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Clinical Utility of Anti- Granulocyte Macrophage-Colony Stimulating Factor Antibodies in Inflammatory Bowel Disease

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Abstract

Background and Aims: Anti-GM-CSF Ab have been recently identified in IBD but there are still few studies on their clinical interestand especially on their prognostic value in IBD.The main purpose of our study was to evaluate the clinical value of anti-GM-CSF Abs in a cohort of IBD patients.

Methods: IBD patients were enrolled at the Department of Gastroenterology of Saint-Etienne's University Hospital. For each patient, clinical and therapeutic characteristics were systematically recorded. Anti-GM-CSF Abs was assessed by ELISA (Elabscience® kit).

Results: 187 patients were included (103 CD, 84 UC) and 47 healthy blood donors. Concentrations of anti-GM-CSF Abs were higher in CD and UC *vs* in healthy controls (158.3 µg/ml et 157.6 µg/ml vs 92.5 µg/ml, p<0.0001.Patients with severe CD course had higher level of anti-GM-CSF Ab.UC patients with anti-TNFα therapeutic response had higher level of anti-GM-CSF Ab than non-responders (196.7µg/ml *vs* 90.5 µg/ml, p = 0.0157 respectively).

Conclusions: Indeed, anti-GM-CSF Ab could constitute an interesting therapeutic monitoring tool in UC but more prospective studies are required to confirm these data.

Keywords: Crohn's disease (CD); Ulcerative colitis (UC); Inflammatory Bowel Disease (IBD); Granulocyte Macrophage Colony Stimulating Factor (GM-CSF); Antibodies (Abs); Anti-Tumor Necrosis Factor α (anti-TNFα)

Introduction

Inflammatory bowel diseases (IBD) are chronic diseases including Crohn's disease (CD) and Ulcerative colitis (UC). The etiology is still only partially known but three factors seem to be involved in the pathogenesis of the disease: environment, genetic susceptibility factors and immune dysregulation. The presence of environmental factors in a particular genetic background will lead to the activation of an inappropriate immune response inducing destruction of the mucosa and an increased intestinal permeability. This is one of the mechanisms that may explain the presence of antibodies (Abs) frequently found in IBD and which constitute biomarkers [1–4]. Increased intestinal permeability is reversible under anti-Tumor Necrosis Factor alpha (anti-TNFα) antibodies treatment [5].In some cases, treatment of IBD could fail, so it is necessary to find predictors of non-response [6] and algorithms that could help in therapeutic optimization.

Several antibodies have been identified in IBD but their role in prognosis and therapeutic monitoring is still unclear [7–9]. Because of low specificity and sensitivity of specific antibodies detection, lack of target values, no national, European or international guideline recommends the detection of antibodies for the diagnosis or prognosis and therapeutic monitoring of IBD to date.

Recently, anti-Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) antibodies have been identified in IBD. These antibodies are directed against GM-CSF which is a cytokine that is necessary for anti-microbial functions of myeloid cells and that have an important role in the regulation of intestinal mucosal damage, in intestinal immunity and inflammatory responses [10–14]. Mice deficient in GM-CSF had defects in mucosal barrier function with more severe intestinal and systemic infection after enteric infection, more severe colitis after enteric exposure to dextran sodium sulphate or developed transmural ileitis following non-steroidal anti-inflammatory drug (NSAID) exposure [10–14]. GM-CSF therapy improved mucosal repair and could have a role in clinical improvement in CD [10–13, 15]. Jurickova *et al* found that anti-GM-CSF Ab were produced by *lamina propria* mononuclear cells isolated from CD ileal resection specimens and that peripheral blood contains GM-CSF neutralizing capacity [16]. High levels of anti-GM-CSF Ab are associated with a reduced bioactivity of GM-CSF, of neutrophil bacterial killing, and an increase in intestinal permeability [14, 16]. Furthermore, these antibodies are used in the diagnosis of autoimmune pulmonary alveolar proteinosis (PAP) [17]. To date, there are still few studies on their clinical interestand especially on their prognostic value in IBD. The main purpose of our study was to evaluate the clinical impact of anti-GM-CSF Abs in a cohort of IBD patients.

Methods

Subject enrolment

Patients with IBD referred to the Department of Gastroenterology of Saint-Etienne's University Hospital were enrolled over a 12-month period between September, 2009 and October, 2010. Diagnosis of IBD was assessed on common endoscopic and histological evidences of the disease, according to the Lennard-Jones criteria. The healthy control group (n=47) was consecutive healthy volunteers selected from blood donors. Ethical approval was obtained from the Ethics Committee of the University of Saint-Etienne and fully written informed consent was obtained from all patients.

Assessment of clinical and therapeutic characteristics

A complete medical chart review of the past history and physical examinations were systematically recorded at inclusion. The following additional clinical data were collected at baseline, including date of birth, gender, disease duration, age at onset, disease location and behavior according to the Montreal classification, concomitant perianal disease, extra-intestinal manifestation, smoking history, previous history of IBD-related surgery. Prior drug-response history (e.g., steroid dependency and/or resistance, as previously defined by the European Crohn's and Colitis Organization (ECCO)) was also recorded by reviewing the medical charts.

The presence of at least one of the following composite criteria in the IBD-related past medical history was defined as severe disease course:

- For patients with CD: uncontrolled active disease requiring adjunction of anti-TNF after failure with conventional immunosuppressant's, two or more CD-related previous surgeries or bowel resection longer than 70 cm, concomitant active perianal disease with complex fistulas or spread bowel disease.
- For patients with UC: pancolitis associated with increased C-reactive protein levels and steroid treatment at UC diagnosis, early use of immunosuppressant during the first year of the disease due to steroid refractory, use of anti-TNFα after failure with immunosuppressant, acute severe UC or colectomy.

Therapeutic response under anti-TNFα treatment (at least one anti-TNFα) was defined as clinical remission under treatment and without any change in the treatment. Clinical remission was defined as clinical score of UC activity (Lichtiger score) lower to 4 and Crohn's Disease Activity Index (CDAI) lower to 150 at inclusion.

Serum analysis

All patients and healthy controls provided a venous blood sample at inclusion. Serum was rapidly transported to the laboratory, separated from blood by centrifugation and kept frozen at -80°C until use. Serum concentrations of anti-GM-CSF Abs were quantified by enzyme-linked immunosorbent assay (ELISA) with Elabscience® kit. Clinical data analyses and serologic assessments were performed in a blinded manner without knowledge of the patients' diagnosis and medical history. Briefly, serum samples were diluted 16000 fold and incubated on microtiter plates coated with specific antigens at 37°C. The unbound antibodies were removed. Then detection antibodies were added to the wells and the plates were incubated at 37°C. The wells were then washed by washing buffer for 3 times and then 100 µl of HRP conjugate was added to each well. After incubation, the plates were washed and 100 µl of the substrate was added. After short incubation in the dark, the stop solution was added to block color development after the recommended times and the optical densities (OD) at 450 nm were measured by an ELISA-microplate reader (EL808®, Biotek).

Statistical analysis

For quantitative data, mean with confidence interval 95%, median value with 1st and 3rd quartiles (Q1 and Q3) were documented. Qualitative data were reported as numbers and percentages. Univariate analyses were generated to identify potential associations with anti-GM-CSF Abs and clinical or therapeutic characteristics. To determine the accuracy of serum anti-GM-CSF Ab measurements in detecting therapeutic response, test receiver operating characteristics curves were drawn by plotting sensitivity against 1-specificity. Overall accuracy of the marker in detecting therapeutic response was represented by area under the curve with 95% confidence interval (CI). The best cutoff was defined as the maximum sum of sensitivity and specificity. Correlation analyses were performed using correlation coefficient r (Spearman). A p value < 0.05 was considered statistically significant. All statistical analysis were performed using GraphPad Prism® (version 5.02) and XL-STAT 2014.3.01® (Addinsoft™).

Results

Clinical and demographic characteristics

187 patients with IBD (103 CD, 84 UC) were included in the study. Clinical and demographic characteristics of all the patients have been reported in Table 1. The median time between diagnosis and the enrolment into the study was 5 years [2.00-13.00] in patients with CD and 8 years [3.00-14.75] in patients with UC. 56% of CD patientshadcolonic (L2) orileocolonic (L3) disease and 42% had a complicated disease behavior (B2orB3) according to the Montreal classification. 46% of UC patients had pancolitis(E3). 66 of 103 CD patients (64%) and 42 of 84 UC patients (50%) had severe disease. Of the 187IBD patients, 105 (56.1%) were treated with anti-TNF Ab (infliximab/adalimumab), 128 (68.4%) had corticosteroid, 47 (25.1%) asteroid refractory and 53(28%) had a surgery.

Among 65 CD patients treated with anti-TNFα, data concerning the response to anti-TNFα treatment were available for 52 patients. Among these patients, 47 were responders.

Among 40 UC patients treated with anti-TNFα, data concerning the response to anti-TNFα treatment were available for 34 patients. Among these patients, 28 were responders.

Characteristics of patients according to response to anti-TNFα (n= 52 CD and 34 UC) are reported in Table 2.There were no significant differences between responders and non-responders.

Serum GM-CSF Ab higher levels are associated with CD and UC

The median concentration of anti-GM-CSF Ab was significantly higher in CD (n=103) and UC (n=84) patients *vs* healthy blood donors (n=47) (158.3 µg/ml, 157.6 µg/ml and 92.5 µg/ml respectively, p<0.001). Median concentrations obtained were not significantly different between UC patients and CD patients (p =0.643) (Figure 1a).

Table 1: Demographic and main baseline characteristics of patients and controls

	CD	UC	Controls
Number	103	84	47
Sex ratio M/F	48/55*	54/30	
Current smokers (n, %)	44 (42.7)*	3 (3.6)	
Mean age at inclusion (± SD, years)	40.9 ± 15.9*	49 ± 17.6	
Median disease duration at the inclusion (years [IQ range])	5 [2-13]	8 [3-14.75]	
Age at diagnosis • A1 : ≤ 16years • A2 : 17-40 years • A3 : > 40 years	 26 (26%) 71 (71%) 3 (3%)		
Disease location • Ileal (L1) • Colonic (L2) • Ileo-colonic (L3) • Ulcerative proctitis (E1) • Left-sided colitis (E2) • Pancolitis (E3)	 44 (44%) 12 (12%) 44 (44%)	 14 (17.5%) 29 (36.3%) 37 (46.3%)	
Disease behavior • No stricturing, no penetrating (B1) • Stricturing (B2) • Penetrating (B3) • Perianal disease	 58 (58%) 27 (27%) 15 (15%) 22 (22%)		
First medication • Anti-TNF • Corticosteroïds • Immunosuppressive • 5-ASA	 1 (1%) 71 (69%)* 7 (7%) 24 (23.3%)*	 0 (0%) 35 (41.7%) 1 (1.2%) 48 (57.1%)	
Concomitant medication during the first year • Anti-TNF • Corticosteroïds • Immunosuppressive	 18 (17.5%)* 83 (80.6%)* 48 (46.6%)*	 6 (7.1%) 50 (59.5%) 20 (23.8%)	
Steroid dependency **Steroid refractory**	71 (68.9%) 18 (17.5%)*	57 (67.9%) 29 (34.5%)	
IBD related Surgery	41 (39.8%)*	12 (14.3%)	
Severe disease	66 (64.1%)	42 (50%)	
Anti-TNF Ab treatment(one anti-TNF or both) • Infliximab • Adalimumab	65 (63.7%)* 57 (55.9%) 19 (18.6%)	40 (48.2%) 39 (47%) 9 (10.8%)	

*p<0.05 *vs* UC

Relationships between anti-GM-CSF Ab and IBD phenotype and severity

No significant difference of anti-GM-CSF Ab levels was observed depending on the location (median anti-GM-CSF concentration: 147.2 µg/ml for L1, 178 µg/ml for L2, and 106.4µg/ml for L3, p=0.610), the disease behavior (median anti-GM-CSF concentration 158.6 µg/ml for B1, 182.1 µg/ml for B2, and 156.2 µg/ml for B3, p=0.728) or the age at diagnosis of CD(median anti-GM-CSF concentration 170.7 µg/ml for A1, 159.7 µg/ml for A2, and 75.6 µg/ml for A3, p=0.141), or depending on the extent of the disease in UC(median anti-GM-CSF concentration 145.6 µg/ml for E1, 166 µg/ml for E2, and 157 µg/ml for E3, p=0.965). However, the median concentration of anti-GM-CSF Ab was significantly higher in patients with severe CD (169 µg/ ml*vs*135.1µg/ ml, p = 0.0363)(Figure 1b).There was no significant difference for UC according to the severity of the disease (median anti-GM-CSF concentration 142.7 µg/ml for severe UC vs 164.5 µg/ml, p=0.651).

Relationships between anti-GM-CSF Ab and IBD treatment

Median concentration of anti-GM-CSF Ab was significantly lower in CD patients with corticosteroid dependence (n=71) *vs* patients with no steroid dependency (n=32) (156.2µg/ml *vs* 184.8 µg/ml, p = 0.0414) (Figure 1c). However, there was no significant difference between CD patients with steroid resistance or not (158.5 µg/ml *vs* 158.3 µg/ml respectively, p=0.640).

There was no significant difference of anti-GM-CSF Ab concentration in IBD patients treated with anti-TNFα (n=105) *vs* patients without anti-TNFα (n=80) (median 158.3 µg/ml and 160.5 µg/ml respectively).

The median concentration of anti-GM-CSF Ab was not different in CD responder's vs non-responder. However, the median concentration was significantly lower in UC patients not responding to treatment with anti-TNFα *vs* responders (90.5 µg/ml *vs*196.7µg/ml, p = 0.0157) (Figure 1d).

The optimal threshold of anti-GM-CSF Ab to predict response to anti-TNFα therapy in UC was ≥ 102µg/ ml, with a sensitivity of 92.96% [76.5-99.1] and a specificity of 66.67% [22.3-95.7] and an AUC 0.82 (Figure 2).

Relationships between anti-GM-CSF Ab and anti-glycan and anti-GP2 Abs

Anti-GM-CSF Abs were associated with AMCA (spearman r = 0.315, 95% confidence interval [0.178-0.442], p<0.0001), and also with anti-GP2 IgA (spearman r = 0.152, 95% confidence interval [0.004-0.293], p=0.0383) but not with anti-GP2 IgG, ACCA or ALCA.

Table 2: Demographic and main characteristics of patients according to response to anti-TNFα (n=52 CD and 34 UC 47 CD)

	Responders to anti-TNFα		Non-responders to anti-TNFα	
IBD	**CD (n=47)**	**UC (n=28)**	**CD (n=5)**	**UC (n=6)**
Sex ratio M/F	22/25	18/10	2/3	4/1
Current smokers (n,%)	25 (53.2)	1(3.6)	3 (60)	0
Mean age at inclusion (± SD, years)	36.2 (12.7)	44.6 (15.5)	28.5 (4.2)	56.6 (14.2)
Median disease duration at the inclusion (years [IQ range])	4 [2-12]	7 [4-10]	8 [8-11.8]	3 [2-8.5]
Age at diagnosis				
• A1 : ≤ 16years	16 (34%)		0	
• A2 : 17-40 years	29 (61.7%)		4 (100%)	
• A3 : > 40 years	2 (4.3%)		0	
• No data	0		1	
Disease location				
• Ileal (L1)	14 (29.8%)		2 (50%)	
• Colonic (L2)	8 (17%)		0	
• Ileo-colonic (L3)	25 (53.2%)		2 (50%)	
• Ulcerative proctitis (E1)		3 (11.5%)		2 (33.3%)
• Left-sided colitis (E2)		9 (34.6%)		1 (16.7%)
• Pancolitis (E3)		14 (53.8%)		3 (50%)
• No data	0	2	1	0
Disease behavior				
• No stricturing, no penetrating (B1)	25 (53.2%)		1 (25%)	
• Stricturing (B2)	14 (29.8%)		3 (75%)	
• Penetrating (B3)	8 (17%)		0	
• Perianal disease	14 (29.8%)		1 (25%)	
• No data	0		1	
First medication				
• Anti-TNF	1 (2.1%)	0	0	0
• Corticosteroïds	34 (72.3%)	13 (46.4%)	3 (60%)	3 (50%)
• Immunosuppressive	2 (4.3%)	0	0	0
• 5-ASA	8 (17%)	15 (53.6%)	2 (40%)	3 (50%)
Concomitant medication during the first year				
• Anti-TNF	14 (29.8%)	4 (14.3%)	1 (20%)	2 (33.3%)
• Corticosteroïds	38 (80.9%)	20 (71.4%)	4 (80%)	5 (83.3%)
• Immunosuppressive	26 (55.3%)	9 (32.1%)	2 (40%)	3 (50%)
Steroid dependency	35 (74.5%)	24 (85.7%)	4 (80%)	5 (83.3%)
Steroid refractory	9 (19.1%)	15 (53.6%)	1 (20%)	3 (50%)
IBD related Surgery	22 (46.8%)	4 (14.3%)	3 (60%)	1 (16.7%)
Severe disease	40 (85.1%)	23 (82.1%)	5 (100%)	5 (83.3%)
Anti-TNF Ab treatment				
• Infliximab	47 (100%)	28 (100%)	5 (100%)	5 (83.3%)
• Adalimumab	9 (19.1%)	5 (17.9%)	0	1 (16.7%)

Discussion

In recent studies, anti GM-CSF Ab levels in patients with IBD were correlated with location and extent of intestinal involvement. Thus, higher concentrations were found in ileocolonic CD and damages extended to the left colon in UC [18]. In another study of Gathungu *et al*. an association was found between high levels of anti-GM-CSF Ab and stricturing and penetrating behaviors of CD [19]. In contrast to these studies, no significant difference was observed depending on the location, the disease behavior of CD, or depending on the extent of the disease in UC in our study. These discrepancies could be due to different methodology used for example for the evaluation of phenotypical characteristics and different populations studied. However, patients with a severe CD had higher median anti-GM-CSF Ab concentrations.

There are conflicting results about the association of anti-GM-CSF Abs and treatment. In the study of Däbritz *et al*. levels of anti-GM-CSF Ab were significantly lower in patients without medication *vs* patients treated with oral corticosteroids,

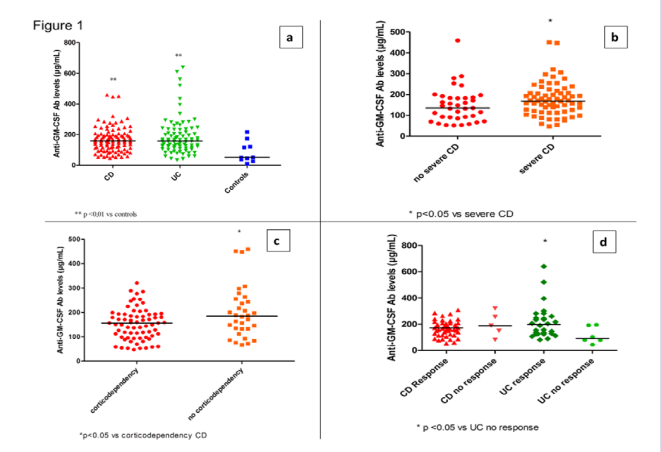

Figure 1: Anti-GM-CSF Ab levels in different groups: a. inCD (n=103), UC (n=84) and in controls (n=10); b.in patients with severe or no severe CD (n=37 and n=66 respectively); c.in steroid dependency and non-steroid dependency CD patients (n=71 and n=32 respectively); d.in studied groups according anti-TNFα therapy response(CD responders n= 60, CD non responders n=5, UC responders n=34, UC non responders n=6); Horizontal lines represent median anti-GM-CSF concentration

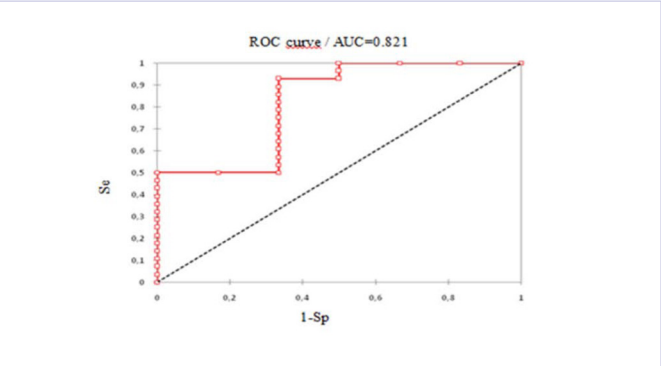

Figure 2: Receiver Operating Curve of anti-GM-CSF Ab in anti-TNFα treatment response in UC. Se: sensitivity, Sp: specificity, AUC: Area under Curve

5-aminosalicylates, azathioprine, anti-TNF or methotrexate [18]. 58 % of patients on medical therapy at the time of the inclusion had low Ab levels and 38 % showed elevated concentrations during the follow-up of patients on medical therapy [18]. Nylund *et al*. showed significant higher GM-CSF Ab in pediatric patients treated with infliximab [20]. However, in a study of Han *et al*., median concentrations were higher in patients who had received 6-mercaptopurine, but there was no variation with corticosteroid or infliximab exposure [14]. Nevertheless, there are no data on the association of anti-GM-CSF with therapeutic response to date.

In a recent study, Däbritz *et al*.showed that anti-GM-CSF Ab were correlated with disease activity and could help to predict and detect a relapse in both CD and UC. High concentrations of anti-GM-CSF Ab > 1.7 µg/ml in CD and > 0.5 µg/ml in UC are correlated with a clinical relapse from two to six months before. The sensitivity and specificity of these antibodies for the detection of relapse 2-6 months were respectively 88% and 95% in CD, and 62% and 68% in UC. Moreover, a baseline concentration > 1.7 µg/ml was predictive of relapse within 18 months in CD [18]. These data suggest that serum Anti-GM-CSF Ab in patients with IBD may be an interesting tool for monitoring disease activity in order to optimize therapy, but their potential interest in therapeutic monitoring remains unknown.

In our study, lower concentrations of anti-GM-CSF Ab were found in UC patients who did not respond to anti-TNFα treatment. To date, no study has yet shown this relationship in UC. However, this result is only based on very few patients and has to be confirmed with other studies. The preliminary hypothesis that could rise about this association would be an increase in anti-GM-CSF Ab linked to increased production of GM-CSF. This could be related to increased apoptosis of macrophages in the *lamina propria* secondarily to anti-TNFα [21].

Concentrations of anti-GM-CSF Ab obtained in this study were greater than observed in previous studies concentrations [14,16,18–20,22] which could be related to a lack of specificity of the technique used or better sensitivity. Our study has several limitations as the cohort of IBD patients comes from a tertiary referral single-center leading to a selection bias; the relative small sample size of the population, that may have limited the identification of some associations with disease behavior; the criterion defining a severe disease course are composite, including heterogeneous parameters, and may be the subject of discussion; the determination of anti-GM-CSF Abs is based on a unique serum sample and hypothesized that these antibodies remain stable over the time. Longitudinal analysis would be interesting to determine if this marker is stable over time.

Moreover, Jurickova *et al* showed that anti-GM-CSF Ab were produced by *lamina propria* mononuclear cells isolated from CD ileal resection specimens and that peripheral blood contains GM-CSF neutralizing capacity (16). Some other studies have shown that anti-GM-CSF Abs could be neutralizing antibodies or not(23-25).Indeed, it would also be interesting to evaluate GM-CSF bioactivity in parallel in order to determine if anti-GM-CSF Abs found in our cohort are neutralizing Abs.

In conclusion, these findings support the notion that anti-GM-CSF Ab could be an interesting therapeutic monitoring tool especially in UC in which there are currently few predictors of treatment response. However, more prospective and longitudinal studies are required to confirm these data.

Authorship Statement

Guarantor of the article: Stéphane PAUL

Specific author contributions: JB, AD, CG, have performed the research. JB, XR and SP collected and analysed the data. JB, SP and XR designed the research study and wrote the paper. All authors approved the final version of the manuscript.

Conflicts of interest

The authors disclose the following:

For S.P., M.R., G.B., E. P., J.B., A.M., L.C., C.G., J.M.P.: No conflict of interest.

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