The Role of Combining Biochemical Markers in Assessing the Endoscopic Activity in Ulcerative Colitis

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Ulcerative colitis (UC) is a chronic, idiopathic and recurrent inflammatory bowel disease (IBD), characterized by periods of activity and remission whose monitoring requires invasive explorations associated with discomfort for the patient and important costs. Mucosal healing became one of the most important therapeutic targets in UC. The aim of our study was to identify a score, made up of noninvasive, available, used in current clinical practice biochemical markers, which should correlate with endoscopic activity in UC. We conducted a prospective study on 114 patients with UC. All patients were assessed both for biological inflammatory markers: erythrocyte sedimentation rate (ESR),C- reactive protein (CRP), fibrinogen, platelets, albumin, fecal calprotectin (FC) and by colonoscopy to estimate the endoscopic activity using Mayo score. By linear regression, we tried to identify a biochemical score correlated with endoscopic activity. Out of the serological markers,ESR (p=0.014), CRP(p= 0.021) and fibrinogen (p=0.035) correlated with the endoscopic activity of the disease. The best sensitivity to determine the endoscopic activity was given by FC (96.05%) with a predictable negative value of 91.1% (p=0.001). The score determined by linear regression: 1 (ESR>15mm/1h) x 0.305 + 1(fibrinogen>340.5mg/dL) x 0.309+1 (CRP> 5 mg/L) + 1 (calprotectin>200 μg/g) had an increased positive predictive value compared to each and one biomarker, nevertheless, with a sensitivity and specificity inferior to that of FC. Up to now, it is the first attempt to achieve a score made up exclusively of biological markers. The obtained score, although with an increased accuracy, has proven to have a lower predictability in comparison with FC used individually and cannot entirely replace colonoscopy.

Key words: inflammatory biomarkers, score, fecal calprotectin, ulcerative colitis, Mayo score

Ulcerative colitis (UC) is a chronic disease characterized by periods of activity alternating with those of remission. In recent years, the progress made has imposed new therapeutic targets, beyond the induction and maintaining clinical remission: normalization of inflammatory tests, mucosal healing (absence of endoscopic lesions) and even histologicalhealing [1]. It has been demonstrated that mucosal healing is correlated with decreasing the number of hospitalizations, of corticosteroids, of surgical procedures, reduction of disability and increase in patients' quality of life [2].

Many aspects of IBD have remained challenges for doctors: diagnosis, prognostic, assessment of activity and severity as well as the response to treatment. For each of these aspects, there is no one single standard *golden* test, only a combination of symptoms, laboratory parametric signs, radiology, endoscopy and histology. The activity IBD is assessed by scores of clinical and endoscopic activity. The score of clinical activity estimates indirectly the activity of the disease and is a poor predictor of the activity of endoscopic inflammatory activity. Repeated endoscopic assessments are invasive and costly, with discomfort for the patient.

In this context, it is justified to be concerned with finding a biomarker or a group of biomarkers which should correlate with endoscopic activity of the disease, to allow a personalized monitoring and treatment and to anticipate relapses. The serologic markers of inflammation, used in current clinical practice (platelets, ESR, fibrinogen, CRP, albumin) represent an objective, non invasive method to put inflammation into light, but they are non specific, occur in any inflammatory process of the body and correlate to a small extent to endoscopic activity. In recent years, fecal inflammation markers have gained a well deserved role in assessing patients with IBD, the most studied being FC. It is a protein binding calcium and zinc, and represents 60% of the cytosolic proteins of granulocytes [3]. It is resistant to bacterial degradation and remains stable in stools at room temperature up to one week. The concentration of FC is an indirect marker of the number of neutrophils which infiltrate bowel mucosa. It is the most frequently used marker in differentiating BII and SII, in assessing endoscopic activity and therapeutic monitoring. The great advantage of FC is that it can be detected in low values of inflammation, that are insufficient to induce an increase in ESR or CRP and is not influenced by other extra bowel conditions [4,5]. Calprotectin cannot differentiate the period of activity from other conditions which could increase bowel inflammation (bacterial suprainfection, colo-rectal cancer, use of non steroid anti-inflammatory drugs,etc). The combination of serologic markers with the fecal ones could lead to diagnosis performance. In this context, our study tries to identify a non invasive biochemical score which should correlate with endoscopic activity in UC.

Experimental part

A prospective study was conducted on 114 patients with UC, hospitalized in the Institute of Gastroenterology and

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Hepatology in Iasi, in the period of time 1st January 2016 – 1stJune 2017. There were included patients with confirmed diagnosis of UC, both in periods of activity and in remission, which had colonoscopy. There were excluded from the study patients with Crohn's disease or indeterminate colitis, colo-rectal cancer, bacterial suprainfections, colon diverticulosis, non steroid anti-inflammatory drugs use, associated systemic diseases which could influence studied parameters.Before colonoscopic examination, venous blood was taken to assess laboratory parameters: platelets, ESR, fibrinogen, CRP, seric albumin.FC was measured by Elisa method. For the endoscopic activity evaluation,we used Mayo endoscopic score, considering Mayo score d" 1 remission and > 1 activity.

Ethical considerations

The protocol was approved by the local ethics committee. All participants signed an informed consent.

Statistical analysis

Statistical analysis was performed in SPSS 18.0, with nominal significance defined as $p<0.05$. Continuous variables were described using ANOVA test. Relations between laboratory markers and Mayo score are reported by Pearson correlation coefficients. ROC curves were analyzed to assess the optimal cut-off values of markers. Sensitivity, specificity, positive predictive value and negative predictive value were calculated in 95% confidence intervals for this cut-off value. The multiple linear regression analysis was used for the score.

Results and discussions

In the study group mean age was 43.6±14 years, with a preponderance of male cases (54.3%). More than half of the patients presented left colitis(53.5%), followed in frequency by pancolitis(28.9%), and proctitis (17.5%).One third of the patients had a first diagnosis. Duration of the disease was extremely varied with a mean duration of 8 ± 3 years.

38 patients (33.33%) had endoscopic remission (Mayo endoscopic score ≤ 1).

The analysis of serological inflammatory parameters demonstrated significant statistical correlations between CRP, ESR, fibrinogen and endoscopic activity. On the contrary, platelets and albumin did not correlate with endoscopic activity.The strongest statistical correlation was recorded between FC and Mayo endoscopic score ($p=0.001$) (table 1).

For each parameter we analyzed positive predictive value, negative predictive value, sensitivity, specificity, accuracy at the following cut-off values: ESR 15 mm/1h, CRP 5 mg/L, fibrinogen 340.5 mg/dL, FC 200 μg/g. We

Table 1
THE CORRELATION BETWEEN INFLAMMATORY BIOMARKERS AND ENDOSCOPIC ACTIVITY IN UC PATIENTS

Activity	N	Medium	Std. deviation	Std. error	Confidence interval		Min	Max	p t-Student test
					- 95%CI	+95%CI			
Albumin (mg/dL)									
Remission	38	46.79	3.64	1.82	42.00	51.58	42.97	51,70	0.136
Activity	76	40.87	8.37	1.13	37.68	44.06	18.16	52,89	
Platelets(n x 10^3 /mm^3)									
Remission	48	305130	94810	19769	264132	346129	202000	553000	0.279
Activity	55	342759	157275	17695	307532	377987	134000	992000	
ESR (mm/1 h)									
Remission	38	17.52	19.93	4.16	8.90	26.14	1.00	63,00	**0.014**
Activity	76	27.79	25.39	2.84	22.14	33.44	1.00	108,00	
Fibrinogen (mg/dL)									
Remission	38	351.18	62.17	13.29	318.78	383.58	234.00	501.00	**0.035**
Activity	76	401.48	114.12	12.60	369.43	433.53	209.00	678.00	
CRP (mg/L)									
Remission	38	10.7	1.67	0.35	0.52	1.62	0.03	8.18	**0.021**
Activity	76	3.23	4.45	0.61	2.47	4.17	0.03	26.00	
Calprotectin (μg/g)									
Remission	38	35.93	3.39	1.35	32.5	39.36	5	42	**0.001**
Activity	76	402.16	48.0	3.48	348.5	455.82	38	535	

Remission= Mayo 0,1; Activity= Mayo 2,3

Tabel 2

PREDICTIVE VALUES, SENSITIVITIES, SPECIFICITIES AND ACCURACY FOR CRP, ESR, FIBRINOGEN, FC AND CALCULATED SCORE OF ENDOSCOPIC INFLAMMATION FOR ACTIVE ENDOSCOPIC DISEASE IN UC

Marker	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy	p
CRP	63.7	76.2	62.5	62.06	62.2	0.089
ESR	57.1	34.8	62.1	51.0	59.7	0.105
Fibrinogen	62.5	56.5	64.0	50.0	59,3	0.050
FC	73	91.1	96.05	83.78	96.9	0.001
Calculated score	76.3	71.05	86.41	81.81	85.08	0.001

found that FC had the best predictability for endoscopic activity (area under the ROC curve 96.9%), followed by PCR (62.2%), ESR (59.7%) and fibrinogen (59.3%) (Table 2).The model of multiple correlation shows inter-correlations between parameters: FC, CRP, ESR and fibrinogen, being significant from a statistical point of view, facilitated the elaboration of predictability score for endoscopic inflammation.

Score of endoscopic inflammation (SEI) (Mayo > 1) = 1 (ESR > 15 mm/1h) x 0.305+ 1 (fibrinogen>340.5 mg/dL) x 0.309 +1 (CRP> 5 mg/d:)+1 (FC>200 μg/g).

The SEI calculated on the basis of higher cut-off value of markers : ESR, fibrinogen, CRP and FC of our patients proved a good predictability (86.41%) of Mayo activity with 85.08% accuracy (tabel 2). The ROC curve analysis revealed that the sensitivity and specificity of calculated SEI and FC are superior to ESR, fibrinogen and CRP but without significant differences between FC and calculated SEI (fig.1).

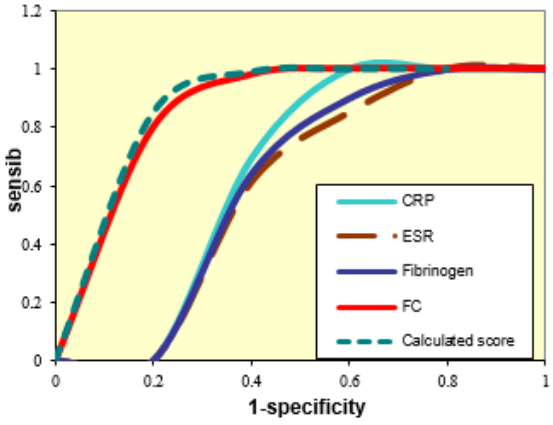

Fig. 1. The ROC curve analysis on the abilities of CRP, ESR, fibrinogen, FC and calculated score to make a difference between endoscopic active UC and inactive UC

In UC evaluation of endoscopic activity besides the colonoscopy remains a challenge in clinical practice. UC is a lifelong disease so repeated colonoscopies are associated with discomfort, increased costs, and impairment of quality of life. On the other hand it has been proved that the assessment of endoscopic inflammation is determinant for treatment monitoring [6]. Starting from these remarks, it is justified the interest for non-invasive evaluation of endoscopic activity. A lot of new biomarkers (genetics, metabolomics, proteomics, gut microbiota biomarkers) have been studied, but they are not yet ready for clinical practice [7, 8]. We have tried to make a score consisting of non invasive, available, quantifiable markers, which could estimate endoscopic activity in UC.

The most commonly used markers to evaluate the activity of UC are the acute phase reactants: CRP, ESR, fibrinogen, platelets and albumin, but they have a reduced sensitivity and specificity [9]. Our study shows significant correlations between serological inflammatory markers (fibrinogen, CRP, ESR, not platelets and albumin) and endoscopic activity.Fibrinogen over the value of 340.5mg/dL is a predictable marker for endoscopic UC activity, with a sensitivity of 64%, but it is to be noted that in 43.5 % of subjects in remission we found more increased values than thecut-off limit of this parameter. ESR over 15 mm/1h joins the predictable markers of UC endoscopic activity with a sensitivity of 62.1%, but there is also a high negative predictable value: 34.8%. CRP over cut-off value (5 mg/L), with 62.2% accuracy,includes in a correct way 63.7% of our patients with Mayo activity. Our data are in accordance with other studies from the literature. CRP is considered to be superior to ESR, fibrinogen and albumin in assessing inflammation from IBD, but its role is smaller in UC in comparison with Crohn's disease [10].CRP is increased in 50%-60% of patients with active UC, but it can be normal in 5-10% of patients with severe activity [9].In Yoon et al. study a cut-off value of CRP ≤ 8 mg/L showed a sensitivity between 50.5 and 53.5% and specificity between 85.1% and 87.2% in detecting remission [11].Chen et al. found also significant correlation between CRP (r= 0.634), ESR (r = 0.644) and Mayo score [12]. However, in Miranda – Garcia study, the same with our study, none serological marker had an area under the ROC curve > 0.70 [13].

FC level over 200μg/g showed the greatest sensitivity (96.05%) and specificity (83.78%). Nevertheless, 27% of cases, although they had levels of calprotectin under the established cut-offvalue had endoscopic activity. 8.9 % of patients, although with high levels of FC, were in endoscopic remission.The ROC curve analysis reveals significantly higher values compared to the rest of the parameters analyzed. The efficacy of endoscopic discrimination between endoscopic active and inactive UC assessed by the area under the ROC curve was 0.969. Our data are in agreement with numerous studies in literature which demonstrates the positive correlation of FC with the endoscopic activity of UC, although there are variable cut-off values [14]. Ma et al. reported a 0.77 positive predictive value of FC ≤ 250μg/g in identifying mucosal healing [15].In a meta-analysis on 2499 IBD patients the pooled sensitivity and specificity estimates for FC and CRP

were 0.88 (95% CI 0.84-0.90) and 0.73 (95% CI 0.66-0.79) and 0.49 (95% confidence interval (CI) 0.34-0.64) and 0.92 (95% CI 0.72-0.96), respectively [16]. In 2013, Schopfer et al pointed out that the endoscopic disease activity correlated best with FC (Spearman's rank correlation coefficient r= 0. 821), followed by Lichtiger Index (r=0.682), CRP (r= 0.556), platelets (r=0.488, blood leukocytes (r=0.401), and hemoglobin (r=0. 388) [16].In UC FC has stronger correlation with disease endoscopic activity than conventional inflammatory markers (r=0.798 versus r=0.463 for CRP and r=0.467 for ESR)[18]. Going deeper, Mack et al showed that fecal calprotectin < 200μg/g predicted histological remission with a sensitivity of 71% and specificity of 76% [19].

We used the linear regressive method to see if there were correlations between the noninvasive biological markers, trying to find a score with a better diagnosis accuracy for Mayo score. From the literature of the domain, we have found that, up to now, there is no score made up exclusively of quantifiable biological markers, for estimating endoscopic activity. Interesting, adding the clinical activity index to the FC,Yonn et al. showed higher AUC (0.980) for estimating endoscopic activity in UC [11]. Our score, which uses cut-off values of fibrinogen, ESR, CRP, FC, has a positive predictive value, 3.3% greater than that of FC. In spite of this, the score values occurred under the sensitivity and specificity values of FC, because of low sensitivity and specificity of serological inflammatory markers.

Conclusions

We may say that it is the first attempt to make a score made up exclusively of accessible biological markers, used in current clinical practice up to now. The obtained score, although had the greatest positive predictive value in comparison with each of the studied biomarkers, was inferior to FC regarding sensitivity, specificity and accuracy. Further studies are required, which should identify a non invasive biomarker (or that combination of biomarkers), with high sensitivity and specificity, with reduced variability, replicable, that should be accessible for a proper evaluation of endoscopic activity in UC.

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