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FECAL CALPROTECTIN IN CHILDREN WITH SPECIAL REFERENCE TO INFLAMMATORY BOWEL DISEASE

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**Karolinska
Institutet**

Stockholm 2007

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Published and printed by Universitetservice US-AB
Karolinska Institutet, Nanna Svartz väg 4, SE-171 77 Stockholm, Sweden
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ISBN 91-7357-013-3

To my family with all my love

LIST OF PAPERS

The studies will be referred to by their Roman numerals in the thesis.

- I. Fagerberg UL, Lööf L, Merzoug RD, Hansson L-O, Finkel Y
Fecal Calprotectin Levels in Healthy Children Studied With an Improved Assay
Journal of Pediatric Gastroenterology and Nutrition 2003;37:468-472

- II. Fagerberg UL, Lööf L, Myrdal U, Hansson L-O, Finkel Y
Colorectal Inflammation Is Well Predicted by Fecal Calprotectin in Children With Gastrointestinal Symptoms
Journal of Pediatric Gastroenterology and Nutrition 2005;40:450-455

- III. Fagerberg UL, Lööf L, Lindholm J, Hansson L-O, Finkel Y
Fecal Calprotectin - A Quantitative Marker of Colonic Inflammation in Children With Inflammatory Bowel Disease
Submitted

- IV. Fagerberg UL, Lööf L, Lindholm J, Hansson L-O, Finkel Y
Serum Amyloid A, High Sensitivity C-Reactive Protein and Calprotectin as Markers of Inflammation in Pediatric Inflammatory Bowel Disease
Submitted

ABSTRACT

This thesis aims to study the clinical usefulness of fecal calprotectin as a noninvasive marker of colonic inflammation in children with suspected or confirmed chronic inflammatory bowel disease (IBD). Calprotectin, a calcium-binding protein predominantly expressed in neutrophils, is stable in feces for several days, and can be measured by an enzyme-linked immunosorbent assay.

Gastrointestinal symptoms as abdominal pain, diarrhea, bloody stools, and weight loss are common in children presenting with IBD. However, the symptoms can be vague, or even similar to the symptoms of other more common gastrointestinal disorders and functional complaints. Early recognition of IBD is important to prevent adverse effects such as delayed onset of puberty, impaired growth, and unnecessary suffering. The routine investigations include blood tests, fecal cultures, endoscopy, and radiological examinations. Endoscopy with histological examinations of biopsy specimens is the gold standard for diagnosis. It is also used for objective estimation of disease activity and to monitor the efficacy of treatment. However, endoscopy is unsuitable for frequent use as it is an invasive and costly procedure requiring careful bowel preparation and, in children, general anesthesia.

Study I establishes *reference values for fecal calprotectin* by analyzing fecal samples from 117 healthy children and adolescents. The conclusion was that the upper reference value for fecal calprotectin concentration is $<50 \mu\text{g/g}$ in boys and girls from 4 through 17 years of age.

Study II evaluates the *feasibility of fecal calprotectin to detect colorectal inflammation in children*. Fecal samples were collected from 36 children with gastrointestinal symptoms suggestive of IBD before undergoing colonoscopy. Elevated fecal calprotectin concentrations strongly predicted the presence of IBD or other colorectal inflammation, and the test had a sensitivity of 95% and specificity of 93%. Thus, fecal calprotectin can be used as a diagnostic tool to facilitate selection of children who should undergo diagnostic colonoscopy.

Study III aimed to *evaluate fecal calprotectin as a quantitative marker of inflammatory activity in pediatric IBD*. Thirty-nine children with IBD delivered fecal samples and underwent colonoscopies. The results demonstrated that fecal calprotectin is a valid surrogate marker for quantitative estimation of colonic inflammation in pediatric IBD. Normalized fecal calprotectin concentration seems to indicate complete, histological mucosal healing.

Study IV compared *plasma calprotectin, high sensitivity C-reactive protein, and serum amyloid A with fecal calprotectin and routine blood tests* as markers of histological inflammation in 32 children with IBD. Fecal calprotectin measurement was found to be a more reliable test for estimation of histological inflammatory activity in the colon.

In conclusion, the present thesis demonstrates that fecal calprotectin is a simple and noninvasive method that can be used as a sensitive diagnostic tool to detect colorectal inflammation and IBD in children with gastrointestinal symptoms. Further, the fecal calprotectin method was shown to be useful as a quantitative, surrogate marker of colonic inflammatory activity. The simplicity of obtaining and analyzing fecal calprotectin will facilitate the care of children with gastrointestinal symptoms as well as the monitoring of inflammatory activity in pediatric IBD.

Keywords: biological markers, calcium-binding proteins, Leukocyte L1 Antigen Complex, calprotectin, acute-phase proteins, serum amyloid A, C-reactive protein, ELISA, feces, blood, children, adolescent, colitis, inflammatory bowel disease, colonoscopy, reference values, diagnosis.

ISBN 91-7357-013-3

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LIST OF ABBREVIATIONS

AUC	Area under curve
CD	Crohn's disease
CI	Confidence interval
CRP	C-reactive protein
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ESPGHAN	European Society of Pediatric Gastroenterology Hepatology and Nutrition
ESR	Erythrocyte Sedimentation Rate
hsCRP	High sensitivity C-reactive protein
IC	Indeterminate colitis
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IL	Interleukin
MMP	Matrix metalloproteinases
NK-cells	Natural killer cells
NSAID	Non-steroidal anti-inflammatory drug
ROC	Receiver operating characteristic
SAA	Serum Amyloid A
TNF-α	Tumor necrosis factor - alpha
URT	Upper respiratory tract
UC	Ulcerative colitis

1 INTRODUCTION

Gastrointestinal symptoms as abdominal pain and diarrhea are common problems in children and adolescents. Different studies report that recurrent abdominal pain affects as many as 9% to 19% of schoolchildren enough to interfere with normal daily activity (1, 2). Abdominal pain is one of the most common reasons for seeking medical help during childhood. Both abdominal pain and diarrhea are frequently seen in functional gastrointestinal disorders in children, but can also be symptoms of various organic disorders (2). Consequently, a thorough diagnostic workup usually has to be performed to find the underlying cause and enable appropriate treatment.

In some children, abdominal pain and diarrhea are the first presenting symptoms of inflammatory bowel disease (IBD), a chronic gastrointestinal disease with unknown etiology. IBD comprises two major forms: Crohn's disease (CD) and ulcerative colitis (UC). Most patients with CD and UC have an intermittent course with periods of relapse and remission and commonly need lifelong follow-up. In recent decades, pediatric IBD has become more common in several Western countries, including Sweden (3, 4). An increased incidence has been reported from Stockholm, Sweden where 5.2 IBD cases /100 000 children (aged 0 - 15 years) were detected in year 1990 through 1992, compared to 10.5 / 100 000 children in year 1999 through 2001 (3). Because symptoms can be vague and insidious, it is not unusual to find a long delay between the first appearance of symptoms and diagnosis of IBD (5). Often, the disease is first detected when symptoms such as bloody stools, delayed puberty, weight loss, or impaired growth have appeared. In most children the onset of IBD occurs around the time of puberty. This is a vulnerable period in life, involving psychosocial and physical changes. Hence, early detection of IBD is essential to avoid adverse effects and unnecessary suffering.

Several tests and investigations, e.g. blood tests, fecal cultures, endoscopy, and radiology are needed to diagnose IBD in children. Endoscopy with histological assessment of biopsy specimens is considered to be the gold standard method for diagnosing IBD and for differentiation into UC or CD. This method is also used for macroscopic and microscopic assessments of the extent and severity of mucosal inflammation and to evaluate the efficacy of treatment. However, endoscopy cannot be used frequently as it is an invasive and expensive procedure. Furthermore, endoscopy is a difficult investigation for the patient since it requires careful bowel preparation and, in children, also sedation or general anesthesia. Inflammatory markers in peripheral blood, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), orosomucoid, albumin, and blood count are routine analyses in IBD. These tests can, however, show a normal result despite the presence of mucosal inflammation. Hence, reliable noninvasive methods are needed.

Calprotectin is a calcium and zinc-binding protein, which is abundant in neutrophil granulocytes but also in monocytes and macrophages (6). The protein can be measured in feces, in plasma, and in other body fluids. Increased concentrations of

fecal calprotectin were first reported in adults with IBD or gastrointestinal cancer (7). Prior to the work initiated in this thesis, no pediatric studies had been presented on fecal calprotectin. Furthermore, existing studies in adult IBD patients were based on an original enzyme-linked immunosorbent assay (ELISA) method. To evaluate fecal calprotectin as a marker of IBD or other colonic inflammation in children, the following 4 studies were performed with a new and improved fecal calprotectin ELISA:

Study I aimed to establish reference values for fecal calprotectin in healthy children and adolescents. *Study II* evaluated the role of fecal calprotectin as a diagnostic tool of gastrointestinal inflammation and inflammatory bowel disease (IBD) in children. *Study III* investigated the validity of fecal calprotectin as a surrogate marker for quantitative assessment of endoscopic and histological colonic inflammation in pediatric IBD. *Study IV* compared plasma calprotectin and the two plasma acute phase proteins, high sensitivity C-reactive protein and serum amyloid A, with fecal calprotectin and routine blood tests as markers of histological inflammation in pediatric IBD.

2 BACKGROUND

2.1 CALPROTECTIN

2.1.1 Molecular structure and nomenclature

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 (8). This protein was later shown to be a calcium-binding heterocomplex with a total molecular mass of 36.5 kDa (9) consisting of one light chain ($L1_L$) and two heavy chains ($L1_H$) (10, 11). The name calprotectin was proposed when the protein was found to have antimicrobial properties and thereby a putative protective function (12).

In this thesis we use the name calprotectin consistently. However, independent research groups have studied this protein under various names in recent decades (Table 1). The light chain 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 (13-15). Other groups have used additional names for the light and heavy chains as Calgranulin A and B (16, 17) or myeloid-related protein 8 and 14 (MRP-8/14) (18). Currently, the name S100A8/S100A9 is frequently used for the heterocomplex to demonstrate that the protein belongs to the calcium-binding S100 protein family (14, 19, 20). The nomenclature of the S100 proteins was established according to the organization of the S100 genes (21). The complex form of the 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 (22).

Table 1. Nomenclature of calprotectin.

Nomenclature	References
L1 (L1 light chain and L1 heavy chain) = Calprotectin	(8-12)
Cystic fibrosis protein (P8,14)	(13-15)
Calgranulins A/B	(16, 17)
MRP 8/14	(18)
S100A8/S100A9	(14, 19, 20)

2.1.2 Cells of the immune system

The cells of the immune system arise from pluripotent hematopoietic stem cells through two main lines of differentiation; the lymphoid and the myeloid lineage. In the lymphoid lineage the cells differentiate into T cells, B cells, or natural killer cells. The myeloid lineage produces phagocytes (monocytes, macrophages, and granulocytes) and other cells as megakaryocytes for platelet production. Three different kinds of granulocytes exist; neutrophils, eosinophils, and basophils. These cells have cytoplasmic granules whose staining gives these cells a distinctive appearance in blood smears. Because of their irregularly shaped nuclei the granulocytes are also called polymorphonuclear leukocytes. In healthy adults, about 1×10^{11} granulocytes are released daily from the bone marrow to replace normal losses, but the number can increase 10-fold during severe infections. Granulocytes have a transit time of 6 to 7 hours in circulation before they reach the tissue, and their total lifetime is 2 to 3 days. The granulocytes enter tissues only at sites of infection or inflammation by migration through the vessel wall. The neutrophils are recruited to phagocytose bacteria, while the eosinophils and basophils are recruited to the sites of allergic inflammation. Circulating monocytes also enter the tissues, where they differentiate into phagocytic macrophages. Monocytes and macrophages may live for months or years (23, 24).

2.1.3 Neutrophils

This section describes the neutrophil granulocyte in greater detail since calprotectin is expressed predominantly in this cell. The major storage organ for mature neutrophils is the bone marrow, which contains about 7 times the intravascular pool of neutrophils. In response to cytokines, i.e. inflammatory messenger substances, and other mediators of inflammation the neutrophils are released from the bone marrow into the blood stream. In the blood the neutrophils constitute most of the leukocytes (about 60%-70% in human adults and 40%-60% in children and adolescents) (23, 24).

The neutrophils are the body's first-line defense against microorganisms and other infectious agents. When stimulated by different chemotactic agents (e.g. complement C5a, products of other leukocytes, platelets, and certain bacteria) they migrate from the vessels into the tissue where they eliminate pathogens either within the cell following phagocytosis, or outside the cell by releasing toxic mediators. Neutrophils have a large arsenal of mediators, i.e. enzymes and antibacterial proteins stored in azurophilic (primary) granules, specific (secondary) granules or other compartments of the neutrophils (Figure 1). The neutrophils also secrete cytokines to recruit other inflammatory cells. Eventually the neutrophils undergo programmed cell death, i.e. apoptosis. Apoptosis is critical for maintaining cellular homeostasis, and the accumulated neutrophils need to be safely removed to resolve the inflammation. However, there is increasing evidence that defective phagocytic clearance of apoptotic neutrophils and/or aberrant or delayed apoptosis may contribute to the pathogenesis of IBD and other autoimmune disorders. Consequently, the tissue is damaged because of neutrophil accumulation and uncontrolled release of toxic substances into the tissue (25-27).

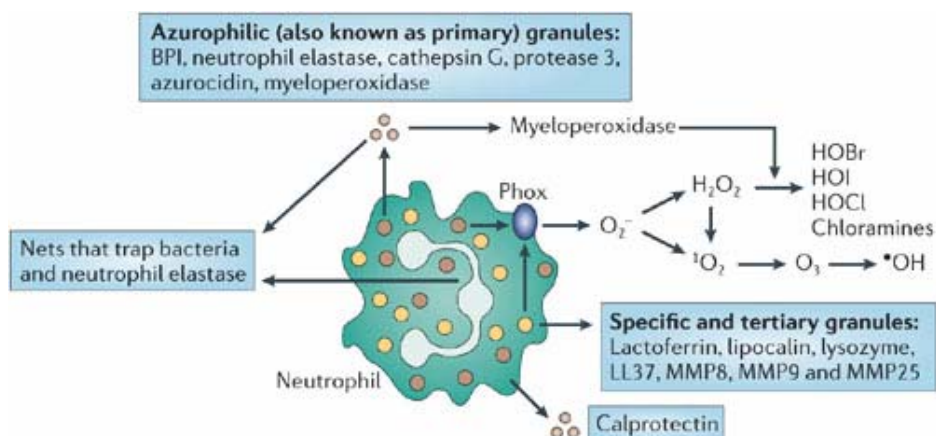


Figure 1. Neutrophils deliver multiple antimicrobial molecules including calprotectin.

Microbicidal products arise from most compartments of the neutrophil: azurophilic granules, specific granules and tertiary granules, plasma and phagosomal membranes, the nucleus and the cytosol. BPI = bactericidal permeability increasing protein; H_2O_2 = hydrogen peroxide; HOBr = hypobromous acid; HOCl = hypochlorous acid; HOI = hypoiodous acid; MMP = matrix metallo-proteinase; 1O_2 = singlet oxygen; O_2^- = superoxide; O_3 = ozone; OH = hydroxyl radical; phox = phagocyte oxidase. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews / Immunology (27).

2.1.4 Origin of calprotectin

The genes for calprotectin and the other proteins from the S-100 protein family have been mapped to chromosome 1, q12-q21 (19). Calprotectin is found primarily within cells derived from the myelomonocytic cell lineage, i.e. predominantly in neutrophils, monocytes, and macrophages but not in resting B or T-lymphocytes (6, 28-31). It is also a keratinocyte protein found in squamous epithelia, except for normal skin (32).

Calprotectin is present both in the cytoplasm and on the plasma membrane in neutrophils and monocytes, also those in a resting state (6). In the neutrophils, calprotectin constitutes 5% of the total proteins and approximately 60% of the cytosolic proteins (8, 10). Each neutrophil cell contains 5 to 25 picogram calprotectin per cell (33, 34). In the monocytes, calprotectin accounts for approximately 1.6% of the total protein content (35).

Several research groups have stated the possibility of an extracellular secretion of calprotectin from stimulated neutrophils (6, 36, 37) and monocytes (38), but calprotectin is also released as a result of cell disruption or death (37, 39). Calprotectin can be measured in plasma (40), synovial fluid (41), cerebrospinal fluids (42), oral fluids (43), urine (44), and feces (7). Elevated calprotectin concentrations have been found in recruitment of inflammatory cells because of an ongoing infection, inflammation, or malignant disorder (40, 45, 46)

2.1.5 Biological functions

The biological properties of calprotectin are not fully known. Existing data show that calprotectin participates in the regulation of inflammatory processes in several ways. Table 2 lists some of the postulated biological activities of calprotectin.

Several studies have demonstrated antimicrobial and antifungicidal activities of calprotectin (12, 47, 48). Calprotectin is known to be both a calcium- and zinc-binding protein (9, 49). By binding to zinc, calprotectin can reduce the local concentration of zinc. Thereby, calprotectin deprives the microorganisms of zinc (50, 51) and also inhibits many zinc-dependent enzymes (52). 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.

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 (53-56). These properties suggest a regulatory role of calprotectin in inflammatory processes through its 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 (57). 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 (58).

Table 2. Biological functions of calprotectin.

Biological functions	References
Immunoregulatory function	(58, 59)
Inhibition of immunoglobulin synthesis	(60)
Antimicrobial activity	(12, 47, 48)
Fungistatic activity	(12, 47, 48)
Chemotactic factor	(61, 62)
Intracellular signal transduction	(48)
Apoptosis-inducing activity	(53, 56)
Growth inhibitory effect	(54, 55)
Cytotoxic effects on various tumor cell lines	(53)

2.1.6 Plasma calprotectin

Fagerhol et al used a radioimmunoassay to analyze plasma calprotectin concentrations for the first time in healthy adults in 1980. 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) (33, 63). Plasma calprotectin can now be measured with an ELISA method, but reference values have not yet been established. EDTA plasma samples have been recommended since EDTA stabilizes the cell membranes and effectively inhibits the release of calprotectin and other proteins from leukocytes (8, 10, 63). Furthermore EDTA prevents, at least partly, proteolytic cleavage of proteins.

The half-life of plasma calprotectin is calculated to be 5 hours (34). Elevated plasma calprotectin concentrations are found in patients with various inflammatory or malignant disorders and seem to reflect an increased leukocyte turnover (10, 35, 40, 64), or possibly the release of calprotectin at activation or cell death of these cells (6, 33). In patients with severe bacterial infections the plasma calprotectin levels can rise up to 40 to 130 times the normal, while viral infections show normal or only slightly elevated calprotectin levels (40). At least in bacterial conditions, the plasma calprotectin concentrations tend to remain elevated for 2 or 3 weeks after tissue damage. This can be explained by the involvement of neutrophils and macrophages in the tissue repair processes, which will continue long after cessation of the inflammatory activity (40). Plasma calprotectin is considered to be less reliable as a marker of gastrointestinal inflammation, but comparative studies are missing.

2.1.7 Fecal calprotectin

Roseth et al first described the original ELISA method for fecal calprotectin in 1992 (7). Polyclonal rabbit calprotectin antibodies were used in the ELISA, and the results were provided in “milligram calprotectin per liter of fecal homogenate”. In a group of 33 healthy adults, the median value of fecal calprotectin was 2 mg/L (range 0.5 - 8 mg/L) (7). A reference interval of 0.9 - 6.7 mg/L was calculated (i.e. between the 5th and 95th percentile), but for convenience 10 mg/L was chosen as the upper reference limit (65). The fecal calprotectin concentrations in healthy adults were approximately 6 times that of normal plasma calprotectin concentrations. Measurement of fecal calprotectin in a spot sample was found to reflect the average daily excretion of calprotectin. An earlier paper reported the calcium-calprotectin complex to be resistant to both heat and proteolytic enzymes (10). Calprotectin in feces was shown to be stable up to 7 days at room temperature (7), making it possible for the patient to take the sample at home and send it to the laboratory by ordinary mail.

In adults, elevated fecal calprotectin levels were detected with the original method in chronic IBD (7, 66, 67) and in gastric cancer, colorectal cancer, and colonic polyps (65, 68). Kristinsson et al found elevated fecal calprotectin concentrations in 87% of the patients with colorectal cancer, but the excretion of calprotectin was not related to the size, localization, stage (Dukes A-D), or histopathological grading of the tumor (69). The excretion of calprotectin in feces seems to be related to the flux of neutrophils and mononuclear cells into the gut wall, their turnover, and their migration into the gut

lumen (66, 70). This theory was supported by the finding of a correlation between excretion of indium-111-labelled neutrophils and calprotectin concentration in feces (66, 67).

The first methodology paper about the improved ELISA assay for fecal calprotectin was published in year 2000 (71). A better extraction yield was achieved by using dissociating agents in the extraction solution in conjunction with a higher dilution of the sample. A 1- to 6-fold increase in the calprotectin concentration was noted in samples with a normal calprotectin value (<10 mg/L original method), whereas samples with high fecal calprotectin values showed a higher increase. Consequently, the separation between normal and pathological values is better with the improved method, and there is, on average, approximately a 5-fold increase in fecal calprotectin concentrations. Additionally, the sample size has been reduced from 5 g to 120 mg feces, and the results are now expressed as micrograms of fecal calprotectin per gram wet feces. When the improved ELISA assay for fecal calprotectin was studied in 59 healthy adults, the median fecal calprotectin concentration was 26 µg/g (range 4-262 µg/g) and the cutoff was suggested to be <50 µg/g for adults (71). In studies I-IV we used the improved ELISA assay.

2.2 INFLAMMATORY BOWEL DISEASE

This section presents a brief overview of chronic inflammatory bowel disease (IBD) with special reference to pediatric IBD.

2.2.1 Definitions

Inflammatory bowel disease is a group of disorders with chronic and relapsing inflammation of unknown etiology. Diagnosis of the two main forms, i.e. Crohn's disease (CD) and ulcerative colitis (UC), is based on clinical presentation, endoscopic and histological features, and radiological abnormalities (72). Endoscopy with mucosal sampling is the gold standard method of diagnosis. Although CD and UC have many similarities, there are also several clinical and pathological differences. The distribution pattern and the macroscopic and histological profiles differ as described below.

Crohn's disease

Crohn's disease is named after Dr Burrill Crohn (1884-1984), an American gastroenterologist, who made his first observation of "regional ileitis" in a 17-year-old boy in 1932 (73). Today, CD is known to be characterized by segmental, discontinuous, and transmural inflammation involving any part of the intestinal tract, from the oral cavity to the anus. In some individuals CD is complicated with perianal abscess, fistula formation, or fibrostenosing processes. The histopathological finding of granuloma is pathognomonic for CD. Granulomas can be found in 25%-70% of CD cases, and the frequency is higher in children than in adults (74, 75).

Ulcerative colitis

The term "ulcerative colitis" was first used by Sir Samuel Wilks (1824-1911), Guy's Hospital, London, in a postmortem case report about a young girl in 1859 (76). Per definition, UC is restricted to the colonic mucosa and the distribution is typically continuous, involving the rectum and to a variable extent the colon in the oral direction. No certain characteristics of the inflammatory reaction are specific for UC.

Indeterminate colitis

A diagnosis of indeterminate colitis is used when a distinct diagnosis of CD or UC cannot be established. In most IBD populations this is the case in 10%-20% of the patients (75, 77).

2.2.2 Epidemiology

Inflammatory bowel disease primarily affects young adults aged 15-35 years. However, in up to 25% of all IBD cases the initial disease manifests during childhood (<18 years). An increased incidence (number of new cases in a year) of IBD, especially CD, has been reported in recent decades, with the highest IBD incidences reported from

developed, urbanized countries, e.g. European countries and North America. Sweden is one of the countries where an increased incidence of IBD, and especially CD, has been observed in both children and adults (3, 78). In 1999-2001 the overall incidence of IBD was 10.5/100.000 children (0-15 years of age) in northern Stockholm, with an incidence of CD in 8.4/100.000 children, UC in 1.8/100.000 children, and IC in 0.2/100.000 children. A 5-fold increase in the incidence of CD was noted from 1990 to 2001. The prevalence of IBD is estimated to be 0.5% in Sweden, i.e. approximately 50 000 individuals.

2.2.3 Etiology and pathogenesis

The etiology and pathogenesis of IBD remains obscure, but the onset of disease seems to be the result from interactions of several factors, e.g. environmental triggers, genetic predisposition, and dysregulation of the gastrointestinal immune system (79-83).

Environmental triggers

The pathogenic cascade of inflammation usually begins with the exposition of an antigen. In IBD the antigen or antigens are unknown, but may be an offending agent such as a bacteria, virus, protein, or other nutritional components in the diet. Normally, at antigen presentation the cells of the immunological defense in the intestinal wall are activated, but in IBD an aberrant immune response causes an abnormal cytokine response and excessive activation of inflammatory cytokines.

Genetics

There is likely a genetic defect that affects how the immune system functions and how the inflammation is turned on and off in response to an antigen in individuals who develop IBD. This genetically determined susceptibility is probably triggered by one or more environmental factors. In CD, the NOD2/CARD15 gene has been established as a genetic susceptibility factor (84), but no specific genes have yet been linked to UC. However, twin studies support the genetic contribution to disease susceptibility in UC and particularly in CD (85).

Immune response

Several immunological mucosal abnormalities have been described in IBD patients. These can be grouped into those that involve the epithelial barrier, those that involve the innate immune response (nonspecific defense against pathogens by phagocytes, dendritic cells, and NK cells), and defects in the adaptive immune response (T and B cells).

CD4⁺ T cells represent the vast majority of activated mononuclear cells that infiltrate the gut wall in IBD. In the lamina propria the CD4⁺ T cells undergo apoptosis or functional differentiation. They predominantly differentiate into T-helper type 1 lymphocytes (T_H1 cells) in Crohn's disease, and in the T_H1 immune response there is production preferably of the cytokines IFN- γ , TNF- α , and IL-12. By contrast, the immune response in UC is characterized by a T_H2 response with increased production of IL-4, IL-5, and/or IL-13 (86). The production of proinflammatory cytokines (IL-

IL-1 β , IL-6, IL-8, TNF- α) by activated macrophages and T lymphocytes seem to be critical in both UC and CD, resulting in the recruitment of effector cells (neutrophils, cytotoxic T lymphocytes) that contribute to development of bowel inflammation and tissue damage (79). Figure 2 demonstrates a working hypothesis regarding the role of cytokines in the pathogenesis of CD (87).

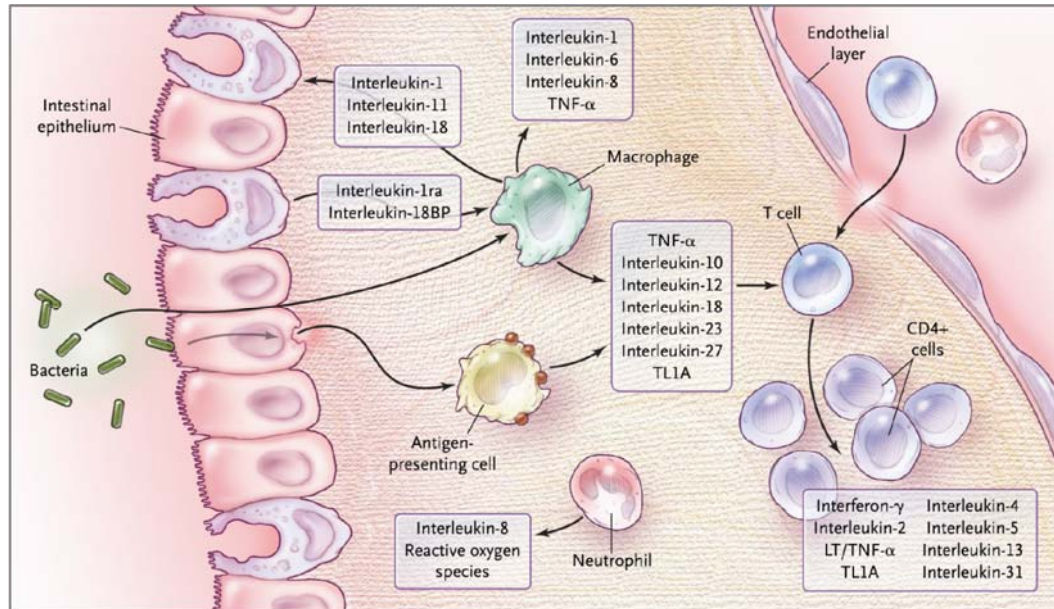


Figure 2. Working hypothesis regarding the role of cytokines in the pathogenesis of CD.

When the mucosal immune system in patients predisposed to the development of Crohn's disease is first exposed to an initiating antigenic stimulus, a dysregulated and overly aggressive cytokine-mediated T-cell response is mounted. Cytokines involved in innate immune responses, such as TNF- α , IL-1, IL-6, and possibly IL-12 and IL-18, may play a key role in this phase. Once CD4+ T cells are activated, effector cytokines involved in the adaptive immune response, including TNF- α and IFN- γ , as well as IL-4 and IL-13, mediate the effector phase of the intestinal inflammatory response. Novel cytokines such as TL1A and IL-23, IL-27, and IL-31 may also contribute to the effector phase.

BP = binding protein, ROS = reactive oxygen species, LT = lymphotoxin.

Reprinted by permission from New England Journal of Medicine (87)

2.2.4 Pediatric IBD

Inflammatory bowel disease is recognized as one of the most significant chronic gastrointestinal disorders to affect children and adolescents. Although IBD in adults and children share many similarities in clinical presentation and treatment it is important to remember that children are not small adults (88). The anatomical location of the inflammation may be different in children compared to adults. For example, UC in children is more likely to be extensive than adult-onset disease, and only a minority of the children present with proctitis (5). Furthermore, pediatric IBD is often diagnosed in the preadolescence or early adolescence, which is a vulnerable time of development and growth (5). Mental and emotional strain is common in children with IBD, and there is a risk for impaired psychosocial development in these children (89).

Unique to pediatric IBD, and to CD in particular, are complications of growth impairment and delayed puberty resulting from malnutrition and persistent inflammation (5). Bone demineralization is another common complication (89). Hence, various clinical, therapeutic, and psychosocial concerns specific to pediatric IBD must be considered. Accordingly, the challenges for pediatric gastroenterologists and IBD teams are to ensure prompt diagnosis and appropriate therapeutic management. Furthermore, optimal care and nutrition are essential if children with IBD are to achieve potential physical development and maximal growth, the best possible mental development, and a good quality of life. It is also important to reduce the risks for long-term complications, e.g. development of fibrostenosis, strictures, epithelial dysplasia, and cancer in the gastrointestinal tract.

2.2.5 Clinical presentation

The site and extent of intestinal involvement and the severity of inflammation can explain variations in the clinical presentation and course of IBD. However, the presenting symptoms tend to be essentially the same in adults and children. Abdominal pain, diarrhea, weight loss, and perianal lesions are typical symptoms for CD, and diarrhea and bloody stools are typical for UC (5). The main differentiating features are growth retardation and delayed puberty, symptoms that occur particularly in pediatric CD (5, 90). Extraintestinal manifestations occur in approximately one third of the patients, but may not be present at onset of disease (Table 3). Occasionally symptoms are discrete even in cases with pronounced gastrointestinal inflammation. Diagnosis is delayed especially in CD with small bowel disease and in younger children (5).

2.2.6 Differential diagnosis

Several differential diagnoses need to be considered in investigating children with gastrointestinal symptoms before the IBD diagnosis can be confirmed. Differential diagnoses to consider are, e.g. infective colitis, celiac disease, allergic or cow's milk colitis, autoimmune colitis, microscopic colitis, chronic granulomatous disease, appendicitis, Henoch Schönlein purpura, and Behcet's disease (91). Symptoms of functional gastrointestinal disorders, e.g. irritable bowel syndrome, can also mimic the clinical presentation of IBD.

2.2.7 Laboratory tests and diagnostic procedures

Several investigations, e.g. blood tests, fecal cultures, endoscopy, and radiology are necessary to rule out differential diagnoses before the IBD diagnosis can be confirmed (Table 3) (92). Some of the diagnostic procedures are resource-demanding and troublesome for the child, and they usually need to be repeated if IBD is diagnosed. Noninvasive and painless diagnostic procedures are of considerable value in the pediatric population and should be used whenever possible. A specific, noninvasive, routine method is not yet available to diagnose IBD.

Table 3. Clinical workup in children with suspected IBD.

Clinical workup		
History	Family history of IBD Daily activity and school absence	
Clinical symptoms	Gastrointestinal: Abdominal pain Tenesmus Diarrhea / Stool pattern Bloody stools Nausea Vomiting Fistulae Perianal abscess Oral ulcerations	Systemic or Extraintestinal: Poor weight gain/weight loss Growth retardation Delayed puberty Deficiencies of vitamins and minerals Osteoporosis Fever, anorexia Joint involvement Skin manifestations Eye manifestations Liver and kidney manifestations Vascular involvement
Laboratory	Blood tests: Full blood count ESR Albumin Electrophoresis with orosomuroid CRP Liver function tests Urea, creatinine Tissue transglutaminase antibody Serology for Yersinia, Campylobacter, Salmonella	Stool cultures: (Salmonella, Shigella, Campylobacter, Yersinia, Clostridium difficile/toxin, E. Coli etc.). Fecal samples: Amoeba, Giardia Lamblia, other parasites. Other tests: consider tuberculosis, CMV and other viruses.
Investigations	Gastroduodenoscopy and ileocolonoscopy including biopsies Small bowel enema (SBE) or Small bowel follow through (SBFT)	Additional: Abdominal ultrasonography Leukocyte scintigraphy Abdominal computerized tomography Magnetic resonance imaging Capsule endoscopy Dexa scan (Dual Energy x-ray Absorptiometry)

Inflammatory markers in peripheral blood

Inflammatory markers in peripheral blood, including ESR, CRP, orosomucoid, albumin and blood count, are routine analyses but all these tests can show normal even though mucosal inflammation exists (93). In many studies the concentrations of the inflammatory markers have been compared to various clinical disease activity indices, which may explain the diverging results about the validity of the different routine blood tests (94, 95). However, none of these laboratory tests are specific and sensitive enough to be used as a surrogate marker of gastrointestinal inflammation and, thus, endoscopy is required for evaluation of the endoscopic and histological disease activity (93).

Full blood count

Anemia and thrombocytosis are common changes in IBD. In one study in children suspected of IBD, a combination of anemia and thrombocytosis had a positive predictive value of 90% for IBD and a negative predictive value of 81% (96). The platelet count reflects a nonspecific response to inflammation. As the platelet count has a fairly wide normal range and a small range of abnormality, and as other compounding factors such as hemorrhage of any sort can raise the platelet count, this parameter is not widely used in clinical practice. The pathogenesis of anemia is usually multifactorial and often a result of iron deficiency and intestinal inflammation with blood loss.

Erythrocyte sedimentation rate

Erythrocyte sedimentation rate (ESR) refers to the rate at which erythrocytes fall through plasma (millimeter per hour). This depends largely on the plasma concentrations of fibrinogen, which is an acute phase protein. The result can be greatly influenced by the size, shape, and number of erythrocytes and by other plasma constituents such as immunoglobulins. The ESR changes relatively slowly when the inflammatory activity changes (97). An increase of ESR with age has been demonstrated. In children with possible IBD the optimal threshold level was found to be ≤ 10 mm/h, i.e. lower than the established reference value in children. At this level, sensitivity was 82% and specificity 78% to detect IBD (96).

Acute-phase proteins

An acute-phase protein has been defined as one whose plasma concentration changes by at least 25% during inflammatory disorders. The purpose of acute-phase reaction is to counteract the underlying challenge in order to restore homeostasis as soon as possible. Albumin, orosomucoid, C-reactive protein (CRP), and serum amyloid A (SAA), belong to the approximately 40 known acute-phase proteins (97). Changes in the acute-phase proteins indicate an inflammatory process, although not necessarily IBD (98, 99).

Albumin

Serum albumin levels decline in active disease (100), and the albumin concentrations can be influenced by protein loss from the gut and by malnutrition secondary to inadequate intake, malabsorption, or increased requirements.

Orosomucoid

Orosomucoid may increase 4- to 5-fold in severe inflammation. The half-life of orosomucoid is about 4 to 5 days in serum. Increased values of serum orosomucoid have been reported in several IBD studies including pediatric studies (101, 102).

C-reactive protein

In 1930, Tillet and Francis described systemic changes, the acute-phase response, in the plasma of patients with pneumococcal pneumonia. They discovered the C-reactive protein (so named because it reacted with pneumococcal C-polysaccharide). By attaching to the polysaccharide structure on the bacteria, CRP activates the classical pathway in the complement cascade, leading to opsonisation and phagocytosis of infectious agents and damaged cells. CRP has been widely used in pediatrics as an indicator of the acute-phase response to inflammation or tissue damage (103). On the individual level CRP has also been used as an indicator of the disease course.

Production of CRP in the hepatocytes is modulated by circulating cytokines as interleukin 1b, interleukin 6, and tumor necrosis factor. However, the production of CRP, as well as cytokines, may differ between individuals because of genetic polymorphism, i.e. individual variability within the genes (104).

The conventional CRP method, which measures concentrations from 5 or 8 mg/L and up, has been frequently studied and used in IBD (102, 105, 106). A high-sensitivity CRP method is now available for measurements of CRP from 0.2 mg/L. The CRP concentrations are shown to be lower in healthy children (geometric mean 0.37 mg/L) compared to healthy adults (geometric mean 0.98 mg/L) (107).

Serum amyloid A

Serum amyloid A has been studied less extensively, and its actions are largely unknown. However, commercial assays are now available (108). One study that compared healthy newborn infants to adults found that SAA concentrations increased with age (109). A reference interval <10 mg/L was suggested for the age groups, but well-established reference values in children are still missing. In a study of familial Mediterranean fever, SAA was shown to be a better marker than CRP, ESR, fibrinogen, and ferritin in monitoring of subclinical inflammation and response to therapy. Factors like age, gender, age at onset, age at diagnosis, or duration of treatment had no significant effects on the SAA level in this population (110).

Study IV investigated hsCRP and SAA as inflammatory markers in comparison to plasma and fecal calprotectin in children with IBD. Table 4 presents characteristics of these two acute-phase proteins and calprotectin.

Table 4. Characteristics of the inflammatory proteins CRP, SAA and calprotectin.

	C-reactive protein	Serum Amyloid A	Calprotectin
Belongs to	Pentraxin superfamily	Apolipoprotein family	S100 family of calcium-binding proteins
Origin	Synthesized by hepatocytes	Mainly synthesized by hepatocytes	Abundant in neutrophils
Encoded by	Chromosome 1 (1q 23-24)	Chromosome 11 (11:p15.1)	Chromosome 1 (1q 12-21)
Consists of	Five monomers	Three isotypes in plasma (SAA1, SAA2, and SAA4)	Heterocomplex – Light and heavy chain
Molecular weight	118 kDa	12 kDa	36 kDa
Increase with infectious diseases	100 -1000 fold	100 -1000 fold	40 – 130 fold
Time for up-regulation	Within hours	Within hours	Within hours

2.2.8 Endoscopy and histopathology

In the 1970s endoscopic examination of the gastrointestinal tract became feasible for routine use in children with improvements in the technology and a reduction of instrument diameter. Advancements in pediatric endoscopy have contributed to current knowledge about many gastrointestinal diseases in children, including IBD. Endoscopy is considered the gold standard for diagnosing IBD and is also a tool for estimating disease activity and the efficacy of therapy. In IBD, endoscopic investigation of both the upper and lower gastrointestinal tract is recommended including intubation of the terminal ileum (111). The assessment of inflammation is based on the macroscopic findings from the procedure and the histopathological appearance in multiple biopsy specimens. These must be taken from the mucosa in each investigated segment of the gastrointestinal tract and placed in separate containers to identify localization and the extent of inflammation, and to facilitate differentiation between UC and CD. However, endoscopy is clearly a laborious, time-consuming, and expensive procedure, and the preparatory colonic cleansing can be a practical problem in children. Furthermore, in children the procedure requires sedation, or most often general anesthesia. Hence, careful selection of patients is essential. Until now, the decision to go through with colonoscopy has been based on medical history, physical examination, and routine blood tests.

Diagnostic criteria

The macroscopic findings from endoscopy differ considerably according to the diagnosis, the stage of the disease, and its severity. The level of severity may vary from subtle (e.g. loss of the vascular pattern and edema) to severe (e.g. inflammation with ulcerations and bleeding). The mucosal changes may involve variable parts and length of the gastrointestinal tract. Macroscopic colonic inflammation in UC is by definition continuous, but in CD it can also be patchy. Aphthoid ulceration, cobblestoning, and fissuring ulceration are classical features of CD, while UC is characterized by a diffuse symmetric colitis with a granular mucosa and loss of haustration. However, the endoscopic profile of the mucosa can be similar in CD and UC. Histological abnormalities can exist although macroscopic appearance is normal (112). Neutrophil infiltration of crypt epithelium (cryptitis) and, crypt lumina (crypt abscesses) is common in IBD. Diagnostic findings during endoscopy and histology for UC and CD has been summarized by the ESPGHAN working group on pediatric IBD (adapted in Table 5) (113).

Table 5. Endoscopy and histology in IBD

	Crohn's disease	Ulcerative colitis
Endoscopy	Ulcers (aphthous, linear, or stellate) Cobblestoning Skip lesions Strictures Fistula Abnormalities in oral region Abnormalities in perianal region Segmental distribution	Ulcers Erythema Loss of vascular pattern Granularity Friability Pseudopolyps Spontaneous bleeding Continuous
Histology	Submucosal or transmural involvement Ulcers, crypt distortion Granulomas (non-caseating, non-mucin) Crypt abscess Focal changes (within biopsy) Patchy distribution (biopsies)	Mucosal involvement Crypt distortion Mucin granulomas (rare) Crypt abscess Goblet cell depletion Continuous distribution

2.2.9 Treatment

As with adults, the clinical course and responsiveness to treatments vary widely among children with IBD, and predictive markers of future clinical relapse do not yet exist. Medical treatment of pediatric CD or UC is based mainly on evidence from studies in adult IBD patients with dosages extrapolated from adult dosages. Many therapeutical studies have evaluated the effect of medication by measuring clinical disease activity indices to define disease activity and remission (114) and not by endoscopic or histological assessment. Additional randomized, double-blind, placebo controlled clinical trials are needed, as are better-defined treatment guidelines for pediatric IBD. Further understanding the pathogenesis of IBD is essential for developing more efficient therapies with as few side effects as possible. Hopefully, the advancements in knowledge will lead to a cure for the disease. Briefly, the following treatments are currently used in children (Table 6) (115):

Table 6. Examples of treatment options for pediatric IBD

Treatment	Comments
Sulfasalazine and mesalazine	For induction of remission and maintenance.
Corticosteroids (predniso(lo)ne and budesonide)	For induction of remission.
Antibiotics (metronidazole and ciprofloxacin)	For induction of remission and maintenance in CD and fistulous disease.
Azathioprine or 6-mercaptopurine (6-MP)	Immunosuppressive treatment for maintenance.
Enteral nutrition	For induction of remission and maintenance especially in growth retardation and/or delayed puberty in CD
Methotrexate	For induction of remission and maintenance in CD
Anti-TNF-α treatment	For induction of remission in severe, refractory disease Fistulous disease
Granulocyte and monocyte apheresis	More data needed
Surgery	In CD if fibrostenosis or localized inflammation. In UC colectomy in severe cases.

2.2.10 Assessment of disease activity in IBD

Clinical disease activity

Clinical indices have been developed for longitudinal estimation of disease activity and for evaluation of antiinflammatory therapies in IBD, in particular in therapeutic trials (116). Disease activity indices are usually based on routine blood tests, the patient's reported symptoms, extraintestinal manifestations, and physical signs. Albumin, hematocrit, and ESR are the blood tests included in the pediatric version of Crohn's Disease Activity Index (PCDAI), and growth has been added to clinical signs, as it is an important long-term indicator of successful treatment in children (117). However, neither height nor signs of perirectal disease actually change rapidly enough to make them useful for assessing clinical outcome during therapy (118). Furthermore, symptoms can be neglected or underreported, especially if the child or adolescent is afraid of therapies or investigations associated with discomfort. Hence, clinical disease activity and indices do not necessarily reflect the degree or extent of mucosal inflammation (102, 119, 120). In addition, compilation of clinical disease indices is time-consuming, making them less applicable to daily clinical practice.

Endoscopic and histological disease activity

The invasive nature of endoscopy means that the method cannot be used frequently for routinely assessing inflammation. Further, there is no simple and widely used endoscopic scoring system available, and macroscopic assessment is also subjective and dependent on the endoscopist's experience. Macroscopic assessment could also be problematic in cases with unsatisfactory bowel cleansing.

Histology as a tool for measuring disease activity was introduced for UC in 1956 (121). Crypt abscesses, crypt destructions, erosions, and ulcerations are microscopic indicators of tissue injury that can be used as markers of disease activity. Interobserver variation in microscopic scoring has been studied and found to be minor and infrequent, in one study occurring in less than 10% of the biopsy samples (122). However, when using a scoring system in studies, it is preferable that a single histopathologist performs all of the microscopic assessments (123). Several microscopic scoring systems have been introduced, but no system seems to be favorable (123). Microscopic signs of activity can persist despite clinical and endoscopic remission at medical treatment. The importance of subclinical inflammation has yet to be studied extensively, but there may be an increased risk for relapse (124).

3 AIMS

The specific aims of the different studies in this thesis were:

- I** To establish reference values for the improved, quantitative analysis of fecal calprotectin in healthy children aged 4 through 17 years.
- II** To evaluate the feasibility of fecal calprotectin to detect colorectal inflammation in children with gastrointestinal symptoms.
- III** To evaluate fecal calprotectin as a quantitative marker of macroscopic and microscopic colonic inflammatory activity in children with inflammatory bowel disease and to evaluate fecal calprotectin concentrations at complete, microscopic remission of inflammation.
- IV** To evaluate the usefulness of the two plasma acute-phase proteins, serum amyloid A and high sensitivity C-reactive protein and plasma calprotectin as markers of microscopic inflammation in pediatric inflammatory bowel disease and to compare them with fecal calprotectin and routine blood tests.

4 MATERIAL AND METHODS

A brief summary of the materials and methods for studies I-IV is presented here along with short descriptions of the methods we used in these papers. For more detailed information we refer the reader to the individual papers.

4.1 CHILDREN IN STUDIES I - IV

Study I involved healthy children recruited from day nurseries and school health services in Västerås, Sweden and from the families of the hospital staff. Studies II-IV were performed at the Department of Gastroenterology, Astrid Lindgren Children's Hospital, Stockholm, and the Department of Pediatrics, Central Hospital, Västerås, Sweden. Children with gastrointestinal symptoms suggestive of IBD were included in Study II and children with confirmed IBD were studied in studies III-IV together with controls. Differentiation between Crohn's disease, ulcerative colitis, and indeterminate colitis was made in the children with IBD according to accepted clinical, endoscopic, microscopic, and radiological criteria for diagnosis (113).

Table 7. Summary of the participating children in Studies I-IV

Study	Number of Children at Start	Excluded	Included	Number of Controls and Cases
Study I	139	22	117	Cases = 117 healthy children (1 child developed proctitis later)
Study II	40	4	36	Controls = 14 noninflamed Cases = 22 inflamed* *20/22 with IBD (including the child from Study I who developed proctitis).
Study III	58	7	51	Controls = 12 noninflamed (from Study II) Cases = 39 IBD** **12/39 were included also in Study II.
Study IV	41	1	40	Controls = 8 noninflamed (from Study II) Cases = 32 IBD*** ***10/32 were included also in Study II 32/32 were included also in Study III

4.2 DESIGN OF STUDIES I - IV

Study I

In study I, fecal samples were obtained from healthy children aged 4 through 17 years, and the fecal concentration of calprotectin was analyzed. A health questionnaire was used to ensure that these children did not have abdominal pain, diarrhea, other intercurrent disease, nose or menstrual bleeding or nonsteroidal anti-inflammatory drug medication before the sampling period. The study included 117 children (52 girls and 65 boys), and they were categorized into four age groups (4-6, 7-10, 11-14, and 15-17 years). Children with fecal calprotectin values $>50 \mu\text{g/g}$ were asked to deliver an additional sample.

Study II

Study II was based on 36 children with gastrointestinal symptoms suggestive of IBD. The decision to perform a colonoscopy was made by a pediatric gastroenterologist after evaluation of the child's medical history, physical examination, and routine laboratory blood tests. Fecal cultures and/or serology were used to exclude bacterial gastroenteritis. Serological markers for celiac disease or tissue samples from the duodenal mucosa were checked to rule out celiac disease. The children delivered a fecal spot sample and blood samples before undergoing colonoscopy. Depending on the outcome from the colonoscopy the children were grouped into two categories: one with histopathological findings of colonic inflammation ($n=22$) and one without inflammation of the colon mucosa ($n=14$). The concentrations of fecal calprotectin and routine inflammatory markers in blood (such as ESR, CRP, orosomucoid, albumin, platelet count) were compared between the groups.

Study III

At the outset, Study III was comprised of 51 children with suspected or previously confirmed IBD. They were asked to deliver a fecal spot sample before they underwent a planned colonoscopy with multiple colonic biopsy specimens. Twelve of the children had a noninflamed colonic mucosa and constituted a control group. Thirty-nine children fulfilled the inclusion criteria for the IBD study group (CD $n = 27$, UC $n = 10$, and IC $n = 2$). Macroscopic and microscopic assessments of the colonic inflammation were performed and converted into extent and severity scores of inflammation. Clinical assessment of IBD activity was based on the patient's history, clinical examination, and routine laboratory tests by the clinicians. Furthermore, the patients were grouped into two categories: symptomatic ($n = 23$) and asymptomatic ($n = 16$). The following criteria had to be fulfilled for inclusion in the asymptomatic group; no reported symptoms, no abdominal mass, no glucocorticoid therapy, and normal blood tests with hemoglobin $<115 \text{ g/L}$, ESR $\leq 12 \text{ mm/hour}$, CRP $<8 \text{ mg/L}$, orosomucoid $<1.15 \text{ g/L}$ and albumin $\geq 37 \text{ g/L}$. The fecal calprotectin concentrations were compared between these groups and also correlated to the macroscopic and microscopic extent and severity scores of the inflammation.

Study IV

In Study IV, fecal and blood samples were obtained from children with suspected or previously confirmed IBD when investigated with colonoscopy. Microscopic assessments of colonic inflammation were performed in multiple biopsy specimens and converted into a combined microscopic extent and severity score. In 8 cases the IBD diagnosis was refuted, as they had neither microscopic colonic inflammation nor other signs of IBD at investigation. These children comprised a control group. Children with newly confirmed IBD (n = 10) and children with previously established IBD (n = 22) constituted the “IBD study group”. Serum Amyloid A and high sensitivity CRP were analyzed in plasma, and calprotectin was measured in plasma and in feces. The concentrations of these inflammatory markers and routine blood tests (such as hemoglobin, ESR, CRP, orosomucoid, albumin, platelet count) were correlated to the microscopic, combined, extent and severity scores of inflammation. The children with IBD were classified into two categories based on the histopathological examination: a) microscopic remission, or b) active colonic inflammation. The concentrations of the inflammatory markers were compared between the groups.

4.3 FECAL CALPROTECTIN

In studies I-IV, the stool samples were prepared and analyzed according to the manufacturer’s instructions (Calprest®, Eurospital SpA, Trieste, Italy). Stool was collected in screw-capped plastic containers and sent the same or next day by mail to the laboratory. The weight of each sample (40-120 mg) was measured, and an extraction buffer containing citrate and urea was added in a weight/volume ratio of 1:50. The samples were mixed for 30 seconds, by means of a vortex, and homogenized for 25 minutes. One milliliter of the homogenate was transferred to a tube and centrifuged for 20 minutes at 10 000 g. Finally the supernatant was collected and frozen at –20°C. The supernatants were thawed and calprotectin was analyzed with the quantitative calprotectin ELISA method. Calprotectin was expressed as µg/g feces.

4.4 ANALYSES OF BLOOD SAMPLES

In studies II and IV, routine laboratory tests from peripheral blood were analyzed according to the recommendations of the manufacturer, including blood count (ADVIA 120, Bayer Diagnostics, Terrytown, NY USA), erythrocyte sedimentation rate (BD Seditainer™ ESR Tube, Becton-Dickinson, NJ USA), albumin, and orosomucoid (Image, Beckman Coulter, CA USA). A Dade Behring Nephelometer (BNII analyzer, Dade Behring Diagnostic, GmbH, Marburg, Germany) was used for analyses of serum Amyloid A (N Latex SAA®) and high sensitivity CRP (hsCRP®) in plasma with particle-enhanced nephelometric assays, and the concentrations were expressed as mg/L. Calprotectin was measured in plasma with an enzyme-linked immunosorbent assay method (Calprest®, Eurospital SpA, Trieste, Italy) and expressed as µg/L.

4.5 ASSESSMENT OF MACROSCOPIC INFLAMMATION

The endoscopies in studies II-IV were performed under general anesthesia by experienced pediatric endoscopists. In Study III the macroscopic appearance of the mucosa was converted into regional scores in eight colonic segments for each patient according to a previously used model to permit comparison of results (i.e. normal appearance = 0, loss of vessel pattern or edema = 1, contact hemorrhage = 2, ulceration or surface mucopus = 3) (125, 126). The predefined colonic segments were cecum, ascending colon, right flexure, transverse colon, left flexure, upper and lower part of descending colon, upper and lower part of sigmoid and rectum.

The macroscopic severity score was equivalent to the highest regional score in any colonic segment (with a possible range 0 - 3). The macroscopic extent score was defined as the number of colonic segments with a regional score ≥ 1 (with a possible range 0 - 8). Finally, a combined macroscopic extent and severity score was calculated from the sum of the 8 regional scores (with a possible range 0 - 24).

4.6 ASSESSMENT OF MICROSCOPIC INFLAMMATION

In studies II-IV, multiple biopsy specimens were taken from the terminal ileum (when intubated) and colon at colonoscopy for histological analysis by experienced gastrointestinal histopathologists. The inflammation was evaluated according to accepted conventional criteria for diagnosis of IBD with differentiation into CD, UC, or IC. In studies III-IV, the microscopic assessments of inflammation in the crypts, enterocytes, and cellularity of the lamina propria (mononuclear cells and neutrophils) were graded in biopsy specimens from the 8 predefined colonic segments according to a previously used model to enable comparison of results (Table 8) (125, 126).

Table 8. Microscopic grading system in each colonic biopsy specimen.

Crypts		Enterocytes	
Normal	0	Normal	0
Single inflammatory cells	1	Loss of single cells	1
Cryptitis	2	Loss of groups of cells	2
Crypt abscesses	3	Frank ulceration	3
Neutrophils in lamina propria		Mononuclear cells in lamina propria	
Normal	0	Normal	0
Slight increase	1	Slight increase	1
Moderate increase	2	Moderate increase	2
Marked increase	3	Marked increase	3

The possible sum of grades varied from 0 to 12 per segment. According to the model, the sum of grades was then converted into a regional microscopic score to define the

severity of inflammation for the segment. Thus, grades 0 - 1 were converted to score 0, grades 2 - 4 to score 1, grades 5 - 8 to score 2 and grades 9 - 12 to score 3. Finally the individual's 8 regional microscopic scores were transformed into a microscopic extent, a microscopic severity, and a microscopic combined extent and severity score in the same way as was previously described for the macroscopic scores in section 4.5. The possible total score of the microscopic combined extent and severity score ranged from 0 to 24 in each patient. To define mucosal healing in the IBD group, a cutoff level of ≤ 2 for the microscopic combined extent and severity score was used. This cutoff was established from the control group in studies III-IV where all the children had combined scores ≤ 2 and a noninflamed colonic mucosa. To analyze the effects of the conversion steps explained above as a possible source of error in Study III, all the available colonic biopsy specimens taken from the patient were graded according to Table 9. These grades were then summarized and divided directly by the number of biopsy specimens taken.

4.7 STATISTICS

Statistical analyses were performed using the SPSS (Statistical Package for Social Sciences Inc. Chicago, IL, US) version 10.1 (study I - II) and 11.0 (study III - IV) for Windows. The descriptive statistics were calculated as median values with ranges given as the minimum and maximum values or with 95% confidence interval (CI). The Kruskal-Wallis and the Mann-Whitney U tests were used for comparison between groups. Statistically significant differences were assumed when $p < 0.05$.

In Study I, simple regression analysis was used to assess the correlation between fecal calprotectin concentration and age.

In study II, sensitivity, specificity, positive and negative predictive values, and observed agreements were calculated with the laboratory values categorized as normal and abnormal. The following values for the different tests were regarded as abnormal: fecal calprotectin $\geq 50 \mu\text{g/g}$, platelet count $> 400 \times 10^9/\text{l}$, albumin $< 37 \text{ g/l}$, orosomucoid $\geq 1.15 \text{ g/l}$, ESR $\geq 16 \text{ mm/h}$ and CRP $\geq 7 \text{ mg/l}$. Ninety-five percent confidence intervals were determined for the observed agreements of the fecal calprotectin and the blood tests. A discriminant analysis was also used to study the relation between histopathology and laboratory variables.

In studies III - IV, the nonparametric Spearman rank order correlation test was applied for correlations between variables. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) with 95% CI were calculated for the inflammatory markers. An AUC of 0.5 means that the test is not better than chance alone to discriminate, whereas a test with an AUC approaching 1.0 indicates perfect discriminative capability.

In Study IV, a method according to Hanley et al was used for comparison of the AUC for different inflammatory markers derived from the same cases (127).

4.8 ETHICS

Ethical approval was obtained from the Regional Research Ethics Committees at Karolinska Institutet, Stockholm and Uppsala University, Uppsala, Sweden. The children were included in the study after verbal informed consent from the children and their caregiver(s).

5 RESULTS AND DISCUSSION

5.1 RESULTS IN STUDIES I - IV

Study I - Fecal Calprotectin Levels in Healthy Children Studied With an Improved Assay

In 117 healthy children, the median fecal calprotectin concentration was 13.6 $\mu\text{g/g}$ (95% confidence interval 9.9 – 19.5 $\mu\text{g/g}$) and the 95th percentile was found to be 105.1 $\mu\text{g/g}$. In the different groups, aged 4-6, 7-10, 11-14, and 15-17 years, the median calprotectin concentrations were 28.2, 13.5, 9.9, and 14.6 $\mu\text{g/g}$, respectively. No significant correlation was found between age and fecal calprotectin concentration ($r = 0.17$) (Figure 3). The median value for boys (13.4 $\mu\text{g/g}$) and girls (15.5 $\mu\text{g/g}$) did not differ significantly either. In 104/117 (89%) children, the fecal calprotectin concentration was below the recommended reference value for adults (<50 $\mu\text{g/g}$). The remaining 13 children had a fecal calprotectin concentration >50 $\mu\text{g/g}$, and they all delivered additional fecal sample from 4 to 14 months later. All but 3 children had a concentration of <50 $\mu\text{g/g}$ in the follow-up sample. One case, 10 months later, showed an increase from 60.7 $\mu\text{g/g}$ to 240 $\mu\text{g/g}$ in the fecal calprotectin concentration. This boy had noticed bloody diarrhea for some months, and at investigation a distal ulcerative proctitis was found. When his fecal sample was excluded, and the results from the follow-up samples ($n = 12$ children) were used instead of their first samples, the 95th percentile was 43.4 $\mu\text{g/g}$.

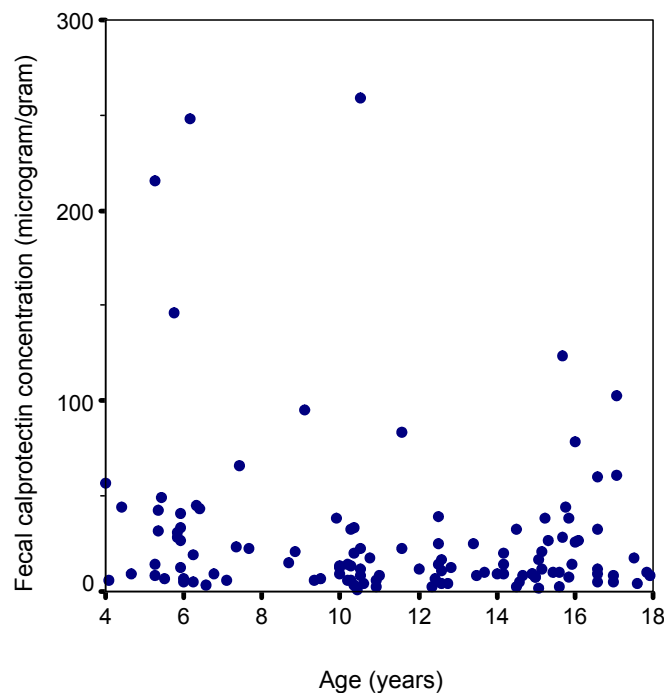


Figure 3.

Fecal calprotectin concentration and age in 117 healthy children aged 4 through 17 years.

Study II - Colorectal Inflammation is Well Predicted by Fecal Calprotectin in Children With Gastrointestinal Symptoms

Children with gastrointestinal symptoms were grouped into two categories: one with histopathological findings of colonic inflammation (n=22) and one without inflammation of the colon mucosa (n=14). In 20/22 children with colonic inflammation the diagnosis was inflammatory bowel disease. Most of the children in the noninflamed group suffered from functional bowel disorders (5/14) or food intolerance (4/14). Median fecal calprotectin concentration was 349 $\mu\text{g/g}$ (95% confidence interval, 213-440 $\mu\text{g/g}$) in the group with colonic inflammation compared to 16.5 $\mu\text{g/g}$ (95% confidence interval, 6.9-28.2 $\mu\text{g/g}$) in the group without colonic inflammation. The difference in median fecal calprotectin concentrations was statistically highly significant (p-value <0.00001). Figure 4 presents the individual concentrations of fecal calprotectin for the two groups.

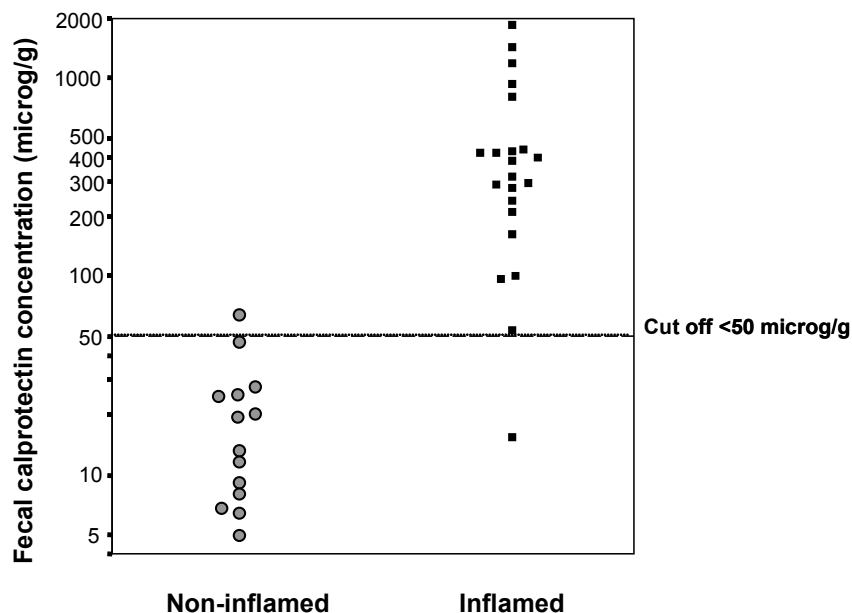


Figure 4.

Individual concentration of fecal calprotectin (log scale) in 14 children without colorectal inflammation [•] and 22 children with colorectal inflammation [■] at colonoscopy (p-value <0.00001). Dotted line represents cutoff level for fecal calprotectin (<50 $\mu\text{g/g}$).

The fecal calprotectin test had a sensitivity of 95% for detection of colorectal inflammation and a specificity of 93%, a positive predictive value of 95% and a negative predictive value of 93% when <50 $\mu\text{g/g}$ was used as the upper reference limit. The observed agreement between the fecal calprotectin test result and colorectal histopathology was 94%. The associations between histopathology and platelet count, albumin, ESR, CRP, and orosomucoid were also analyzed by using the recommended reference and cutoff values for the tests (Table 10). Completely normal results in all of these blood tests were observed in 5 of the 22 children with colonic inflammation.

Blood test and fecal calprotectin analyses were also evaluated together in a discriminant analysis with log₁₀-transformed values. Fecal calprotectin was the single most valuable variable in the model after step-wise inclusion (p<0.001). The receiver operating characteristic (ROC) curve for fecal calprotectin showed that the optimal cutoff was 50.5 µg/g and the area under curve was 0.97.

Table 10.

Diagnostic accuracy of fecal calprotectin and blood tests to detect colorectal inflammation in children with gastrointestinal symptoms.

Laboratory test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Observed Agreement
FECAL TEST					
Calprotectin	95%	93%	95%	93%	94%
BLOOD TESTS					
Albumin	59%	100%	100%	61%	75%
Platelet count	50%	100%	100%	56%	69%
Orosomuroid	41%	100%	100%	52%	64%
ESR	41%	100%	100%	52%	64%
CRP	36%	100%	100%	50%	61%

Study III - Fecal Calprotectin - A Quantitative Marker of Colonic Inflammation in Children With Inflammatory Bowel Disease

The median fecal calprotectin concentration was found to be 264 µg/g (95% CI 101 to 382 µg/g) in the IBD group compared with 16.5 µg/g (95% CI 6.9 to 28.2 µg/g) in the control group (p < 0.001). A significant difference in fecal calprotectin concentrations was also found within the IBD group when the IBD cases were grouped into two categories; in patients with IBD symptoms the median concentration was 392 µg/g (95% CI 278 to 440 µg/g), and in asymptomatic IBD patients the corresponding value was 32.9 µg/g (95% CI 9.4 to 237 µg/g) (p < 0.001).

The fecal calprotectin concentrations correlated significantly to the scores for both macroscopic and microscopic inflammatory activity in the IBD group. Table 11 presents the correlations and median values of the scores. The strongest correlation was found between fecal calprotectin and the microscopic combined extent and severity score (r = 0.75 with 95% CI 0.57 to 0.86, p < 0.001). A similar correlation was found when testing our own model for calculation of microscopic inflammation (r = 0.79 with 95% CI 0.63 to 0.88, p < 0.001).

Table 11:

Median values of the macroscopic and microscopic scores and the correlations between fecal calprotectin and the various scores.

	Macroscopic			Microscopic		
	Extent Score*	Severity score**	Extent & Severity score***	Extent Score*	Severity score**	Extent & Severity score***
Median (range)	5 (0-8)	1 (0-3)	6 (0-24)	6 (0-8)	2 (0-3)	7 (0-18)
Correlation f-calprotectin	r = 0.61	r = 0.52	r = 0.65	r = 0.71	r = 0.72	r = 0.75
Significance	p<0.001	p=0.001	p<0.001	p<0.001	p<0.001	p<0.001

* The extent score was defined as the number of colonic segments scoring ≥ 1 .

** The severity score was equivalent to the highest regional score in any colonic segment.

***The combined extent and severity score was calculated from the sum of the 8 regional scores.

In the group of asymptomatic IBD patients 9/16 (56%) children appeared to have a complete microscopic healing of the colonic mucosa. Their median fecal calprotectin concentration was 9.9 $\mu\text{g/g}$ (95% CI 5.9 to 41.9 $\mu\text{g/g}$) with fecal calprotectin concentrations $<50 \mu\text{g/g}$ in 8 of the children and a borderline concentration (i.e. 50 – 100 $\mu\text{g/g}$) at 84.7 $\mu\text{g/g}$ in 1 child. Subclinical colonic inflammation was present among the remaining asymptomatic IBD patients (7/16). For these children the median value of the microscopic, combined extent and severity score was 7 (range 6 - 11) and the median fecal calprotectin concentration was 237 $\mu\text{g/g}$ (95% CI 11.9 to 368 $\mu\text{g/g}$). Accordingly, the fecal calprotectin concentrations differed significantly ($p = 0.004$) between asymptomatic patients with noninflamed and inflamed mucosa, respectively.

Using $<50 \mu\text{g/g}$ as upper reference limit for fecal calprotectin and ≤ 2 as cutoff for the combined microscopic extent and severity score we calculated the accuracy with which the fecal calprotectin method detected microscopic colonic inflammation in children with IBD. We found that the sensitivity was 93% (95% CI 0.76 to 0.99), the specificity 73% (95% CI 0.39 to 0.94), the positive predictive value 90% (95% CI 0.73 to 0.98), and the negative predictive value 80% (95% CI 0.44 to 0.97). The observed agreement between the microscopic scorings and the fecal calprotectin concentrations was 87% (95% CI 0.73 to 0.96). The ROC curve showed that the area under the curve was 0.87 (95% CI 0.72 to 1.0).

The maximal sum of sensitivity and specificity for fecal calprotectin was achieved at 85.7 µg/g, which indicated that this concentration was the optimal cutoff for discrimination between inflamed and noninflamed colonic mucosa in children with IBD. Active inflammation in the terminal ileum could be suspected in ten symptomatic CD patients. Two of them had elevated fecal calprotectin concentrations (278 µg/g and 491 µg/g, respectively) although their microscopic extent and severity score was ≤ 2 at colonoscopy.

Study IV - Serum Amyloid A, High Sensitivity C-Reactive Protein and Calprotectin as Markers of Inflammation in Pediatric Inflammatory Bowel Disease

In the IBD group the combined microscopic extent and severity scores correlated significantly to the plasma concentrations of SAA, hsCRP, calprotectin, orosmucoïd, platelet count, albumin, and ESR (Table 12). However, the strongest correlation to the combined extent and severity score was found for fecal calprotectin.

Table 12.

Correlations between inflammatory markers and the microscopic extent and severity scores in children with IBD.

Inflammatory markers	Correlation to microscopic extent and severity score	Significance
	(r-value)	(p-value)
Hemoglobin	- 0.24	0.186
Orosomucoïd	0.37	0.036
Serum Amyloid A	0.40	0.024
High-sensitivity CRP	0.41	0.020
Plasma calprotectin	0.51	0.003
Platelet count	0.52	0.002
Plasma albumin	- 0.56	0.001
ESR	0.67	<0.0001
Fecal calprotectin	0.77	<0.0001

Table 13 demonstrates the median values of ESR, SAA, hsCRP, plasma, and fecal calprotectin together with the median values of the combined microscopic extent and severity score for the children in the control group, the IBD patients in remission, and the IBD patients with active colitis.

Table 13.

Microscopic scores of inflammation in colon and concentrations of inflammatory markers in groups of patients with IBD and controls.

	Controls - no colitis	IBD - healed mucosa	IBD - colitis	Comparison between IBD groups
	(n = 8)	(n = 10)	(n = 22)	(p-value)
Microscopic extent and severity score	0 (0 - 2)	0.5 (0 - 2)	10 (7 - 13)	<0.0001
ESR (mm/h)	5 (2 - 14)	3 (2 - 9)	12 (7 - 26)	<0.001
Serum Amyloid A (mg/L)	2.1 (0.2 - 4.1)	1.0 (0.5 - 11.5)	2.6 (1.3 - 8.2)	0.17
High-sensitivity CRP (mg/L)	0.37 (0.15 - 0.89)	0.35 (0.09 - 1.3)	0.62 (0.31 - 2.7)	0.21
Plasma calprotectin (µg/L)	345 (167 - 694)	216 (104 - 464)	404 (259 - 1163)	0.05
Fecal calprotectin (µg/g)	22.4 (6.5 - 65)	18.5 (8.5 - 278)	336 (213 - 440)	<0.001

Values expressed as medians (with 95% confidence interval).

The area under curve (AUC) in the ROC curves were 0.66 (95% CI 0.43 to 0.88) for SAA, 0.64 (95% CI 0.43 to 0.85) for hsCRP, 0.72 (95% CI 0.52 to .0.92) for plasma calprotectin, and 0.87 (95% CI 0.71 to 1.0) for fecal calprotectin. The AUC for fecal calprotectin was significantly larger compared with the AUC for SAA ($p = 0.007$), hsCRP ($p = 0.027$) and plasma calprotectin ($p = 0.046$). No significant difference was found between the AUC of SAA, hsCRP, or plasma calprotectin respectively. The optimal cutoff for distinction between complete mucosal healing and mucosal inflammation, defined by the highest sum of the specificity and sensitivity, was determined for each test. For SAA the optimal cutoff was 1.25 mg/L, for hsCRP 0.41 mg/L, for plasma calprotectin 243 µg/L, and these cutoff values were well below the upper limit of the recommended reference values for the inflammatory markers in plasma. The optimal cutoff for fecal calprotectin was calculated to be 86 µg/g, i.e. slightly above the recommended reference value for children from 4 years of age and up (<50 µg/g) (128).

5.2 STUDY I - REFERENCE VALUES OF FECAL CALPROTECTIN

This section discusses the results from Study I, comparing them to current knowledge about reference values of fecal calprotectin in different age groups. When we initiated Study I, no data were available on fecal calprotectin in children. Our overall intention was to study fecal calprotectin with special reference to pediatric IBD, and for this purpose we first wanted to establish pediatric reference values in ages where pediatric IBD commonly appears. Based on the results in Study I, we suggested the cutoff level for adults (<50 µg/g) to be used also for children aged 4 through 17 years regardless of sex. Further, we recommended fecal calprotectin concentrations ≥50 µg/g to warrant follow-up.

In recent years much attention has been paid to inflammatory markers in feces, especially to fecal calprotectin. Several minor control groups have been presented in pediatric studies on fecal calprotectin, shown in Table 14 together with the results from Study I.

Table 14. Studies with data on fecal calprotectin concentrations in healthy children and adolescents.

Reference	Year	Individuals, number	Age, median years (range)	Fecal calprotectin concentration median µg/g (range)
Bunn et al (129)	2001	31	6.8 (1.5 - 15.3)	Original method 2.1mg/L* (0.5 - 6.3)
Olafsdottir et al (130)	2002	24	5.3 (1 - 13)	40 ± 28 (Mean±SD)
Fagerberg et al (128) Study I	2003	117	11.6 (4.0 - 17.9)	13.6 (1.2 - 259)
Carroccio et al (131)	2004	10	3.5 (1 - 10)	15 (10 - 40)
Berni Canani et al (132)	2004	76	6.6 (1.1 - 18.0)	28 (1 - 113)

* Approximately 11 µg/g (2.5 - 31.5) with improved method

The results from these studies are in accordance with our Study I, but nevertheless our study on fecal calprotectin is the most comprehensive work on reference values in children and adolescents. In addition, we have further evaluated the suggested cutoff from Study I by performing studies II - IV.

Apparently, healthy children aged 4 through 17 years exhibit similar patterns of fecal calprotectin excretion as adults, and the following threshold values seem to apply also in children aged 4 through 17 years: negative test when $<50 \mu\text{g/g}$, weakly positive test when between 50 and $100 \mu\text{g/g}$, and strongly positive when $>100 \mu\text{g/g}$ (133).

In infants, however, the fecal calprotectin concentrations seem to be age-dependent. Surprisingly high concentrations have been found, especially in healthy infants 0 through 3 months of age with a median fecal calprotectin concentration of $265 \mu\text{g/g}$ (130, 134). The explanation for this observation could be a migration of neutrophils through the mucosal membrane during the development of oral tolerance and regulation of the microbial flora (7). In adults, an increase of fecal calprotectin concentrations has been observed with age in studies of healthy subjects aged 50 to 70 years (135).

5.3 STUDY II - FECAL CALPROTECTIN AS A DIAGNOSTIC TEST

In Study II we applied the cutoff $<50 \mu\text{g/g}$ to evaluate the feasibility of the fecal calprotectin method to detect colorectal inflammation in children with gastrointestinal symptoms suggestive of IBD. The sensitivity of fecal calprotectin (95%) was found to be superior to any of the other routine inflammatory markers in peripheral blood. Consequently, we proposed the fecal calprotectin method as a useful tool to select patients who should undergo diagnostic colonoscopy for investigation of colorectal inflammation.

Several research groups have reached the conclusion that the fecal calprotectin method is a useful diagnostic test in adults and children. However, the results and suggested cutoffs vary to some extent in certain studies. The different study designs and purposes and the variations in ages of the study populations could explain this, at least in part.

In adults, Tibble et al used the original fecal calprotectin method to study 602 individuals with symptoms suggestive of irritable bowel syndrome or organic intestinal disease. The sensitivity and specificity of fecal calprotectin for organic disease were 89% and 79%, respectively (136). Limburg et al evaluated the improved method for fecal calprotectin as a screening test for colorectal inflammation in adults with chronic diarrhea (133). The study included patients with previously diagnosed chronic colitis and patients with chronic diarrhea of unknown origin. The sensitivity for any colorectal inflammation was then 83% with a cutoff level at $100 \mu\text{g/g}$. For IBD, sensitivity was 94%, and for microscopic colitis it was 64%.

In 19 children with recurrent abdominal pain, Olafsdottir et al reported normal fecal calprotectin concentrations ($18 \pm 24 \mu\text{g/g}$) with a significant difference at comparison with the elevated concentrations in 17 children with IBD (130).

By now there are also several pediatric studies evaluating the improved method of fecal calprotectin as a diagnostic tool for inflammatory disorders in the whole gastrointestinal tract. Carrioco et al evaluated fecal calprotectin as a marker of "organic causes" of chronic diarrhea in adults and children (131). The enrolled children were aged 8 months

through 10 years with a median age of 3.5 years. However, the same cutoff ($<50 \mu\text{g/g}$) was used in all ages. Among these children, 7/13 with celiac disease and 11/20 with cow's milk protein intolerance or multiple food intolerance had a positive fecal calprotectin concentration while 2 children with intestinal giardiasis had a concentration $<50 \mu\text{g/g}$. Compared to the adult study population the diagnostic accuracy was superior in children, with a sensitivity of 70%, specificity of 93%, and positive and negative predictive values of 96% and 56%, respectively. In the adult population, false positive tests were found in patients with liver cirrhosis and medication with NSAID and aspirin, resulting in a sensitivity of 64%, specificity of 80%, and positive and negative predictive values of 70% and 74%, respectively.

Berni et al studied the fecal calprotectin concentrations in 281 children, aged 1.1 through 18 years, with gastrointestinal symptoms (132). Compared to controls, the fecal calprotectin concentrations were significantly elevated in groups of children with diseases characterized by gastrointestinal mucosa inflammation, i.e. active allergic colitis, active celiac disease, acute gastroenteritis, gastroesophageal disease, polyposis, and pouchitis. However, the median fecal calprotectin concentration was more elevated in children with active IBD than in any other group. This study reports an optimal cutoff at $103 \mu\text{g/g}$ in calculating the ROC curve.

Certainly, the cutoff that we suggested is lower, but nevertheless $<50 \mu\text{g/g}$ seems to be a reasonable cutoff in children from 4 years and up when using the fecal calprotectin method to detect IBD in suspected cases. With this cutoff we found a very high sensitivity, which is essential if a diagnostic test is to assure that most cases will be detected. However, we must remember that test results are only part of the patient evaluation. Obviously, the severity of symptoms must be considered when making decisions about further investigation, e.g. colonoscopy. Fecal calprotectin is an unspecific marker of inflammation, and conditions other than IBD may also cause concentrations above this cutoff. Consequently, when the fecal calprotectin concentration is between 50 and $100 \mu\text{g/g}$, and suspicion about inflammation is low, an acceptable option might be a new fecal calprotectin test and follow-up examination.

5.4 STUDY III - FECAL CALPROTECTIN AS AN INFLAMMATORY MARKER IN IBD

In Study III, we found a significant correlation between the fecal calprotectin concentrations and the extent and the severity scores of macroscopic and microscopic inflammation in children with IBD. Consequently, the fecal calprotectin method seems to be a useful quantitative surrogate marker for estimating colonic inflammation in pediatric IBD. Our results also indicated that the fecal calprotectin method might be used for recognizing subclinical mucosal inflammation and mucosal healing.

Scoring of colonic inflammation in IBD is a delicate task, and unfortunately no standardized methods exist. The model we chose has been used in other studies. However, there are limitations with scoring models, especially if the inflammation is discontinuous like in CD. Nevertheless, the correlations we found were in agreement with the results from two similar studies. Both of these studies used the original fecal

calprotectin method. Another model for scoring colorectal inflammation was assessed in the first study, which included 62 adult UC patients. The fecal calprotectin concentrations correlated to the microscopic inflammation ($r = 0.7$) and to the macroscopic inflammation ($r = 0.57$) (70). The second study included 13 children with IBD (UC $n = 9$, CD $n = 2$, IC $n = 2$). Fecal calprotectin correlated also in this study to the microscopic inflammation ($r = 0.74$) and to the macroscopic inflammation ($r = 0.65$) when defined as combined extent and severity scores (126).

The excretion of fecal calprotectin has also been compared to assessments of disease activity with methods other than endoscopy. Roseth et al found the correlation between fecal calprotectin and the 3-day excretion of Indium-111 labeled granulocytes to be significant ($r = 0.8$) in adults with IBD (66). Fecal calprotectin excretion also correlated to the scorings of disease activity from technetium-99-labeled white cell scanning in 14 children (CD $n = 10$, UC $n = 3$, allergic colitis $n = 1$) (126). Several of these patients were known to have inflammation in the small bowel. The results were analogous ($r = 0.80$) between the two studies. Additionally, in 35 adult CD patients the correlations between the fecal calprotectin concentrations and the inflammatory scores from radiolabeled white cell scanning were examined (137). The correlation to the combined extent and severity score was found to be significant ($r = 0.71$) whereas no significant correlation was found between the fecal calprotectin concentrations and the CDAI ($r = 0.33$, $p = 0.06$). These studies further support the assumption that fecal calprotectin is a valid marker of gastrointestinal inflammation in IBD and superior to clinical activity indices in predicting the existence of intestinal inflammation.

Our study found slightly elevated fecal calprotectin concentrations in the group of asymptomatic children with mild microscopic inflammation. The quality of fecal calprotectin being able to detect subclinical inflammation has previously been reported only in adults (138, 139). The consequences of low-grade inflammation in the long term is not known, but will be further discussed in Chapter 7 - *Clinical implications and future aspects*.

Moreover, the results in Study III indicated that the fecal calprotectin method may be used as a marker of mucosal healing. These results are supported by a report on adult IBD patients in clinical remission who were investigated with colonoscopy when their fecal calprotectin concentration was $<50 \mu\text{g/g}$ (140). Mucosal healing was achieved macroscopically in 44/45 and microscopically in 38/45 of these patients. The remaining 7 patients had low-grade infiltration in the lamina propria.

Elevated fecal calprotectin concentrations were discovered in Study III in 2 symptomatic CD patients although they had noninflamed colonic mucosa. But in both of these cases the fecal calprotectin could be suspected to originate from inflammation in the small intestine. Accordingly, the calculated specificity and negative predictive value of fecal calprotectin, to distinguish mucosal healing from microscopic inflammation in the colon, may be even better in patients with isolated CD colitis or UC than what we found in Study III. Bremner et al also reported elevated fecal calprotectin concentrations in isolated intestinal CD in 11 children (141). This means that further investigations of the small intestine should be considered in cases with an elevated fecal calprotectin concentration, but normal gastroscopy and ileocolonoscopy.

Previously there has been no suitable fecal marker of inflammatory activity and mucosal healing for routine use. However, measurement of fecal calprotectin concentrations seems to be a promising method for monitoring disease activity in IBD and for follow-up in therapeutic trials.

5.5 STUDY IV - PLASMA CALPROTECTIN AND OTHER BLOOD TESTS IN IBD

In the absence of gastrointestinal infection, fecal calprotectin has been viewed as a better marker of gastrointestinal inflammation than plasma calprotectin in IBD, but we were unable to identify published comparative studies. Hence, we wanted to evaluate plasma calprotectin and two acute phase proteins, SAA and hsCRP, as alternatives to fecal calprotectin, for those children who are reluctant to deliver fecal samples. Our results in Study IV indicated the fecal calprotectin method to be a more reliable and sensitive method for noninvasive estimation of microscopic colorectal inflammation in pediatric IBD when compared to SAA, hsCRP, plasma calprotectin, and routine inflammatory markers in blood.

To our knowledge, these inflammatory markers have not been studied previously in pediatric IBD and established reference values do not exist for plasma calprotectin or SAA in children. However, in a pediatric study of cystic fibrosis the median plasma calprotectin concentration was 700 (range 320 - 1570 $\mu\text{g/L}$) in a small control group of healthy children (142). In comparison with these data the plasma calprotectin concentrations found in Study IV were relatively low, both in the controls and in the IBD children. The noninflamed controls and the IBD children with healed mucosa in Study IV had median hsCRP concentrations similar to what has been described for healthy children (107). However, a comparison between the group of IBD children with healed mucosa and active inflammation, respectively no significant difference was found for either hsCRP or for SAA. Consequently, it appears that only a subtle systemic, acute phase response is induced in pediatric IBD, at least when a mild to moderate histological inflammation is present, as was the case in most patients in Study IV.

In adults, CRP concentrations seem to correlate with clinical disease activity indices and seem to be significantly higher in CD than UC at the same level of disease severity (143). In CD patients, CRP is also considered to correspond closely to clinical remission, response to therapy, and clinical relapse (102). The CRP concentrations in CD were also more elevated than in UC when the inflammatory activity was defined by fecal excretion of Indium-111-labeled granulocytes (144). However, when conventional CRP concentrations (cutoff 8 mg/L) were compared with histological inflammation in UC and CD patients, significance was found only for severe inflammation in CD (145). This has also been confirmed in children with IBD where the conventional CRP concentrations are often within the established reference values despite active ongoing gastrointestinal inflammation (93).

In one study in adult IBD patients with acute episodes, the SAA concentrations were markedly elevated in both CD and UC, whereas the CRP response was more pronounced in CD (146). The observed difference in the CRP response in CD compared to UC has raised the question if the cytokine stimulation differs between UC and CD, or if UC patients might be constitutionally different (144). However, in the study by Niederau et al, the pattern of cytokine release did not differ between UC and CD. Neither the pattern of disease involvement (colitis versus ileitis) nor the type of medical therapy showed any influence on the behavior of the studied inflammatory mediators and acute phase reactions (146). Polymorphisms in the CRP gene have been discussed and interestingly a substantial genetic contribution to baseline CRP and SAA concentrations was found in studying healthy monozygotic and dizygotic twins (104). The baseline CRP is also known to be age dependent (107). Hence, it might be more informative to compare the patient's hsCRP concentrations with previous values than to use a particular cutoff. However, the reliability of acute phase proteins as markers of gastrointestinal inflammation in IBD seems to be limited, as an increase in the acute phase proteins concentrations may originate from infections and various extraintestinal conditions. Fecal measurement of calprotectin apparently more directly reflects histological inflammatory activity. Hence, it is a preferable method for estimating microscopic disease activity.

6 CONCLUSIONS

The following conclusions can be drawn from the studies presented.

- Excretion of fecal calprotectin is not influenced by sex or age in healthy children aged 4 through 17 years (**I**).
- Fecal calprotectin concentration <50 $\mu\text{g/g}$ can be used as reference value for children aged 4 through 17 years (**I**).
- Fecal calprotectin concentrations ≥ 50 $\mu\text{g/g}$ strongly predict the presence of colorectal inflammation in children with gastrointestinal symptoms suggestive of inflammatory bowel disease (**II**).
- A negative test indicates a low probability of colorectal inflammation, and other diagnoses may be considered first if the child has vague symptoms of disease (**II**).
- The fecal calprotectin method may be used as a diagnostic tool to select patients who should undergo diagnostic colonoscopy for investigation of colorectal inflammation including IBD (**II**).
- Fecal calprotectin may be used as a quantitative surrogate marker for estimating macroscopic and microscopic colorectal inflammation in pediatric IBD (**III**).
- Normalized fecal calprotectin concentrations seem to indicate complete microscopic mucosal healing in children with IBD (**III**).
- Fecal calprotectin seems to be a more reliable test compared to plasma SAA, hsCRP, calprotectin in plasma, and routine inflammatory markers in blood for estimating colonic inflammatory activity in pediatric IBD (**IV**).
- Fecal calprotectin is a useful, noninvasive and sensitive marker of colorectal inflammation and can be used as a “CRP-test of the gut” (**I–IV**).
- Fecal calprotectin has the potential to facilitate the diagnostic workup in children with gastrointestinal symptoms and has also the potential for monitoring disease activity in pediatric IBD (**I–IV**).

7 CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

In this thesis, the usefulness of the fecal calprotectin method is proposed. The test can be used to detect colorectal inflammation and IBD in suspected cases, but also to estimate the endoscopic and microscopic colonic disease activity in pediatric IBD. Because of the promising results in Study II we started to use the fecal calprotectin method as a routine diagnostic method already in the beginning of 2004, and we now have considerable clinical experience with the fecal calprotectin method.

The fecal calprotectin method is a helpful tool in the clinical workup in children with gastrointestinal symptoms. The sensitiveness of the test has made it easier to select patients for invasive investigations such as endoscopy and to avoid performing endoscopy in doubtful and probably noninflamed cases. Initially we offered other hospitals in Sweden to submit fecal samples for analysis. However, the method is now becoming a popular, widespread clinical test, whereby several hospitals have introduced the fecal calprotectin method in their own laboratories. The method is also being included in numerous medical studies to evaluate gastrointestinal inflammation. Hence, the number of papers on fecal calprotectin will probably continue to increase in coming years, as will our knowledge of calprotectin.

Although fecal calprotectin is a practicable method, there are some pitfalls to avoid when using fecal calprotectin in clinical practice or studies. First, it is important to be aware of potential factors that may increase fecal calprotectin excretion (Table 15). Several of these factors have been mentioned by different authors, but further studies are needed as indicated in the table. Different mechanisms probably underlie the increased excretion of fecal calprotectin, but these are not fully understood. In the case of non-steroidal anti-inflammatory drug (NSAID), the explanations are thought to be gastrointestinal ulcerations or enteropathy (with inflammation and increased intestinal permeability), i.e. side effects induced by the medication (147, 148). Enteropathy may also be the underlying cause of elevated fecal calprotectin concentrations in cases with over-consumption of alcohol (149). Proton pump inhibitors have also been suspected to give rise to elevated fecal calprotectin concentrations, but controlled studies are needed (149). Nevertheless, in a study by Tøn et al, none of several pharmaceuticals (salazopyrin, azathioprin, steroids etc), nutritional supplements (vitamins, minerals, iron, aluminumhydroxide), or foods (bread, steak) seemed to interfere with the fecal calprotectin assay (71).

Increased concentrations of fecal calprotectin have also been observed in first-degree relatives of CD patients (150) and UC patients (151), but still it is unclear if this is a consequence of genetic predisposition, of environmental factors, or the interaction of both. Further studies are needed to evaluate if these relatives have subclinical inflammation and in that case if they have a greater risk to develop IBD.

Table 15. Potential factors that may increase fecal calprotectin excretion.

Potential factors	Comments	References
Gastrointestinal		
Medication with NSAID	Documented in study	(147, 148)
Over-consumption of alcohol?	Suspect, more data needed	(149)
Proton pump inhibitors?	Suspect, more data needed	(149)
First-degree relative of IBD patient	Subclinical inflammation? More data needed.	(151, 152)
Increased permeability in the intestines?	Early manifestation of IBD? More data needed.	
Profuse gastrointestinal bleeding	Suspect	
Perianal fistula with drainage of pus	Suspect, more data needed	
Obstipation?	Suspect, more data needed	(141)
Extraintestinal		
Liver cirrhosis	Some documentation exists	(131)
Bleeding (menstrual or nose-bleeding)	Suspect, more data needed	
Bacterial infection in the URT, lungs or pharynx	Suspect, more data needed	

The effect of contamination of the fecal sample with blood (menstrual, nose, or gastrointestinal bleeding) or pus (from bacterial infection in the URT or anal fistula) has not been investigated. Logically, however, these conditions should be able to elevate the fecal calprotectin concentrations when an increased amount of neutrophils is added to the feces in these ways. However, it has been estimated that a bleeding volume of at least 100 ml per day is necessary to cause an elevated fecal calprotectin concentration (65). Our results in Study I showed that the median fecal calprotectin concentration in the excluded children with upper respiratory tract infection or tonsillitis was not different from the median in the healthy group of children. However, this observation might be explained by the fact that the infected children probably had nonpurulent infections caused by virus.

Liver cirrhosis is another condition that has been associated with elevated concentration of fecal calprotectin (131). This condition and several others are probably more

common in adults than in children and may explain the lower sensitivity and specificity of the fecal calprotectin method reported in an adult population compared to a pediatric population (131).

A certain variation of calprotectin excretion in feces may also be explained by normal biological day-to-day variability (66, 153). This variation is suspected to be a result of differences in uniformity, number and weight of daily bowel openings, and the slightly uneven distribution of calprotectin in feces. The coefficient of day-to-day variation has been estimated from 29% to 47.5% with the original method and 19% with the improved fecal calprotectin method (67, 130). One study reported a small proportion (3/31) of children with constipation and having fecal calprotectin concentrations >50 $\mu\text{g/g}$ (141). Husebye et al noticed that two populations emerged in adults with normal findings at colonoscopy: one group with remarkably low and stable fecal calprotectin values within the recommended cutoff of 50 $\mu\text{g/g}$ and one group with labile values also beyond this limit (153).

It seems likely that the fecal calprotectin concentration can be elevated not only in colonic inflammation, but also in localized intestinal inflammation. One study reported that 11 children with intestinal CD had elevated fecal calprotectin concentrations (141), and we also observed 2 cases in Study III. Consequently, further investigations of the intestines should be considered when fecal calprotectin concentrations are elevated, even though the gastroscopy and ileocolonoscopy are normal (provided that no other explanation as bacterial gastroenteritis or other conditions from Table 15 could be detected).

Simple methods are needed in clinical studies, but also for routine use in IBD to estimate the inflammatory disease activity in the target organ for treatment – the gastrointestinal mucosal. From Study III and IV we can conclude that the fecal calprotectin method has potential to be an important test for quantifying colonic inflammation and monitoring pediatric IBD. However, the reliability and sensitivity for changes in colorectal inflammatory activity of repeated fecal calprotectin determinations during longitudinal follow-up needs to be studied both in UC and CD. At present, no published studies are available in adults or children.

In routine practice the method seems to be promising in determining whether clinical symptoms originate from disease flares or noninflammatory complications, e.g. fibrostenosis or underlying irritable bowel syndrome (IBS). However, microbial superinfection should also be considered if the patient has a flare with highly elevated fecal calprotectin concentrations. Further, the method may be helpful to guide the physician in finding the optimal time for follow-up colonoscopy, depending on the purpose, i.e. to evaluate the localization and severity of the inflammation in flares, or to perform a control colonoscopy to confirm mucosal healing. Perhaps a follow-up endoscopy can be postponed if the child has no symptoms and the fecal calprotectin concentration indicates good control of disease activity resulting in normalized fecal calprotectin.

In future treatment studies, the fecal calprotectin method may be used as a surrogate marker of intestinal inflammatory activity for evaluating therapeutic response. A recent pediatric study showed the fecal calprotectin concentrations to decline in line with clinical improvement during treatment with steroids, but they seldom fell within the normal range (154). The significance and consequence of subclinical inflammation is not yet fully understood, and there is controversy regarding whether the therapeutic endpoint should be mucosal healing or simply clinical remission (155, 156). Achieving mucosal healing will probably require treatment with more potent immunosuppressive agents, and further studies are needed to evaluate the cost-benefit and the short- and long-term effects before new guidelines about treatment can be presented. Some of the arguments in favor of mucosal healing have been the expectations to alter the disease course, to optimize growth, and to prevent complications, e.g. fibrostenosis, fistulas (155). Chronic bowel inflammation is also known to predispose to malignancy in cases of IBD. Furthermore, in a population of healthy adults aged 50 to 70 years the fecal calprotectin levels were found to be associated with lifestyle risk factors for colorectal cancer, e.g. physical inactivity ($p = 0.01$), obesity ($p = 0.04$), fiber intake ($p = -0.02$), and vegetable consumption ($p = -0.04$). This study speculated that the low-level asymptomatic bowel inflammation might be the link between lifestyle and the pathogenesis of colorectal cancer (135). This further highlights the need of research in subclinical inflammation.

To date, the value of fecal calprotectin as a predictive marker of future clinical relapse has only been studied in adults. Tibble et al used the original method and found a 13-fold increased risk of clinical relapse within 12 months in adult patients with IBD who were in clinical remission and showed elevated fecal calprotectin levels (corresponding to $>250 \mu\text{g/g}$ in the method used by us) (138). At this threshold value, sensitivity was 90% and specificity was 83% for predicting relapse. The predictive value of fecal calprotectin has been investigated in one more study where the test was found to be a stronger predictor of clinical relapse in UC than in CD. When the fecal calprotectin concentration was higher than $150 \mu\text{g/g}$, the relapse risk was two-fold in CD patients and 14-fold in UC patients (139). However 71% of the CD patients in this study had ileitis and just 16% had CD colitis, whereas 68% of the UC patients had proctosigmoiditis. The different localizations of the inflammation may have influenced the subjective symptoms and thereby the clinical disease activity indices, making the results in this study difficult to interpret.

Undeniably, calprotectin is the first inflammatory marker in feces to be available for routine use, but probably not the last because of the advantages of noninvasive tests, especially in pediatric care. Most likely, fecal calprotectin assays will be refined in the future. Already there are several commercial tests for fecal calprotectin on the market. Some of these assays use monoclonal antibodies instead of polyclonal, and the cutoffs may differ. Rapid tests are also on the way. Other neutrophil-derived proteins that can be measured in feces (e.g. elastase, myeloperoxidase, lysozyme, lactoferrin, and protein S100A12) (157-161), and luminal nitric oxide (162, 163), have also been evaluated as inflammatory markers in recent years. Several of them appear to be interesting markers of gastrointestinal inflammation as well, but calprotectin may have some advantages because it accounts for 60% of the cytosolic protein found in neutrophils.

8 SAMMANFATTNING PÅ SVENSKA (SUMMARY)

Kronisk inflammatorisk tarmsjukdom (Inflammatory Bowel Disease – IBD) kännetecknas av inflammatoriska förändringar med rodnad, svullnad och sår i delar av mag-tarmkanalens slemhinna. Under de sista årtiondena har IBD ökat påtagligt bland både barn och vuxna. Orsaken till ökningen är inte känd, men beror troligen på flera samverkande faktorer som t.ex. ärftlighet, miljö och obalans i immunförsvaret. I Sverige finns uppskattningsvis ca 50 000 individer med IBD och många insjuknar redan under barnåren. De vanligaste formerna av IBD är Crohns sjukdom (CD) och ulcerös kolit (UC). Framför allt är det Crohns sjukdom, som har blivit vanligare hos barn. Symptomen vid IBD hos barn är ofta diffusa och det kan ibland ta tid innan misstanke om sjukdom väcks. Barnets näringsstillstånd kan därför bli påverkat innan rätt diagnos ställs, vilket innebär att vikt och längdtillväxt liksom pubertetsutveckling drabbas. Andra vanliga symptom är magont, diarré, blod i avföringen och/eller viktnedgång.

Utredning av misstänkt IBD innebär ofta stora påfrestningar på barn och föräldrar. De blodprover som idag används kan inte på ett tillförlitligt sätt upptäcka pågående inflammation i mag-tarmkanalen. Därför krävs också avancerade undersökningar såsom gastroskopi, koloskopi och röntgen. Gastroskopi innebär att den övre mag-tarmkanalen granskas med hjälp av en böjlig slang och vid koloskopi utförs motsvarande undersökning på tjocktarmen och nedersta delen av tunntarmen. Genom slangen kan man också ta vävnadsprov, för att i mikroskop bedöma om slemhinnan är inflammerad. Skopi-undersökningar på barn utförs i regel under narkos. Hittills har ett betydande antal besvärliga och kostsamma skopi-undersökningar utförts, för att säkert kunna utesluta IBD. Vid känd IBD har upprepade skopi-undersökningar varit nödvändiga, för att bedöma om insatt mediciner haft avsedd effekt, d.v.s. att minska pågående inflammation i tarmslemhinnan.

Calprotectin är ett protein, som finns i neutrofila granulocyter, d.v.s. i en typ av vit blodkropp som ingår i kroppens immunförsvär. Detta protein är mätbart i olika kroppsvätskor, inklusive blod och avföring. Proteinet tål förvaring i rumstemperatur, vilket innebär att avföringsprov kan skickas per post för analys.

Syftet med den här avhandlingen har varit att utvärdera om analys av calprotectin i avföring kan användas som metod, för att upptäcka inflammation i tjocktarmen vid misstänkt IBD och för att mäta pågående sjukdomsaktivitet vid känd IBD.

I **studie I** var avsikten att *fastställa referensvärden för calprotectin i avföring hos barn*. Sammanlagt ingick 117 friska barn i åldrarna 4 till 17 år i studien. Samtliga barn lämnade avföringsprov och en skriftlig hälsodeklaration. Medianvärdet för calprotectin i avföring var 13,6 mikrogram/gram utan påvisbar skillnad i de lägre respektive högre åldrarna eller mellan könen. Ett av barnen, som hade ett värde >50 mikrogram/gram, utvecklade senare ulcerös kolit med inflammation i ändtarmen.

Slutsatsen blev att <50 mikrogram/gram är lämpligt som normalvärde hos både pojkar och flickor från 4 års ålder och att värden däröver bör följas upp.

I **studie II** var syftet att *utvärdera om calprotectin i avföring kan användas som markör, för att upptäcka inflammation vid misstanke om IBD*. 36 barn med mag-tarmbesvär lämnade avföringsprov innan de genomgick rutinmässig koloskopiundersökning. I 95 % av fallen med pågående inflammation var koncentrationen av calprotectin förhöjd (>50 mikrogram/gram). Huvuddelen (93 %) av barnen med frisk slemhinna i grovtarmen, hade normala värden av calprotectin. Calprotectin i avföring var också klart överlägset rutinmässiga blodanalyser, för att påvisa inflammation i tjocktarmen. Metoden kan därmed användas, för att på ett tillförlitligt sätt spåra inflammation såsom IBD i tjocktarmen. Detta underlättar beslut om vilka barn som behöver genomgå koloskopi och vilka av dessa som särskilt behöver prioriteras.

I **studie III** var avsikten att *utvärdera om mängden calprotectin i avföring var proportionell mot grad och utbredning av inflammationen i tjocktarmen vid känd IBD*. Sammanlagt 39 barn med IBD lämnade avföringsprov inför koloskopi-kontroll. Resultaten visade mycket god överensstämmelse mellan calprotectin i avföring och grad, samt utbredning av inflammation i tjocktarmens slemhinna. Barn med läkt slemhinna uppvisade normala koncentrationer av calprotectin i avföringen. Slutsatsen blev att calprotectin i avföring kan användas som markör för att uppskatta pågående tarminflammation vid känd IBD. Därmed kan man få ett mått på pågående inflammation utan att patienten behöver genomgå koloskopi.

I **studie IV** var syftet att *jämföra rutinmässiga och nya inflammationsmarkörer i blod (såsom högkänsligt CRP, serum amyloid A och plasma-calprotectin) med calprotectin i avföring, som mått på mikroskopisk inflammation vid känd IBD*. 32 barn med känd IBD lämnade blod- och avföringsprov inför koloskopi-kontroll. Calprotectin i avföring visade bättre överensstämmelse med mikroskopisk inflammation i tjocktarmen än något av blodproverna.

Sammanfattning:

Dessa fyra studier visar att calprotectin i avföring är en enkel och tillförlitlig markör för inflammation i tjocktarmen hos barn. Vid misstänkt IBD kan metoden användas för att bättre identifiera patienter, där utredning med koloskopi är motiverad. Hos barn med känd IBD verkar metoden kunna mäta graden av pågående inflammationsaktivitet på ett tillförlitligt sätt. Metoden kan därmed komma att bidra till en förbättrad sjukdomskontroll vid IBD, med ökad möjlighet till medicinjustering i ett tidigt skede. Genom att mäta calprotectin i avföring kan antalet koloskopier på barn komma att reduceras, vilket innebär minskat lidande för patienten och ekonomisk vinning för sjukvården.

9 ACKNOWLEDGEMENTS

I would like to express my deep gratitude to each of you who have supported me and contributed to the accomplishment of this work. In particular I would like to thank:

Yigael Finkel, Associate Professor and my clinical mentor and academic supervisor, for introducing me to the field of pediatric gastroenterology, especially IBD and endoscopy, and for your encouraging support and your confidence in me.

Lars Lööf, Professor and my co-supervisor, for many fruitful discussions, and for sharing your wealth of scientific knowledge.

Lars-Olof Hansson, Associate Professor, for introducing me to the field of clinical chemistry and inflammatory markers, and for always being enthusiastic and supportive.

Johan Lindholm, Associate Professor, for sharing your profound knowledge in gastrointestinal histopathology and for your persistence with grading of histology in many hundreds of biopsy specimens.

Rumjana Djilali Merzoug, Biochemist, for technical expertise, splendid laboratory work, and for your shared enthusiasm and interest in this project.

Urban Myrdal, my colleague, for introducing me to the field of pediatric gastroenterology and sharing your broad base of experience.

Jerzy Leppert, Professor, for believing in my ideas about clinical research in the field of pediatric gastroenterology and for including me as part of the team at the Centre for Clinical Research, Västerås.

Kajsa Nyquist, Britta Parwén, Kerstin Aller, and all others in the gastro-team at Astrid Lindgren Childrens Hospital for your generous assistance in performing the studies and for being such a nice team.

Petra Wahlén, Kent Nilsson, and John Öhrvik, for excellent statistical advice and **Gun Nyberg, Katarina Ringström, Maria Dell' Uva Karlsson, and Tony Wiklund** for valuable help in many ways at the Centre for Clinical Research, Västerås.

Mårten Hallberg, my former chief, for encouraging me to start with research and **Jan Eriksson and the rest of my colleagues** at the Department of Pediatrics, Västerås, for taking interest in my research and allowing their children to participate in the project.

Amela Cefo and the staff at the Department of Pediatrics, Västerås and **Inger-Lise Johansson and Åsa Andersson**, nurses in the school health services of Västerås, for excellent assistance with this project.

Rosanne Forberg and the staff at the department of Clinical Chemistry, Västerås, for practical help with handling of samples.

Ulf Andersson, Professor, for believing in and supporting this project.

Hans Hildebrand, Associate Professor, for generously sharing your wisdom and expertise.

Lena Grahnquist, Henrik Arnell, Maja Idestrom, and Kalle Kappinen, my colleagues at the Department of Pediatric Gastroenterology and Nutrition, Astrid Lindgren Childrens Hospital, for your interest in the project and your highly valued support and friendship.

Lennart, my husband, for your love and for standing by my side through the years.

Antonia and Adrian, my lovely children, for inspiration and for being the absolute joy of my life.

Vera and Helge, my parents, for giving me all their love and invaluable and constant support.

Anette and Helena, my sisters, **and their families** for always being appreciated and honest friends.

Julia and Lennart, my parents-in-law, **and the rest of my family** for giving invaluable encouragement in all situations.

My dear friends for all their support and encouragement during these years. I hope to see you more in the future.

The children and their parents who made this thesis possible by participating in the project.

The studies have been supported by:

Bengt Ihre's Foundation, County Council of Västmanland, Freemasons in Stockholm, Mayflower Charity Foundation for Children, Samariten, The Swedish Society of Medicine, Sällskapet Barnavård, AstraZeneca and Svensk gastroenterologisk förening, Ferring and Schering.

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