Correlations Between Inflammatory Biomarkers and Activity in Inflammatory Bowel Diseases

CATALINA MIHAI[1], CRISTINA CIJEVSCHI PRELIPCEAN[1]*, MIHAELA DRANGA[1], OTILIA GAVRILESCU[1], ANCA CARDONEANU[3], CRISTINA LACATUSU[2], BOGDAN MIRCEA MIHAI[2]

[1]Grigore T. Popa University of Medicine and Pharmacy, Discipline of Medical Semiology - Gastroenterology, Sf. Spiridon Clinical Hospital, 16 Universitatii Str., 700115, Iasi, Romania

[2]Grigore T. Popa University of Medicine and Pharmacy, Discipline of Diabetes, Nutrition and Metabolic Diseases, 16 Universitatii Str., 700115, Iasi, Romania

[3]Grigore T. Popa University of Medicine and Pharmacy, Discipline of Rheumatology, Rehabilitation Hospital, 16 Universitatii Str., 700115, Iasi, Romania

There is an increasing interest in non-invasive methods to assess gut inflammation. The data regarding the correlations between inflammatory markers and activity of inflammatory bowel disease (IBD) are still controversial. In the last years faecal calprotectin became the most widely used biomarker in diagnosis and monitoring the IBD activity. We prospectively studied the correlation between the serological inflammatory markers (platelets, erythrocyte sedimentation rate - ESR, fibrinogen, C Reactive Protein -CRP, ferritin, albumin), faecal calprotectin and severity of IBD in a tertiary referral centre in North-East Romania. Our study demonstrated that is a good correlation between serologic inflammatory markers (platelets, fibrinogen and ferritin, not ESR and albumin) and severity of IBD. CRP is a good marker in Crohn's disease (CD) but not in ulcerative colitis (UC). Faecal calprotectin (FC) is the best inflammatory biomarker which correlates with activity both in UC and CD. Inflammatory biomarkers, especially FC are an important tool to evaluate patients with IBD.

Keywords: Crohn's disease, ulcerative colitis, inflammatory biomarkers, faecal calprotectin

Inflammatory bowel disease (IBD) is a common condition of unknown aetiology characterised by an abnormal response of the immune system to different antigens (microbiota, food antigens, etc) in individuals with a genetic predisposition. The assessment of IBD is challenging and complex, involving clinical diagnosis, laboratory investigation, imaging and histopathological assessment. In current clinical practice the most commonly used severity scores are the CDAI (Crohn's disease activity Index) for Crohn's disease (CD) and the Mayo score (UCDAI - Ulcerative Colitis Disease Activity Index) for ulcerative colitis (UC). Although their use and validity has been established over time, these scores represent more the patient's subjective well-being than the degree of mucosal inflammation. We are practicing at a time when therapeutic targets in IBD have evolved from the induction and maintenance of disease remission to more ambitious goals, such as the modification of the natural history of IBD, mucosal and histological healing [1]. There is some evidence, however, that these well-established scoring systems based especially on clinical symptoms do not correlate with inflammation or mucosal healing [2].

Inflammatory biomarkers provide information regarding the activity of the disease and are widely accepted because of their non-invasitivity. The current data about the role of inflammatory markers in monitoring IBD is still controversial. In the era of personalized medicine it is an increasing interest for detection of new biomarkers which can differentiate the subtypes of IBD, predict the disease course and the therapeutic response.

In IBD there is no single best marker of disease activity. The most commonly used markers are the acute phase reactants: CRP, ESR, fibrinogen, ferritin, platelets and albumin. These are accessible, cheap, non-invasive, but have a reduced sensitivity and specificity [3]. Recently, faecal calprotectin (FC), demonstrated its ability to differentiate IBD from irritable bowel syndrome, as well as assess disease activity, facilitates prognosis, predicts mucosal healing, response to therapy, need of surgery [4]. The test is limited by its inability to differentiate between CD and UC or other causes of intestinal inflammation (neoplasia, infections, polyps, non-steroidal anti-inflammatory drugs) [5]. There are a lot of other promising faecal biomarkers to assess the activity of IBD (cathelicidins, osteoprotegerin, beta-glucuronidase, neutrophil gelatinase associated lipocalin) but we still need more researches to validate these findings [6].

Our study aims to correlate IBD disease activity as determined by the CDAI and Mayo scores with inflammatory biomarkers in patients admitted to a tertiary referral centre in North-East Romania.

Experimental part

A prospective study was performed in 196 (48 with CD and 148 with UC) IBD inpatients at the Institute of Gastroenterology and Hepatology in Iasi, Romania, in the period 2014 – 2016. All patients had a confirmed diagnosis of IBD based on clinical presentation, laboratory investigations, clinical imaging (bowel endoscopy, computed tomography, magnetic resonance imaging) and histological assessment. The following inflammatory parameters were analysed: platelets, ESR, fibrinogen, CRP, ferritin, albumin. FC was assayed using a immunochromatography semiquantitative method (CalDetect, Sofar) and categorised as follows: T1 <15 μg/g, T2 15 -60μg/g, T3 > 60μg/g. The severity of the disease flare in CD was determined according to the CDAI score, whilst for UC using the Mayo (UCDAI) scoring system. The correlation of laboratory findings with disease severity was performed using all the episodes of disease flare requiring inpatient admission.

* email: cristinacijevschi@yahoo.com; Phone: +4 0740 058208

The statistical analysis was performed using the SPSS 20.0 software package. Descriptive statistical parameters were calculated for the variables involved in study: frequency distributions, mean, median, standard error, mean standard deviation and variation. In order to identify statistically significant differences Chi-squared testing was used for qualitative variables, whilst ANOVA testing was used for categorical variables. The significance level used was $p < 0.05$.

All patients gave written informed consent for participating in the study. The study was approved by the Ethics Committee of Sf. Spiridon Hospital, Iasi, Romania.

Results and discussions

CD patients

There were 48 CD patients (52.1% male and 47.9% females) with a median age of 42 years. The severity of disease flare was stratified according to the CDAI score: 12 patients (25%) had remission (CDAI < 150), 30 patients (62.5%) had a mild or moderate flare (CDAI 150 -450), and 6 patients (12.5%) had a severe flare (CDAI > 450). The majority of patients had colonic involvement: L1(ileal)- 10.4%; L2 (colonic) - 43.8%; L3 (ileo-colonic) - 37.5%; L4 (upper digestive tract) - 4.2%; L1 + L4 -4.2%. More than half of the patients (54.2%) had non-penetrating non-stenosing disease (B1), 31.3% had stenosing behavior (B2) and 14.6% had penetrating lesions (B3). 3 patients (6.2%) had peri-anal involvement and 14 patients (29.2%) had previous bowel resections. 13 (27%) of patients had extra-intestinal involvement, including 6 cases with arthritis, 2 renal stones, 2 biliary stones, 2 uveitis and 1 featuring erythema nodosum. 4 of the 6 patients with a severe disease activity score had extraintestinal involvement. Only the presence of extraintestinal manifestations correlated statistically significant with CDAI (table 1).

The laboratory findings analysed were collected from all the flare episodes of the 48 patients included in the study (70 flare episodes). Disease severity had a statistically significant correlation with platelets, ferritin, fibrinogen, CRP, FC but not with ESR and albumin (table 2). The mean CRP was 7.11 mg/dL in patients with severe disease compared to 0.61mg/dL in those with remission. Similarly, the fibrinogen was 5.35 g/L in patients with severe disease compared to 3.5 g/L in those with remis- 47.14%; T2- 38.57%; T3 - 14.28%. 86% of patients with a severe flare had a raised FC, being similar in T2 and T3; in contrast, ¾ of patients in remission had a low FC level (T1).

UC Patients

There were 148 UC patients (60.8% male and 39.2% female) with a median age of 46.51 years. Disease severity was assessed using the Mayo score (UCDAI): 21 patients

Parameters	CDAI < 150	CDAI 150-450	CDAI > 450	p
Gender				0.59
Female	7	14	2	
Male	5	16	4	
Age	46.75 (±12.01)	41.06 (± 14.19)	37.16 (±12.22)	0.31
Current Smoker	6	19	3	0.67
Montreal classification				
A2				
A3	5	20	4	0.30
L1	7	10	2	
L2	0	5	0	0.36
L3	8	10	3	
L4	2	13	3	
L1 + L4	1	1	0	
B1	1	1	0	
B2	6	16	4	0.61
B3	5	8	2	
Perianal involvement	1	6	0	
	0	3	0	0.38
Previous surgery	2	10	2	0.54
Extradigestive manifestations	3	6	4	**0.001**

Table 1
CHARACTERISTICS OF CD'S PATIENTS CORRELATED WITH CDAI SCORE

Parameters	CDAI < 150	CDAI 150-450	CDAI > 450	p
Platelets ($10^3/\mu L$)	271.413(±46.194)	345.666(±135.297)	417.857(±116.869)	**0.005**
ESR (mm/1h)	15.24(±11.34)	35.22(±31.09)	30.00(±20.44)	0.059
Fibrinogen (g/L)	350.48(±45.35)	444.50(±101.97)	535(±64.81)	**0.000**
CRP (mg/dl)	0.61(±1)	4.22(±5.86)	7.10(±10.16)	**0.000**
Ferritin(ng/ml)	84.44(±61.25)	113.87(±199.99)	921.31(±1858.52)	**0.029**
Albumin (g/dl)	24.83 (±22.62)	27.93 (±20.57)	16.77(±15.84)	0.06
Faecal calprotectin				**0.001**
T1	21	11	1	
T2	5	19	3	
T3	3	4	3	

Table 2
CORRELATIONS BETWEEN INFLAMMATORY MARKERS AND CDAI

(14.18%) were in remission (Mayo 0 – 2), 37 (25%) had a mild flare (Mayo 3 – 6), 50 (33.78%) had a moderate flare (Mayo 7 – 10) and 40 (27.02%) had a severe episode (Mayo > 10). 33 patients had extra-gastrointestinal disease manifestations (22.29%) involving: joints (16 patients), skin (13 patients), eyes (3 patients), primary biliary cholangitis (1 patient). Disease involvement was E1 (proctitis) in 32 patients (21.6%); E2 (left colitis) in 85 patients (55.4%); E3 (pancolitis) in 34 patients (23%). A statistically significant correlation was found between the extension of the disease, the presence of extra-intestinal involvement and the Mayo score (table 3).

Laboratory findings were determined, as for CD, for the total number of flare episodes: 169 flares in 148 patients studied. There were a statistically significant correlation with platelets, fibrinogen, ferritin but not with ESR, CRP and albumin (Table 4). The strongest correlation was found between disease severity and FC. FC levels were: T1 – 25.44%; T2 – 30.17%; T3 - 44.37%. Thus, 93.1% of patients in remission had a reduced FC level (T1), whilst 95.1% of those with severe forms of the disease had a T3.

Epidemiological data in ***CD*** patients are similar to those reported in the literature. There were no correlations between epidemiological and demographic factors (age, gender, smoking status), disease location, behavior and CDAI score. It is known that young age, active smoking status, ileal and perianal involvement, as well as stenosing lesions are debilitating factors associated with CD in young patients [7]. In this context, our study highlights the limitations of the CDAI score regarding the estimated prognosis of patients with CD. As long as the CDAI score incorporates extra-intestinal manifestations of disease, it is foreseeable that the severity of the flare episode will correlate with these symptoms.

In the patient cohort study, we found normal mean values for platelets and albumin. The levels of ESR, fibrinogen and ferritin were mildly raised. CRP was the single biochemical markers with significantly raised mean values. A rise in CRP is associated with clinical, endoscopic and histological signs of disease activity in CD [8]. ESR is an inferior inflammatory marker compared to CRP, being affected by a number of concurrent factors, such as age, gender, inflammation, neoplasia, plasma protein levels and haeamtocrit, etc [8]. Its prolonged half-time compared to CRP gives it a relative latency in monitoring the severity of inflammation. Low albumin levels are also considered a marker of inflammation, but its specificity is reduced, with serum levels being affected by malabsorption and malnutrition – events that are frequently seen in CD [9]. Fibrinogen, together with other less well known acute phase reactants (sialic acid, orosomucoid, etc), has not been widely researched. All these markers are considered to be inferior to CRP, predominantly due to their prolonged half-lives. In our study inflammatory markers which correlated with CD activity included: CRP, fibrinogen, platelets and ferritin. The role of ESR was borderline significant. CRP is considered the most powerful serum marker which co-ordinates with inflammatory activity, which has been confirmed not only by our study, but also the existing literature [9,10]. Moreover than that, many authors consider CRP not only an indicator of inflammation but an independent biomarker predicting response to biologic therapy, low Infliximab levels, risk of relapse [11,12].

Despite this, there were patients with a normal CRP and increased disease activity, as well as patients with a raised CRP and inactive disease. This is consistent with other published studies. A prospective study over a period of 2 years on 101 patients with CD suggested that a third of patients with active disease had a normal CRP and another third with a raised CRP had inactive disease [13]. Another study by Jones et al on 164 patients cu CD did not find any relationship between CDAI, CRP and FC [14].

Similar to our study, Sipponen et al. founded a positive correlation between FC concentration and the CDAI in 77

Tabel 3

THE CHARACTERISTICS OF UC'S PATIENTS CORRELATED WITH MAYO SCORE

Parameter	MAYO 0 -2	MAYO 3 -6	MAYO 7 -10	MAYO > 10	p
Sex					0.75
Female	10	20	17	11	
Male	11	17	33	29	
Age	49.85 (±15.19)	44.08 (± 12.78)	49.52(±16.16)	43.22 (±13.23)	0.09
Current Smoker	10	12	22	24	0.07
Extension					
E1	4	15	9	4	**0.002**
E2	9	18	34	21	
E3	8	4	7	15	
Extradigestive manifestations	3	5	8	17	**0.001**

Table 4

THE CORRELATION BETWEEN INFLAMMATORY MARKERS AND MAYO SCORE IN UC

Parameter	MAYO 0 -2	MAYO 3 -6	MAYO 7 -10	MAYO > 10	p
Platelets (10^3/μL)	229.821(±85.857)	295.947(±117.002)	293.241(±84.101)	387.500(±163.562)	**0.000**
ESR (mm/1h)	19.00(±14.85)	16.71(±16.66)	28.67(±23.41)	34.74(±32.89)	0.06
Fibrinogen (g/L)	350.00(±57.66)	351.19(±63.32)	412.42(±90.43)	406.18(±106.16)	**0.014**
CRP (mg/dl)	1.00(±1.97)	2.78(±8.81)	3.13(±5.89)	7.65(±18.04)	0.056
Ferritin(ng/ml)	62.66(±71.23)	65.60(±66.06)	91.52(±82.14)	172.85(±127.98)	**0.011**
Albumin (g/dl)	31.85 (±29.76)	35.80 (±30.45)	31.84(±29.47)	34.96(±28.04)	0.70
Fecal calprotectin					**0.000**
T1					
T2	27	9	7	0	
T3	1	18	30	2	
	1	12	23	39	

CD patients [15]. Despite this, almost a third of patients in clinical remission had significantly raised faecal calprotectin levels (T2 and T3), which suggest the possible ongoing presence of intestinal inflammation even in those in remission. This has also been highlighted by other authors [16]. Calprotectin has a positive predictive value of over 90% regarding endoscopic activity, having a better positive correlation than CDAI scoring or CRP [17]. Some studies suggest a lack of correlation between FC levels and clinical factors [18]. Our data, together with the existing evidence, suggest that CRP, as well as FC should be used in assessing the severity of CD.

Similar to CD, in ***UC*** patients no relationship was found between epidemiological factors and disease severity. In contrast to the CDAI score, the Mayo scoring system used in UC correlated with disease extension. The presence of the extra-intestinal involvement, in particular those involving the joints, had a statistically significant correlation with disease activity, which has also been confirmed in other studies [19]. This suggests that the presence of extra-gastrointestinal symptoms should be used in the assessment of patients with UC, in a similar manner to CD.

The inflammatory markers in UC patients had mean values similar to those in CD. Fibrinogen, ferritin and platelets had a statistically significant correlation with disease severity, the strongest association being with thrombocytosis. Although the mean CRP level was greater in patients with UC than those with CD, it did not correlate with disease severity. This proves that CRP is a useful marker of disease activity in CD, but less so in UC – this has also been mentioned by other sources [20]. A possible explanation would be the lower levels of IL-6 in UC compared to CD, as well as the transmural inflammation seen in CD but not in UC (where inflammation occurs at a mucosal level) [9].

Similar to other studies, we found that there was a stronger correlation between FC and disease activity in UC than in CD [21]. In a Korean UC cohort FC level was significantly correlated with the clinical disease activity index, endoscopic indices, and serum inflammatory biomarkers [22]. The combination of FC with clinical activity indices or CRP may improve the prediction of endoscopic active disease or remission [23, 24].

Conclusions

Our study demonstrated that is a good correlation between extraintestinal manifestations, serologic inflammatory markers (platelets, fibrinogen and ferritin, not ESR and albumin) and severity of IBD. CRP is a good marker in CD but not in UC. FC is the best inflammatory biomarker which correlates with activity both in UC and CD. Further studies are needed in order to find the ideal biomarker (non-invazive, cheap, with good sensitivity and specificity) to estimate severity, disease course and response to treatment among individualized patients with IBD.

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