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Clinical, Biological, and Laboratory Parameters as Predictors of Severity of Clinical Outcome and Response to Anti–TNF–Alpha Treatment in Ulcerative Colitis

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1. Introduction

Ulcerative colitis (UC) is a chronic relapsing inflammatory bowel disease (IBD). The pathogenesis of IBD is complex and so far not fully understood. The long-term clinical outcome of UC is hard to predict. Some clinical phenotypes of UC may to some extent predict the severity of the disease, but the clinical impact of these predictors is minor. . The new immunomolecular understanding of the pathophysiological mechanisms behind the disease, and especially the genetic engineering knock out animal models in early 1990's, was the start of a new therapeutic strategy: the targeting therapy. Based on new knowledge of proinflammatory and anti-inflammatory molecules, new therapeutic targets were established to attack specific components of the inflammatory cascade. TNF-alpha was the first molecule to be blocked with effects on the disease activity first described in the animal models and then described in humans in 1995 for Crohns disease (CD). Later on, numerous other targeting molecules have been developed with more or less efficacy. So far the anti-TNF agent infliximab (IFX) is the only targeting agent that has been found to be effective in UC. However, a lot still needs to be done in order to achieve "personalized engineered therapy" in IBD. After some ten years experiences with targeting therapy the major unresolved questions are: which UC patients will have the greatest effect of targeting therapy inducing a "deep, longstanding" remission and prevent severe outcome such as colectomy? Which patients are in the need of long-term maintenance therapy? If and when can target therapy be stopped? Few studies have investigated potential predictors of the disease's severity and future clinical outcome after the introduction of targeting therapy. Therefore, after ten years with targeting therapy of UC, what clinical, biological or histological markers could give some answers to these questions addressed above?

2. Clinical markers in various clinical settings

Definition of a biological marker

Biomarker is defined by National Institute of health (NIH) as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" (Colburn, 2000). An optimal biomarker has to fit into several criteria to be of clinical value. It has to be accurate, reproducibly, acceptable for the patient, and have high sensitivity and specificity for the outcome it is expected to identify (Mendoza and Abreu, 2009). A **prognostic biomarker** should indicate future severe clinical outcomes such as intestinal stenosis and need of surgical resections. This would give valuable information such as priority of treatment options and closer follow-up visits. A **predictive biomarker** should give information of therapeutic effect of agents such as targeting therapy. The ideal biomarker assay from tissue or body fluids should be accurate, reproducible with high sensitivity and specificity. The biomarker concept is old. Despite this, there is a lack of validation of new candidate biomarkers in order to give a useful guidance to the clinicians (2010).

So far no ideal biomarkers have been found in IBD, but several serological and fecal biomarker candidates have been proposed (Mendoza and Abreu, 2009). A biomarker in IBD should predict: the clinical outcome with special emphasis of patients with complicated disease; which therapeutically strategy should be used and especially which patients would be suitable for the high cost treatment of targeting agents; and finally are there biomarkers of resistance to therapy.

In this review we present the candidate biomarkers based on "old", established methods easily performed in a clinical setting, and biomarkers from the new era of high technological methods.

2.1 Demographic factors (clinical phenotypes) as predictors of disease severity and response of treatment

2.1.1 Smoking, age and early onset of disease

In a retrospective study of Roth et al (Roth *et al.*, 2010) one hundred and two UC patients were investigated. In this study charts were reviewed using standardized data collection forms. Disease severity was generated during the chart review process, and non-endoscopic Mayo Score criteria were collected into a composite. They found that UC severity was associated with younger age at diagnosis and year of diagnosis. Previous studies have shown that patients with early onset UC are more prone to colorectal cancer (Eaden *et al.*, 2001). In the study of Roth et al (Roth *et al.*, 2010) disease severity at presentation did not correlate with the severity index over the disease course, nor did delay from diagnosis to treatment. This is in contrast to the literature presented in a review article by Sandborn (Sandborn, 1999) who found that classifying presentations into one of four severity categories was useful for prognosticating disease. Another intriguing result of the study by Roth et al was that smoking status did not predict disease severity (Roth *et al.*, 2010). Multiple previous studies have found that smoking is a protective factor (Calkins, 1989), while non- or ex-smokers have a higher risk of relapsing disease (Hoie *et al.*, 2007). The limitations of the study by Roth et al are that it is retrospective in design and relies heavily on chart review.

2.1.2 ANCAs and ASCA

Perinuclear anti-neutrophil antibody (ANCAs) is associated with chronic inflammatory diseases as Wegener`s granulomatosis, rheumatoid arthritis and UC (Mendoza and Abreu, 2009). In CD the seroprevalence of ANCA ranges from 2-28% and in UC 20-85% (Mendoza and Abreu, 2009). The sensitivity and specificity in diagnosing IBD range from 50-70% and 80-85%, respectively (Mendoza and Abreu, 2009). Anti-saccharomyces cerevisiae antibodies (ASCA) are found in 39%-69% of CD patients, but only in 5-15% of UC patients (Mendoza and Abreu, 2009). The sensitivity and specificity in diagnosing IBD range from 65-70% and 80-85%, respectively (Mendoza and Abreu, 2009).

The clinical value of ANCA or ASCA to differentiate IBD from IBS patients is limited because of low sensitivity. Serologic evaluation of ANCAs and ASCAs may be helpful in patients with indeterminate colitis (Joossens *et al.*, 2002). Interestingly, pANCA in CD patients have been negatively associated with small small bowel disease, fibrostenosis and small bowel surgery (Vermeire *et al.*, 2004), while ASCAs have been associated with several CD clinical phenotypes (Vermeire *et al.*, 2001;Walker *et al.*, 2004). Further, ASCAS have been associated with small bowel disease in CD in addition to stricturing as well as penetrating disease behavior (Mow *et al.*, 2004; Vermeire *et al.*, 2001; Walker *et al.*, 2004). There is also found a strong association between development of pouchitis and high level of ANCAs (Fleshner *et al.*, 2001; Vernier *et al.*, 2004). Though, this has not been confirmed in another study (Aisenberg *et al.*, 2004).

2.2 Biochemical factors reflecting disease activity or responses to specific therapies (C-reactive protein, calprotectin)

2.2.1 C-reactive protein

2.2.1.1 CRP production in ulcerative colitis and Crohn´s disease

C-reactive protein (CRP) is an acute phase protein that increases during inflammation and has a short half life (19 hours) and will therefore rapidly decrease after resolution of the inflammation (Vermeire *et al.*, 2006). Serum CRP as a biomarker in the course of UC has been studied by number of workers but its usefulness as a diagnostic screening test has not been fully assessed. However, CRP is the most sensitive screening biomarker compared to other biomarkers in adult population for detecting IBD, where CD has the strongest CRP response of the two diseases (Lewis, 2011; Vermeire *et al.*, 2006). The reason for this difference between the two diseases is not known. Both CD patients and UC patients have increased levels of cytokines belonging to T-helper-1 response (Olsen *et al.*, 2007; Vermeire *et al.*, 2006). Santos et al investigated 957 subjects and found strong association between CRP and central abdominal obesity (Santos *et al.*, 2005). Positive association between BMI and CRP has also been observed in otherwise healthy adults and children, suggesting a state of low-grade systemic inflammation in obese persons (Visser *et al.*, 1999; Visser *et al.*, 2001). Several groups have investigated adipose tissue and concluded that invasion of inflammatory cells in adipose tissue leads to increased levels of various pro-inflammatory cytokines including IL-6, IL-8, TNF-alpha, IL-10 and IL-18, potentially linking fat and inflammation (Fantuzzi, 2005;Isakson *et al.*, 2009). It has been speculated whether the transmural inflammation in CD and possible involvement of mesenteric fat which is a major site of IL-6 and TNF-alpha synthesis may explain the difference of the two diseases. Colombel et al examined whether small bowel inflammation at CT enterography correlated with endoscopic severity and CRP in 143 patients with CD (Colombel *et al.*, 2006). They

concluded that CRP correlated with radiological findings of perienteric inflammation (increased fat density), but not of inflammation limited to the small bowel wall, underscoring the potential role of perienteric inflammation in CRP response in CD. Solem et al confirmed this finding in a recent study where abnormal small bowel radiographic imaging was not associated with CRP elevation in CD patients (Solem *et al*., 2005). The final conclusions concerning the difference of CRP in UC and CD patients are still not drawn. However, even though the CRP response is strongest in CD, the overlap between CD and UC patients in CRP levels make it difficult to use it for differential diagnosis between the two diseases.

2.2.2 CRP, genetic factors and IBD

Individual genetic factors may also contribute to differences of CRP levels. Recent studies have suggested that polymorphisms in the CRP gene account for the inter-individual differences in baseline CRP production in humans (Carlson *et al*., 2005) but so far results are conflicting (Vermeire *et al*., 2006). Interestingly, Greenfield et al investigated 194 healthy female twins to examine the relationship between CRP, BMI, blood pressure, lipids and apolipoproteins, independent of genetic influences (Greenfield *et al*., 2004). They concluded that CRP was strongly related to total and central abdominal obesity, blood pressure and lipid levels independent of genetic influences (Greenfield *et al*., 2004).

2.2.3 CRP and the role in predicting disease activity in IBD

In a study by Prantera et al (Prantera *et al*., 1988) 60 UC patients were investigated and they found that the disease severity and the presence of signs and symptoms of toxicity seemed likely to be determined by the amount of colonic tissue involved by inflammation, both in depth and in extent. CRP appeared the most reliable factor reflecting activity and extension of lesion. In a study from Mayo Clinic (Solem *et al*., 2005), 43 UC patients were investigated. In this study they concluded that serum CRP levels were associated with increase in biomarkers of inflammation (except platelets) and an active disease at ileocolonoscopy. However, histological activity was not associated with CRP concentrations in UC patients. These results should though be interpreted with caution given the relatively small sample size of 43. In another study Chouhan et al. (Chouhan *et al*., 2006) concluded that measurement of CRP levels is a simple method of assessing disease activity and extent in UC. They concluded that CRP level >12 mg/L is indicative of severe and extensive disease and that a change in CRP following therapy is a good parameter to assess the effect of the drug on the underlying inflammation. A decrease in CRP in response to therapy is objective evidence that the drug has a beneficial effect on gut inflammation even in patients with little change in symptoms. On the other hand, persistently raised CRP indicates failure of the therapy to control mucosal inflammation (Pepys and Hirschfield, 2003).

In an interesting newly published prospective study of Henriksen et al (Henriksen *et al*., 2008) CRP was measured at diagnosis and after 1 and 5 years in patients diagnosed with IBD in southeastern Norway. After 5 years, 454 patients with UC and 200 with CD provided sufficient data for analysis. The authors concluded in line with earlier findings that patients with CD had a stronger CRP response than those with UC. In patients with UC, CRP levels at diagnosis increased with increasing extent of disease. However, in this study 71% of patients with UC still had CRP levels within the normal range and mean CRP values were within the normal range in all UC subgroups at 5 years. Further they found that in patients

with UC with extensive colitis, CRP levels above 23 mg/l at diagnosis predicted an increased risk of surgery (odds ratio (OR) 4.8, p=0.02). In patients with ulcerative colitis, CRP levels above 10 mg/l after 1 year predicted an increased risk of surgery during the subsequent 4 years (OR 3.0, p=0.02). Interestingly, five years later the authors found no difference in CRP levels between 195 patients who underwent colonoscopy and were in endoscopic remission and those with endoscopic inflammation (mean 6 mg/l versus 7 mg/l, p=0.59). This finding is somehow in contrast to the results of increasing extent of UC correlates with increasing CRP levels. The authors conclude that the results may indicate that CRP is of limited value in predicting disease activity during follow-up. The strength of this Norwegian study is that it is prospective in design (Henriksen *et al.*, 2008). In addition the size of the Norwegian study population seems to be appropriate, in contrast to the study from Mayo Clinic. A minor limitation of the Norwegian study is that it is a multicenter study with different clinicians and therefore there may be some grade of inter-observer variations.

2.2.4 CRP and the role in predicting response to therapy

Several clinical studies have investigated the role of CRP for monitoring the effect of treatment. A decrease in CRP in response to therapy could be objective evidence that the drug has a beneficial effect on gut inflammation. In a recent published Cochrane analysis, the authors included all the randomized controlled trials comparing natalizumab to a placebo or control therapy for the induction of remission in CD (Macdonald and McDonald, 2007). Subgroup analyses demonstrated statistically significant differences in clinical response at twelve weeks favoring three infusions of natalizumab (4 mg/kg) over placebo for CD patients with an elevated CRP at baseline (Macdonald and McDonald, 2007). In a Belgian study 153 CD patients were included and treated with infliximab. Baseline CRP>5 mg/l before the start of therapy was associated with a higher response (76%) compared with patients with CRP<5 mg/l (46%). In line with the findings above, Schreiber et al investigated the efficacy of certolizumab in 292 CD patients and concluded that in the subgroup of patients with low CRP the placebo response rates were high (Schreiber *et al.*, 2005). The situation in UC is more unclear since most of the studies performed concerning CRP and prediction of response to anti-TNF-alpha treatment are done on CD patients (Mendoza and Abreu, 2009; Vermeire *et al.*, 2006). However, Ferrante et al investigated predictors of early response to infliximab in 100 patients with UC (Ferrante *et al.*, 2007). They concluded that pANCA+/ASCA- serotype and an older age at first infliximab infusion were associated with a suboptimal early clinical response, while CRP ≥5 was not a significant predictor (Ferrante *et al.*, 2007). Other studies are done concerning which patients are most likely to respond to intravenous corticosteroid therapy for UC. Travis et al demonstrated that after three days intensive treatment with intravenous steroids, patients with frequent stools (> 8/day), or raised CRP (> 45 mg/l) needed to be identified, as most would require colectomy (Travis *et al.*, 1996). However, clinical scores which is based on symptoms are in some studies found to more accurately identify UC patients who do not respond to intravenous corticosteroids than CRP (Turner *et al.*, 2010).

2.2.5 Summary

Taken together, most of the clinical studies conclude that the values of CRP as a marker in detecting IBD range between 50-60% for UC and between 70-100% for CD and of course

depend on the cut off value used (Lewis, 2011;Vermeire *et al.*, 2006). Several study results point in the direction that CRP levels increase with increasing clinical activity of UC. Still it is important to keep in mind that many of the IBD patients have CRP levels within the normal range at diagnosis and this means that measuring CRP does not necessarily differentiate between IBD and functional order (Henriksen *et al.*, 2008;Lewis, 2011). In one Norwegian study CRP levels above 10 mg/l was a predictor of surgery in a subgroup of patients with UC, however few patients with UC underwent surgery in the study and the data should therefore be interpreted with caution. There are also conflicting results in the different studies concerning the correlation between CRP and endoscopic inflammation in UC and CRP and histological inflammation in UC. Further, in CD, a decrease in CRP levels in response to therapy is objective evidence that the drug has a beneficial effect on gut inflammation (Vermeire *et al.*, 2006). In UC the correlation between CRP levels and benefit of treatment is less clear. In conclusion, further studies should be carried out to evaluate whether highly sensitive CRP assays are more sensitive markers of gut inflammation and disease outcome in IBD patients. The conflicting study results concerning endoscopic inflammation and histological inflammation and CRP underscore the fact that measuring CRP in serum is an indirect marker of an inflammation mainly located in the gut and therefore not an optimal biomarker.

2.3 Calprotectin

2.3.1 Introduction

Calprotectin is a protein that removes 60% of the calprotectin protein from the cytosol (Lundberg *et al.*, 2005). Since the first assay was introduced on the market, there has been a change related to the introduction of a new extraction buffer that gives a fivefold better yield of calprotectin during the extraction procedure (Ton *et al.*, 2000). The concentration of calprotectin in feces is an indirect measure of neutrophils infiltrate in the bowel mucosa (Lewis, 2011). Fecal calprotectin levels fluctuate during the course of IBD, but it is well documented that it is persistently elevated during the disease relapse in both adults and children (Berni et al, 2004, Tibble et al, 2000 and 2001). However, calprotectin is not a specific marker. Increased levels are found in other diseases than inflammatory bowel disease, as in colorectal neoplasia, microscopic colitis, food allergy, active celiac disease, allergic colitis, cystic fibrosis, infection and polyps (Carroccio *et al.*, 2003; Tibble *et al.*, 2000a; van Rheenen *et al.*, 2010). It is also found to increase after use of non-steroidal anti-inflammatory drugs, proton pump inhibitor and with increasing age (Carroccio *et al.*, 2003; Tibble *et al.*, 1999; van Rheenen *et al.*, 2010).

2.3.2 Calprotectin as a tool to differentiate IBD from IBS patients

Several studies have been performed investigating the sensitivity and specificity of calprotecting in various clinical settings. In a meta-analysis study by von Roon et al, data from 30 studies was summarized, including 1210 IBD patients, to address whether fecal calprotectin could be used to differentiate IBS patients from IBD patients (von Roon *et al.*, 2007). The calculated sensitivity and specificity values were 0.95 and 0.91 respectively. The diagnostic precision of calprotectin for IBD was higher in children than adults. The fecal calprotectin threshold used in these studies was 50 microgram/gram (von Roon *et al.*, 2007). In another summary by Gisbert et al, 14 different studies were compared (Gisbert *et al.*, 2009). They summarized that the calprotectin sensitivity range from 63-100% and the

specificity from 74-98% for the diagnosis of IBD , calculating mean sensitivity and specificity of 80% (95% CI, 77-82%) and 76% (95% CI, 72-79) (Gisbert *et al.*, 2009). In a recent meta-analysis by van Rheenen et al 13 studies were included, six in adults and seven in children and teenagers (van Rheenen *et al.*, 2010). In the studies of adults, the pooled sensitivity and pooled specificity of calprotectin was 0.93 (95% confidence interval 0.85 to0.97) and 0.96 (0.79 to 0.99) and in the studies of children and teenagers was 0.92 (0.84to 0.96) and 0.76 (0.62 to 0.86). The lower specificity in the studies of children and teenagers was significantly different from that in the studies of adults (p=0.048). All three meta-analysis studies concluded that fecal calprotectin has a good diagnostic precision for separating organic and functional intestinal diseases and is a useful screening tool for identifying patients who need endoscopy for suspected IBD. However, the specificity of calprotectin was significant lower in children and teenagers compared to adults in the meta-analysis by van Rheenen, while in the study by von Roon the opposite result was found.

There is a notably variation in the range in both pooled sensitivity and specificity in the different studies summarized in meta-analysis studies, worthwhile to discuss. There may be several explanations for these variations. When comparing different studies one must ensure that the patient populations for each of the two disease states are equivalent and that the study designs are comparable. In the study by Costa et al for example 71% of CD patients had small intestinal disease alone, with only 31% having colitis (Costa *et al.*, 2005). These values are compared with 47% and 53%, respectively, in another study included in the summary (Tibble *et al.*, 2000a). Thus there may be a possible selection bias influencing the final predicative values. Further, the disease activity in most of the studies was assessed by the Crohn's disease activity index (CDAI), a test that is highly subjective and correlates poorly with inflammatory activity assessed by In111 labelled white cells and endoscopic indices (Saverymuttu, 1986). Finally the calprotectin assay has been changed (Ton *et al.*, 2000) in the period of the performed studies summarized by Gisbert et al (Gisbert *et al.*, 2009). In this summary 10/14 studies have used the new and more sensitive calprotectin assay and that may have influenced the final results, as commented by the authors (Gisbert *et al.*, 2009). In the meta-analysis by van Rheenen et al the study quality was assessed using the QUADAS (quality assessment of studies of diagnostic accuracy included in systematic reviews) checklist and from this checklist they chose seven best differentiating items (van Rheenen *et al.*, 2010). The other meta-analysis studies did not select studies in the same way and this may of course influence the difference in the pooled sensitivity and specificity values (Gisbert *et al.*, 2009; von Roon *et al.*, 2007). In addition, von Roon et al pooled the sensitivity and specificity separately contrary to general recommendations and included studies that featured a control group with healthy people, which leads to overestimation of diagnostic accuracy (van Rheenen *et al.*, 2010; von Roon *et al.*, 2007). The pooled sensitivity and specificity in meta-analysis should be interpreted with caution because of the heterogeneity in the different studies included. In summary, the final range of the sensitivity and specificity of calprotectin is still not settled, but the main conclusion that calprotectin has a good diagnostic precision, especially in adults is well documented.

2.3.3 Calprotectin and differentiating between UC and CD

The test seems not useful for differentiating between the two diseases (Canani *et al.*, 2006; Silberer *et al.*, 2005; Tibble *et al.*, 2002; von Roon *et al.*, 2007). When comparing the sensitivity and specificity of calprotectin with other serological tests as CRP, ESR, ANCA or ASCA,

calprotectin turn out as the best test in level with clinical scores (Gisbert *et al.*, 2009;Tibble *et al.*, 2002). Tibble et al included 602 patients where 263 had organic intestinal disease and 339 had nonorganic disease (Tibble *et al.*, 2002). Interestingly they found that both fecal calprotectin level and the Rome I criteria were significantly better screening discriminates of patients with organic or nonorganic intestinal disease (Odds ratio (OR), 27.8, positive predicative value (PPV), 0.76, negative predicative value (NPV), 0.89 and 13.3, 0.86, 0.69) than some other commonly used laboratory parameters, such as CRP (OR, 4.2, PPV, 0.67, NPV, 0.68) and ESR (OR, 3.2, PPV, 0.62, NPV, 0.69) (Tibble *et al.*, 2002). Other studies have listed comparable values (Canani *et al.*, 2006; Fagerberg *et al.*, 2005; Tibble *et al.*, 2000a). Sensitivity of calprotectin ranged from 92-95% and specificity ranged from 93-98%, while sensitivity of CRP ranged from 36-41% and specificity from 77-100%. For ESR the values ranged from 41-59% and specificity from 77-100%. For ASCA/PANCA, the sensitivity was 77% and specificity was 88% (Canani *et al.*, 2006).

2.3.4 Calprotectin and relapse in IBD

Tibbel et al have addressed whether calprotectin predict relapse in IBD (Tibble *et al.*, 2000b). They observed that at 50 microgram/gram, the sensitivity and specificity of calprotectin for predicting relapse in all patients with IBD were 90% and 83%, respectively. In their study calprotectin levels of 50 microgram/gram or more predicted a 13-fold increased risk for relapse. They did not find CRP or ESR to be useful in predicting relapse of IBD (Tibble *et al.*, 2000b). Other studies have confirmed the results of Tibbel et al (Costa *et al.*, 2005; D'Inca *et al.*, 2008).

2.3.5 Calprotectin and correlation with disease activity in IBD

Several studies have investigated and found a correlation between calprotectin and the severity of IBD evaluated with clinical, endoscopic and histologic parameters (Berni *et al.*, 2004; Langhorst *et al.*, 2005; Tibble *et al.*, 2000a; Tibble and Bjarnason, 2001). However, calprotectin seems to be better correlated with disease activity in UC than in CD (Costa *et al.*, 2003; D'Inca *et al.*, 2007). Though, it is important to have in mind that CDAI score is mostly based on clinical information and not sensitive concerning subclinical inflammation.

2.3.6 Calprotectin as a marker of treatment response in IBD

It is plausible that calprotectin decreases if a treated IBD patient achieves remission, but so far few studies have confirmed this hypothesis. In a small recent study 38 IBD patients were included and calprotectin was measured before and after treatment with topical/or systemic 5-ASA or prednisolon or immunosuppressiva (Wagner *et al.*, 2008). Using calprotectin values below 95th percentile of the normal range as a negative predictor of active disease after 8 week of treatment, they revealed a negative predicative value of 100%. However, using an elevated level of calprotectin as a positive predictor to detect ongoing active disease or treatment failure after 8 week of treatment, they calculated a positive predicative value of 38% in UC and only of 14% in CD (Wagner *et al.*, 2008). Comparable results were demonstrated in another study, including 57 children with IBD treated with prednisolon (Kolho *et al.*, 2006). In their series, the positive predicative value of fecal calprotectin for active IBD judged by colonoscopy was 0.7. They observed that calprotectin levels declined in line with the clinical improvement but seldom fell within the normal range. The authors suggested that this may be caused of an ongoing inflammation in a clinically silent disease

(Kolho *et al.*, 2006). An interesting study by Røseth et al included 45 IBD patients with normal calprotectin levels after treatment and demonstrated that in 44 of the 45 patients the appearance of both the colon and the terminal ileum was completely normal endoscopically (Roseth *et al.*, 2004). Sensitive markers of mucosal healing in IBD are urgently needed and this small study indicates that calprotectin could be such a marker in IBD patients. Future properly powered studies are needed to evaluate calprotectin as a possible marker of successful treatment in IBD.

2.3.7 Summary

As concluded in the systematic review by von Rheenen et al, high concentration of calprotectin in feces is a strong argument to carry out a colonoscopy in order to rule out the presence of inflammatory bowel disease or other organic pathologies. Parallelism between fecal calprotectin levels and inflammatory bowel disease activity has been confirmed, although this fecal marker appears to better reflect the disease activity in UC than in CD. Further, increased calprotectin levels are found to predict increased risk for relapse in IBD. However, the test seems not useful for differentiating between the two diseases. Finally, calprotectin have a good diagnostic precision for separating IBD from non-IBD diagnosis overall, better than classically ESR, CRP, ANCA or ASCA.

3. Histological parameters as predictors of disease severity or treatment response in IBD

To our knowledge there are not many studies done addressing histological parameters as predictors of disease severity or treatment outcome in IBD. After several search in pubmed.com we managed to find only one study published in 1993 (Schumacher, 1993). Schumacher investigated the possibilities of differentiating between inflammatory bowel disease (IBD) and infectious colitis on clinical, microbiological, laboratory and histological grounds, a prospective study of 105 patients with a first attack of colitis was undertaken (Schumacher, 1993). The strongest histological predictor of IBD in this study was basal plasmocytosis, followed by more than two vertical crypt branches/MPF, crypt distortion, villous mucosa, mucosal atrophy, epithelioid granulomas and Paneth cell metaplasia. These signs were rarely or never found among patients with infectious colitis. Their frequency increased with the interval between the initial symptoms and the first biopsy. Lately, researchers have been focusing on other biomarkers in serum, faeces or mucosa in IBD patients searching for possible predictors of both disease activity and treatment response and compared these biomarkers with both histology and endoscopy in IBD patients.

4. Mucosal inflammatory processes: The main source of biomarkers in IBD

4.1 Introduction

The biological tissue factors playing a role in the pro-inflammatory and anti-inflammatory process in IBD have been revealed by the omics technology and described in several overviews (Kaser *et al.*, 2010;Neuman, 2007;Torres and Rios, 2008): microarray and quantitative real-time polymerase chain reaction (RT-PCR) analyses describe the gene expression of IBD-related proteins, the proteomics describe the translation of proteins including small oligopeptides, whereas the metabolomics describe all the metabolic endproducts of gene expressions. This gives a unique option for molecular fingerprinting. In

general, these tissue factors are linked to various immunological pathways belonging to the innate, adaptive and regulatory immune response including molecules such as cytokines, chemokines, adhesion molecules and other markers of activation with corresponding cellular and soluble receptors; immune cells and non-immune cells. Finally, a relatively new field in IBD, the metabolic fingerprints obtained from metabolomics studies (for review, see (Roda *et al.*, 2010; Scaldaferri *et al.*, 2010). So far there are few reports describing prognostic and predictive bioamarkers. In the following we will describe the candidate biomarkers predicting either clinical outcomes and/or response to therapy. Moreover, as there are very few documented biomarkers we will also add some molecules central in the inflammatory process in IBD that is of great interest in the future biomarker fingerprinting based on the new approaches in high technological omics.

4.2 Proinflammatory cytokines

4.2.1 TNF-α

TNF-α is a cytokine well established inflammatory mediator of CD whereas contradictory reports exist in UC (Kaser *et al.*, 2010; Neuman, 2007;Torres and Rios, 2008). Some reports have found positive correlations with the degree of disease activity in UC (Akazawa *et al.*, 2002;Olsen *et al.*, 2007) and CD (Olsen *et al.*, 2007) but not in all for CD (Akazawa *et al.*, 2002) and UC (Dionne *et al.*, 1997).

As far as we know no studies do exist on mucosal expressions of TNF-α and the prediction of the clinical course, whereas there are only few reports on the predictive value of mucosal TNF-α concentrations and the response to therapy. Pretreatment mucosal TNF-α concentrations were negatively correlated to response to Infliximab in CD (Schmidt *et al.*, 2007) and in UC (Olsen *et al.*, 2007). These findings disagree with another report where low expression of TNF-α predicted a poor response to therapy in CD (Arsenescu *et al.*, 2008). Increased levels of TNF-α were also observed in corticosteroid non-responders compared with responders to corticosteroid treatment in UC (Ishiguro, 1999), but not confirmed in another study (Raddatz *et al.*, 2005). Finally, in a preliminary report, normalization of mucosal TNF-α seemed to predict a longstanding remission after stop of anti-TNF therapy both in UC (Olsen T *et al.*, 2011a). Finally, based on microarray technology in UC patients treated with nfliximab expression of TNF-receptor (SF11B), the receptor for the proinflammatory cytokine 13 (IL-13-R) and the anti-inflammatory cytokine IL-11 predicted the clinical response

4.2.2 IFN-γ, IL-1, IL-12

TNF-α exerts its proinflammatory effect through cytokines such as INF-γ, IL-1β and IL-6 (Neuman, 2007). INF-γ is a mediator of inflammation in CD (Strober *et al.*, 2010), whereas contradictory reports exist for UC (Kobayashi *et al.*, 2008; Olsen *et al.*, 2007). Finally, in one report increased levels of INF-γ in intestinal T lymphocyte cultures were observed in relapsing perianal fistulising disease in CD after treatment with infliximab (Agnholt *et al.*, 2003).

IL-1β was increased in CD and UC in one report (Dionne *et al.*, 1998), and IL-1-receptor/IL-1β ratio is negatively associated to the IBD activity (Dionne *et al.*, 1998). IL-12 is a proinflammatory cytokine known for gating (in synergy with IL-18) in a Th1 direction/stimulating the IFN-γ synthesis in naïve T cells and is up-regulated in IBD (Neuman, 2007). IL-12 in addition to INF-γ and IL-6 is more increased in inflamed than in not inflamed mucosa (Leon *et al.*, 2009). However, neither of these cytokines has been studied as potential biomarkers

4.2.3 IL-6

IL-6 is a pleiotropic cytokine with regulatory effects on the inflammation. The cytokine plays an important role in the initial activation of immune response and in combination with the transforming growth factor (TGF-β), plays a pivotal role in TH17 polarization. Increased levels of the cytokine and its soluble receptor are increased in active CD and in UC (for review, see (Atreya and Neurath, 2005)), but their roles as biomarker are so unknown.

4.2.4 IL-17/Il-23

The first reports of increased density of IL-17 cells in the inflamed mucosa of IBD came in 2003 (Fujino *et al.*, 2003) and was confirmed by later studies (Kobayashi *et al.*, 2008; Olsen T *et al.*, 2011b). Both IL-17 and IL-23 are correlated to the severity of UC (Olsen T *et al.*, 2011b) and are positive correlated to the response to infliximab (Olsen T *et al.*, 2010). Homozygous carriers of IBD risk-increasing IL-23 receptor variants are more likely to respond to infliximab (Jurgens *et al.*, 2010).

4.3 Antiinflammatory cytokines

4.3.1 Transforming growth factor-β

Transforming growth factor-β1 (TGF-β1) is a potent regulatory cytokine, and has in the absence of IL-6, an antiinflammatory effect. Increased levels of TGF-β1 are found both in CD and UC (Lawrance *et al.*, 2001; Olsen T *et al.*, 2011b), which are correlated with the severity of disease in CD but not in UC (Olsen T *et al.*, 2011b).

4.3.2 IL-10

IL-10 has anti-inflammatory effects on proinflammatory cytokines (IL-1, IL-6, TNF-α) and its mucosal levels were found increased both in CD and UC (Akagi *et al.*, 2000; Olsen *et al.*, 2007) (for review see(Kaser *et al.*, 2010)) except for a single study(Nielsen *et al.*, 1996). Of interest, mucosal levels of IL-10 and IL-4 in UC did not correlate with the treatment effects of infliximab (Olsen *et al.*, 2009) and were not found reduced after infliximab treatment (Agnholt *et al.*, 2003).

4.3.3 IL-11

IL-11 mediates anti-inflammatory effects by downregulation of LPS-induced NFkB activation preceding transcription of inflammatory genes. In the mucosal gene signature study of Arijs et al (Arijs *et al.*, 2009) (39) IL-11 was one of several expressed genes separating responders from non-responders on infliximab treatment.

4.4 Molecules of activation

4.4.1 Adhesion molecules and chemokines

4.4.1.1 The adhesion molecules play an important role in mediating intestinal injury by T cells in IBD

The endothelium plays a key role in the pathogenesis of IBD acting as "gatekeeper" regulating the leukocyte trafficking through cell adhesion molecules and chemokine secretion. Integrins, selectins and immunoglobulins are receptors on leukocytes, endothelium, and platelets with their respective ligands which are up-regulated by proinflammatory cytokines through a NF-κB dependent mechanism. Integrin α4β7

expressed on lymphocytes and its specific ligand mucosal vascular addressin on the endothelial cells play a pivotal role in the recruitment of leucocytes from blood to the inflamed tissue. An increased expressions of several adhesion molecules such as e-selectin, intracellular adhesion molecule-1 and 2 (ICAM-1, ICAM 2) and vascular cell adhesion molecule (VCAM) have been found in IBD (for review see (Danese *et al.*, 2005). Although the leukocyte recruitment by adhesion molecules plays a role in the initiation and progression of the disease, there is little documentation of their roles as biomarkers. The gene expression of ICAM-1 is elevated in UC but was not correlated with response to steroids (Raddatz *et al.*, 2004). In the same study low expression of the glucocorticoid receptor was correlated to non-response. Of interest, in the study of Nicolaus et al (Nikolaus *et al.*, 2000) relapsers after induction therapy with infliximab in CD were characterized by an increase in the TNF-α secretion capacity and increased mucosal nuclear NF-κB p65 before recurrence of clinical symptoms. Chemokines and their corresponding receptors are responsible for the chemotaxis and the adhesion and directional homing of immune and inflammatory cells from blood to tissue. The exact immunopathogenic role of chemokines in IBD is not clearly understood. However, increased tissue expressions of these molecules in IBD reflect increased trafficking and lymphocytes recirculation and neo-lympho organogenesis in the intestinal mucosa mediating not only inflammatory process but also repair mechanisms (for review see (Zimmerman *et al.*, 2008)).

4.4.2 Markers of tissue injury

Among the effector mechanisms activated during intestinal inflammation are the key mediators such as inducible nitric oxide synthase (INOS), the apoptosis-related Granzyme B (GZNB), and the system of extracellular matrix degradation mediated by metalloproteinases (MMP). The pathophysiological role of nitric oxide in IBD is to some extent controversial. Increased INOS activity has been shown especially in UC (Palatka *et al.*, 2005) and predicted the progression in IBD (Menchen *et al.*, 2004). Some of MMPs are proinflammatory and found dysregulated in IBD (MMP-1,-3,-9, -12, -13) (Ravi *et al.*, 2007; von *et al.*, 2000). The tissue inhibitor of MMPs (TIMP) has reported to correlate both, positively (macrophage TIMP-1) and negatively (epithelial TIMP-3) to the disease activity in CD, whereas MMPs and TIMPs were decreased after treatment of the disease (Makitalo *et al.*, 2009). In one report infliximab reduced a genotype-associated matrix protective phenotype (Meijer *et al.*, 2007).

4.4.3 Genomics

There is increasing number of IBD suspectibility loci/genes discovered. There is beyond the goal of this review of mucosal biomarkers to describe the genomics in IBD. In short, the most powerful tool is the use of genome-wide association (GWA) scanning in genotyping. In 2011, 71 and 47 suspectibility loci/genes have been discovered in CD and UD, respectively, including 28 in common comprising the total number of 99 loci involved. (Anderson *et al.*, 2011;Franke *et al.*, 2010). This genetic polymorphism linked to the innate immunity, autophagy, defective barrier, IL-10 signalling and adaptive immunity. Of these suspectibility loci/genes some have also been candidate biomarkers. Polymorphisms in apoptopic genes (Fas/LFas system and caspace-9 influence the response of infliximab in luminal and fistulising Crohn's disease (Hlavaty *et al.*, 2005). Using GWA technology a loci (21q22,212IBRWDI) remained significant for responsiveness to anti TNF therapy in children (Franke *et al.*, 2010). There are now several IBD studies worldwide using the GWA technology and functional

genomics. Hopefully, these genome analyses together with gene expression analyses can define new subtypes of clinical outcomes and response to various treatment regimes.

4.4.4 Proteomics

Proteomics identifies biomarkers at protein expression levels in tissue and opens for protein dynamic mapping of proteins in the inflammatory pathways and their metabolic end products. So far, no mucosal proteomics have been reported in IBD, whereas 4 biomarkers of acute phase inflammation have been found in serum from patients with IBD (Meuwis *et al.*, 2008a) and the chemokine platelet aggregation factor 4 (PF4) in serum predicted the response to infliximab in CD (Meuwis *et al.*, 2008b). Of special interest is the fingerprinting of oxidative stress in inflammation and modification of proteins and thereby new potential biomarkers. So far, we are waiting for prospective studies relating fingerprints in mucosa to severity of disease and predictors to treatment.

4.4.5 Metabolomics

Metalobomics is the study of chemical fingerprints of all metabolites in the biological organism and represents the end product of the gene transcriptions. By the use of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) simultaneously assessment of numerous of metabolites corresponding to the "metabolome" and its end-points of metabolic products may be done. Thus, in UC different metabolic profiles have been observed in biopsies when compared to controls (Bjerrum *et al.*, 2010). In CD, pathways with differentiating metabolites included those involved in the metabolism and or synthesis of amino acids, fatty acids, bile acids and arachidonic acid, several metabolites were positively or negatively correlated to the disease phenotype (Jansson *et al.*, 2009). Finally, in fecal extracts low levels of butyrate and acetate and amino acids have been observed in IBD compared to the controls (Marchesi *et al.*, 2007). This opens for new insight of the etiopathogenesis of the disease. However, so far we do not know how this technology can contribute to the discovery of new biomarkers.

5. Concluding remarks

There are so far very few documentations of mucosal (by biopsy) or body fluid (non-invasive) biomarkers predicting long-term clinical outcome or response to treatment. The most promising candidate predictors are TNF-α but no validation data exists. In general, there is a need for prospective studies with a broad fingerprinting assay from mucosa as well as from body fluids. From a theoretical point of view, mediators of inflammation and their metabolites correlating with severity of disease are the candidates of greatest interest. After documentation of a candidate biomarker, there is a need of both a validation and adjustments of practical use for the clinicians. Therefore, biomarkers of IBD are still in the start of defining candidates of interest.

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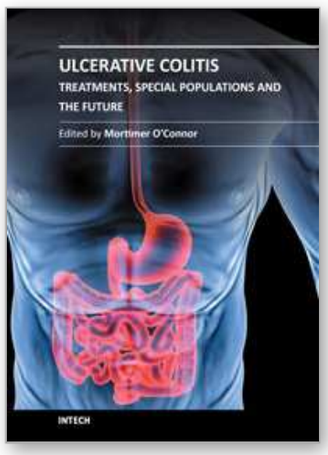

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This book is intended to act as an up to date reference point and knowledge developer for all readers interested in the area of gastroenterology and in particular Ulcerative Colitis. All of the chapter authors are experts in their fields of publication and deserve individual credit and praise for their contributions to the world of Ulcerative Colitis. We hope that you will find this publication informative, stimulating and a reference point for the area of Ulcerative colitis as we move forward in our understanding of the field of medicine.

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