Calprotectin
The emerging Autoimmunity marker for intestinal inflammation

What is Calprotectin?

- Synonyms:
  - L1 protein
  - Human leucocyte protein
  - MRP-8/14
  - Calgranulin (A and B)
  - Cystic fibrosis antigen (CFA)

- Present in normal stool in up to 50 µg/g stool (higher in newborns)

What is Calprotectin?

- Calcium- and zinc-binding protein of the S100-group
- Heterodimeric complex

- Many functions in the cell

- Calprotectin is a major part of the cytoplasm of neutrophil granulocytes, monocytes and epithelial cells
  - 60% of the soluble ingredients and 5% of the total proteins of neutrophil granulocytes

What is Calprotectin?

- In inflammation, the leukocytes migrate through the intestinal wall → increased calprotectin level in the stool

- The concentration of calprotectin correlates with the number of granulocytes in the intestinal lumen

In which diseases does Calprotectin occur in the stool?

- Inflamatory Bowel Diseases

What are the specific features of IBD?

Inflammatory Bowel Diseases (IBD)

- Chronic conditions of the gastrointestinal tract that may occur at various times over a lifetime
- The main IBD entities are Crohn's disease (CD) and ulcerative colitis (UC)
- Accounting for far fewer cases are other forms of IBD, which are not always classified as typical IBD:
  - Collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's disease, indeterminate colitis

Epidemiology of IBD

- Prevalence app. 0.1 % (lower in other ethnic groups – caucasians have about 4x higher risk)
- But:…´The number of cases increased by 3.08 times over the past 10 years´…*
- Incidence: 10/100,000/year
- May affect persons of all ages, peak between 15 years to 35 years

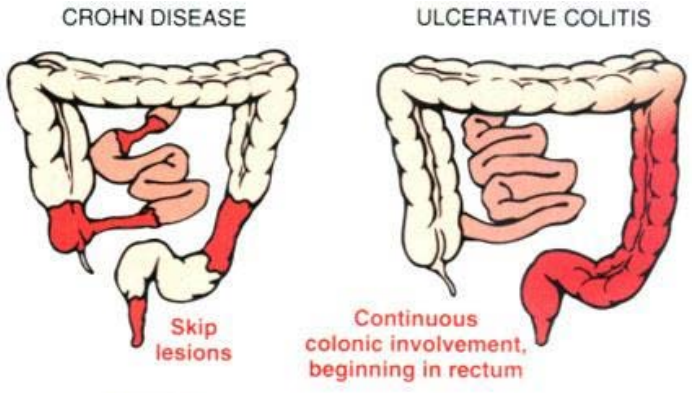
*Jiang XL, Cui HF. An analysis of 10218 ulcerative colitis cases in China. World J Gastroenterol. 2002;8:158–161.

Epidemiology of IBD

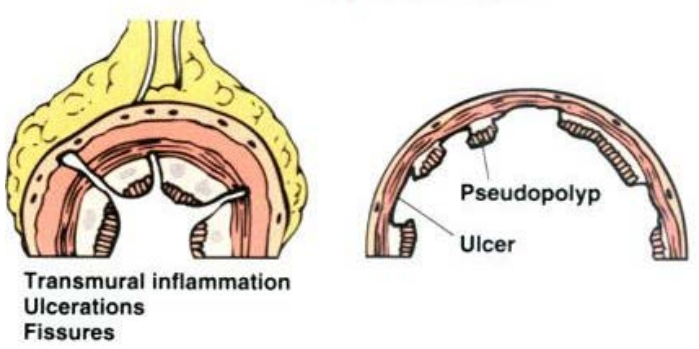
- Males and females are affected equally
- Genetic background may be important
  - about 20 percent of subjects with Crohn's have a blood relative with some form of inflammatory bowel disease
- The risk of developing ulcerative colitis is higher in nonsmokers, but….you know

Main difference between Crohn's disease and UC

- Location of inflammatory changes
  - Crohn's can affect any part of the gastrointestinal tract,
    from mouth to anus (*skip lesions*)
    The majority of cases start in the terminal ileum
  - UC is restricted to the colon and the rectum (as the name suggests)



- Nature of inflammatory changes
  - Crohn’s affects the whole bowel wall ("transmural lesions“)
  - UC is microscopically restricted to the mucosa (epithelial lining of the gut)



Classical symptoms and Crohn‘s disease and UC

- Abdominal pain, vomiting, diarrhea, rectal bleeding, severe internal cramps/muscle spasms in the region of the pelvis, weight loss

- Various associated complaints or diseases like arthritis, pyoderma gangrenosum, and primary sclerosing cholangitis

Main difference between Crohn's disease and UC

- Extra-intestinal manifestations
  - such as liver problems, arthritis, skin manifestations and eye problems occur in different proportions
- Quality of life in patients with Crohn’s disease generally lower than with UC
- Overlap
  - Rarely, a definitive diagnosis of neither Crohn's disease nor UC can be made by biopsy sample presentation (indeterminate colitis)

Diagnosis of IBD

- **Blood tests** - to determine whether there is anemia resulting from blood loss, or whether there is an increased number of white blood cells, suggesting an inflammatory process

- **Stool culture** - to determine whether there is blood loss, or whether an infection by a parasite or bacteria is causing the symptoms

- **Colonoscopy, biopsy**

- **Upper GI x-ray (also called barium swallow)**
  A barium containing fluid coating the inside of organs
  is swallowed and will show up on an x-ray of the GI organs

- **Barium enema** - barium is given into the rectum as an enema

Differential diagnoses

- Infections
  (clostridium, cytomegalovirus, yersinia, bacterial or viral gastroenteritis, giardiasis…)
- Eosinophilic Gastroenteritis
- Food Poisoning
- Intestinal Motility Disorders
- Intestinal Radiation Injury
- Lactose Intolerance
- Food allergy
- Celiac disease
- Irritable bowel syndrome (IBS)

Differential diagnosis – IBS

Irritable bowel syndrome (IBS or spastic colon)

- Most frequent gastrointestinal disorder
- Diagnosis of exclusion
- Functional bowel disorder characterized by chronic abdominal pain, discomfort, bloating, and alteration of bowel habits in the absence of any detectable organic cause
- Diarrhea or constipation may predominate, or they may alternate (classified as **IBS-D**, **IBS-C** or **IBS-A**, respectively)
- IBS may begin after an infection (post-infectious, **IBS-PI**), a stressful life event, or onset of maturity without any other medical indicators
- IBS is **not** inflammatory!

Treatment of IBD

- Mesalazine is the main drug. It is more useful in UC than in Crohn‘s
- Immunosuppression in severe cases to control the symptom (prednisone, TNF inhibition, azathioprine, methotrexate, or 6-mercaptopurine)
- Steroids are used to control disease flares and were once acceptable as a maintenance drug
- Biologicals (such as TNF inhibitors)
- Surgery in severe cases (bowel resection, strictureplasty or a temporary or permanent colostomy or ileostomy)
- Alternative medicine concentrate on controlling underlying pathology in order to avoid prolonged steroidal exposure or surgical excisement

Treatment in development

- "Helminthic therapy" may not only prevent but even cure (or control) IBD: a drink with roughly 2,500 eggs of the *Trichuris suis* helminth taken twice monthly decreased symptoms markedly in many patients. It is even speculated that an effective "immunization" procedure could be developed – by ingesting the cocktail at an early age

- Prebiotics and probiotics and in some studies have proven to be as effective as prescription drugs.
(Furrie E et al. Gut 2005, 54: 242–9; Kruis W et al. Gut 2004, 53: 1617–23)

- Cannabis eases IBD symptoms

Prognosis for IBD patients

- Limits quality of life but is rarely fatal on its own
- Fatalities due to complications such as toxic megacolon, bowel perforation and surgical complications are rare
- Increased risk of colorectal cancer, particular in widespread ulcerative colitis
  - This is usually caught much earlier than in the general population in routine surveillance of the colon by colonoscopy, and therefore patients are much more likely to survive
- Possibly elevated risk of endothelial dysfunction and coronary artery disease

Clinical usefulness of the Calprotectin determination!

Calprotectin is a marker for intestinal inflammation

- Calprotectin is a highly sensitive and specific marker for detection of intestinal inflammation (Vieira et al., 2009)

- Calprotectin is the most accurate tool to asses active mucosal inflammation (Canani et al., 2008)

Calprotectin can differentiate between inflammatory and non-inflammatory intestinal disorders

- Calprotectin is useful to differentiate organic intestinal disorders (means IBD) from functional disorders, e.g. polyps (Gisbert & McNicholl, 2008)

Calprotectin can differentiate between IBD and IBS

- Calprotectin is able to separate inflammatory bowel diseases (IBD), like Crohn's disease (CD) and ulcerative colitis (UC) from irritable bowel syndrome (IBS) (Konikoff & Denson, 2006, Sutherland et al., 2008)

- Calprotectin is an accurate marker of IBD in both children and adult patients (Carroccio et al., 2003)

- Lactoferrin is the only gastrointestinal marker showing comparable good performance like Calprotectin (Sutherland et al., 2008)

Calprotectin is a marker for disease activity in IBD

- Calprotectin levels correlate with the severity/activity of IBD (Sutherland et al., 2008), but Calprotectin better reflects the disease activity in UC than CD (Vermeire et al., 2005; Gisbert & McNicholl, 2008)

- Calprotectin can predict IBD relapses and can be used for monitoring and optimizing therapy in IBD patients (Gaya & Mackenzie, 2002; Angriman et al., 2007; Sutherland et al., 2008)

Calprotectin is a non-invasive first step method to provide guidance in the diagnostic workup of GI distress patients

- Calprotectin detection is a non-invasive method in contrast to endoscopy (Canani et al., 2008)
- Calprotectin is remarkably stable and easy to detect in stool (Adland & Fagerhol, 2002; Angriman et al., 2007)
- Calprotectin is a useful screening tool for identifying patients who are most likely to need endoscopy for suspected IBD (Fagerberg et al., 2005; van Rheenen et al., 2010)
- Measuring Calprotectin levels would result in a 67% reduction in the number of adults requiring endoscopy (van Rheenen et al., 2010)

Attention: Calprotectin occurs to some extent also in intestinal disorders other than IBD!

- Several diseases other than IBD – specially colorectal neoplasia and gastrointestinal infection – can also increase Calprotectin (Gisbert & McNicholl, 2008)

- Calprotectin is increased in a majority of patients with scleroderma (Andreasson et al., 2011)

EliA Calprotectin

Is it inflammatory ?

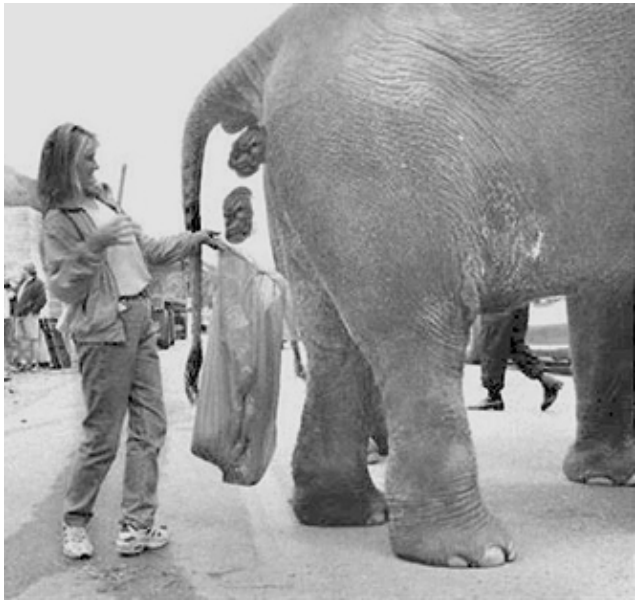

Phadia 100


Phadia 250


Phadia 2500


Phadia 5000

A critical aspect: Sampling



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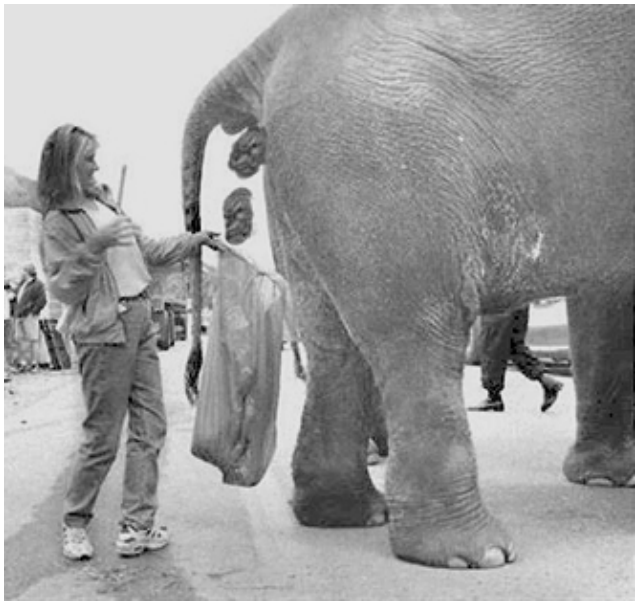

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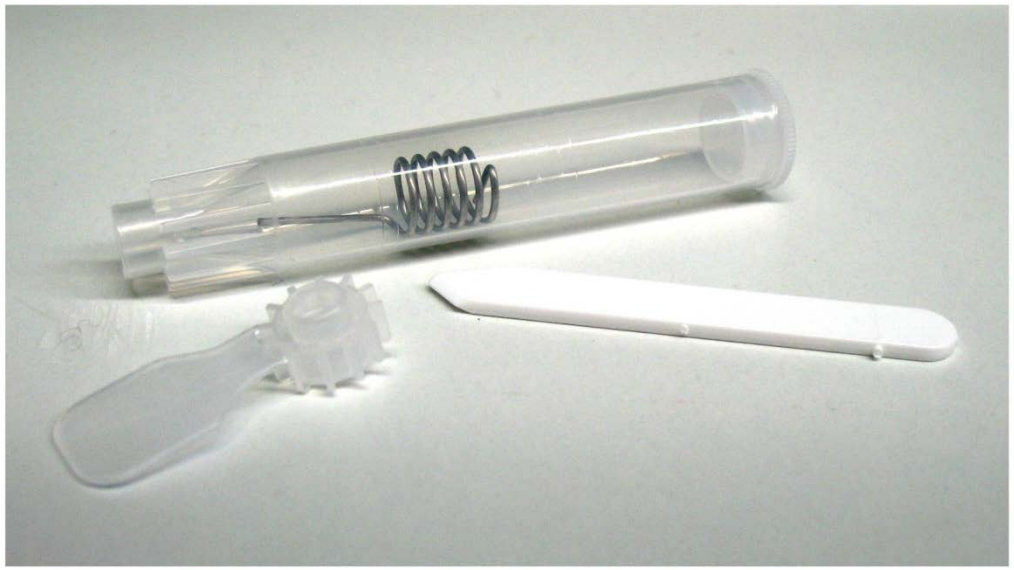
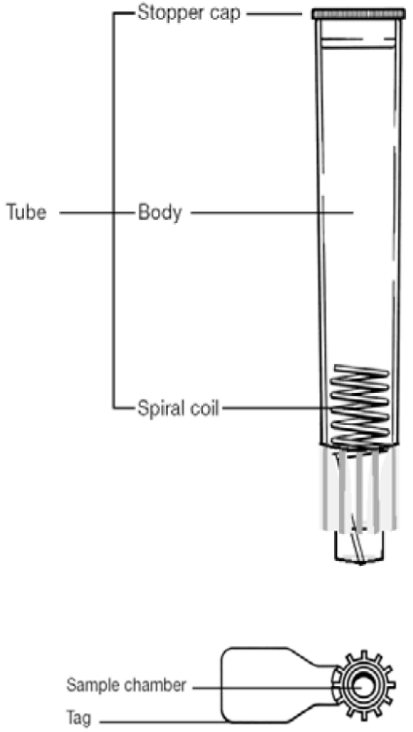
Sample Extraction

- Roche “faecal sample preparation kit” is recommended
- Can be ordered through Phadia (now Thermo Fisher Scientific)
  (Faecal Extraction Device; 50 units/package)



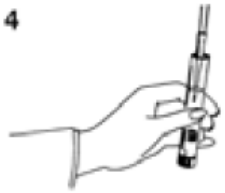


Sample Extraction

Carefully press the sample into the hollow cavity in the base cap and level off the surface

Firmly press the tube onto the base cap, detach the tag, and add 5 ml of Extraction Buffer

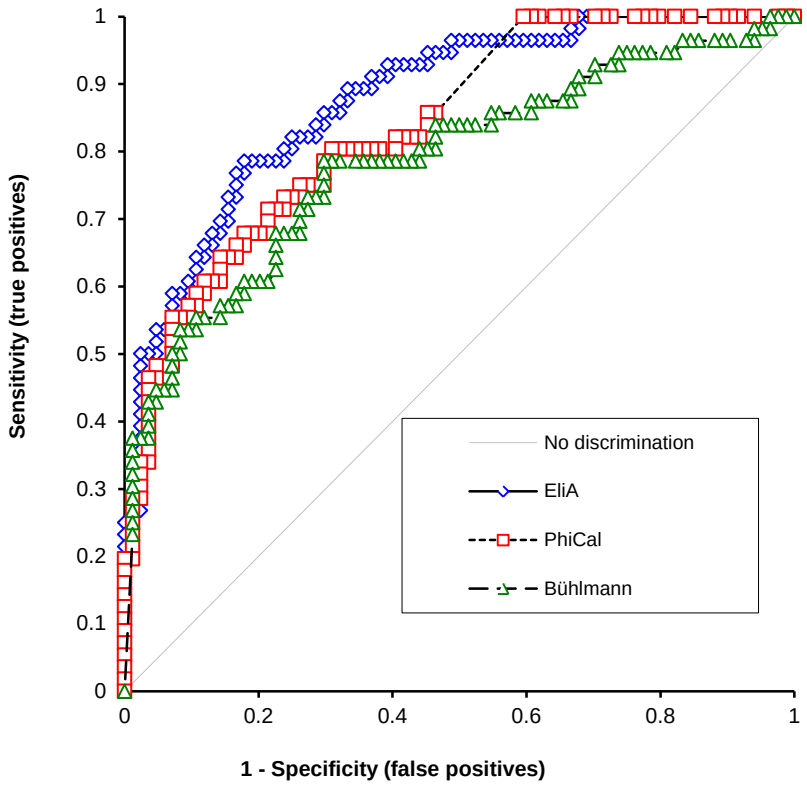
Carefully re-cap the tube and homogenize the sample for about 1 minute using a vibration mixer (e.g. Vortex mixer). Centrifuge for 5 minutes at 3'000 x g (for centrifugation transfer into an Eppendorf tube)



Collect the supernatant and use an appropriate dilution for the quantification of Calprotectin.

- About 100mg of homogenous stool are transferred to the extraction device
- The transferred amount is weighed
- Add EliA Calprotectin Extraction Buffer (50 times the weight volume: 5 ml + 100mg stool sample)
- Homogenize the sample using a vortex mixer and incubate for 5 minutes
- Transfer the homogenate (1-2 ml) to an Eppendorf tube and centrifuge for 5 minutes at 3000-10000 rpm
- Transfer the supernatant into a fresh tube
- Extraction needs about 15 min time
- This extract can be stored long term for at least 4 month

EliA Calprotectin – first results



Comparison with two conventional manual tests (PhiCal and Bühlmann) with following samples:

Ulcerative colitis	20
Crohn‘s disease	13
Celiac disease	18
Disease controls	11
Stool donors	78

Thank you!