weeks after tissue damage. Plasma calprotectin level is also elevated in other inflammatory conditions like sepsis, rheumatoid arthritis, cystic fibrosis, deep abscesses etc.[16] This can be explained by the involvement of neutrophils and macrophages in the

tissue repair processes, which will continue long after cessation of thew inflammatory activity. Plasma calprotectin is considered to be less reliable as a marker of gastrointestinal inflammation but comparative studies are missing.[17,19] Fagerhol *et al.* used a radioimmunoassay to analyze plasma calprotectin concentrations for the first time in healthy adults in 1980.[5] The normal plasma calprotectin concentrations were then found to be significantly higher among males (120 to 660 µg/L) than females (90 to 530 µg/L). Plasma calprotectin can now be measured with an ELISA method.[5]

Cautions and limitations of using faecal calprotectin:

It is important to remember that despite all of this promising data, faecal biomarker quantification as a screening test for IBD cannot fully replace conventional endoscopy and radiology. IBD remains a histological diagnosis requiring intestinal biopsies, so a positive calprotectin will require further tests not only to confirm or refute a diagnosis of IBD, but also to exclude other causes of raised biomarkers including malignancy, polyps, viral or bacterial gastroenteritis, NSAID enteropathy, untreated coeliac disease or gastro-oesophageal reflux disease.[42] Therefore the use of faecal biomarkers should be restricted to situations where the result along with clinical judgement informs a decision about treatment, allows avoidance of invasive tests, or where invasive tests have not provided a satisfactory conclusion-for example, a normal colonoscopy but clinical suspicion of small bowel inflammation. It is also necessary to appreciate that inflammation is only one component of IBD and it is only with regard to this that faecal biomarkers are of clinical use. Symptomatic patients with IBD who have normal biomarkers should not simply be labeled as having IBS without further consideration. Patients may have symptoms due to non-inflammatory mechanical disease such as bile salt malabsorption, fibrotic strictures or adhesions and these are likely to be best treated by identifying the correct problem and giving specific treatment.[30]

Furthermore, symptoms remain the most powerful mediators of patient behaviour and decision making. As such the greatest challenge to incorporating faecal biomarkers into management pathways may be when there is disparity between how a patient feels and what faecal biomarkers inform us regarding their current inflammatory activity. For instance, it may be very difficult trying to encourage a patient with no symptoms and a positive faecal biomarker that they should begin powerful immunosuppressant treatment that poses risk of side effect.[30]

Conclusion and the future for faecal calprotectin in *IBD:*

The outlook for faecal calprotectin in clinical practice appears promising. Potentially in the future faecal biomarker measurement will be standard prior to first gastrointestinal outpatient clinic visit for those with abdominal pain or diarrhoea. This may allow faster triage and assessment of patients and perhaps the avoidance of unnecessary investigation in those with functional diarrhoea or IBS. As surrogate markers of mucosal healing in IBD, calprotectin may allow objective mapping of an individual patient response to treatment and quantify the likelihood of future relapse. If this test can be used to demonstrate an adequate response to biological therapy, they may aid decisions regarding dose escalation, shortening of dosage interval, switching to an alternative agent or ultimately withdrawal of unnecessary immunosuppressant for those in sustained deep remission. Equally future trials may tell us whether consistent suppression or elevation of calprotectin convey prognostic significance in terms of hospitalization, the need for surgery and an effect on quality of life.[30]

In summary, faecal biomarkers will never fully replace colonoscopy and radiology which are necessary to obtain tissue samples and investigate the complications of IBD. However, in a society where patient satisfaction, risk minimization, cost reduction and hospitalization avoidance are a priority, these non-invasive, inexpensive, reproducible and clinically significant measurements are likely to have a greater role in our future diagnostic and therapeutic pathways.

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