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Tissue levels of Tumor Necrosis Factor-alpha as molecular biomarker of inflammation and prediction of sustained treatment response in patients with Ulcerative Colitis.

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BACKGROUND.

Ulcerative Colitis (UC) is a chronic inflammatory disorder involving the large intestine and causing a life-long impact on patient's quality of life. The pathogenesis of UC is only partially known and is the result of a complex interaction between genetic and environmental factors and a dysfunction of the innate and adaptive immune response. (1)

The traditional treatment approach for Inflammatory Bowel Diseases (IBD) include a step-up approach from conventional treatment such as mesalazine and corticosteroids to immunomodulators (azathioprine or 6-mercaptophurine and methotrexate) followed by biotechnological drugs. The introduction of anti-TNF-α biological therapies for the treatment of Ulcerative Colitis and Crohn's disease (CD) more than 20 years ago, represented a breakthrough in the treatment of these two conditions. Anti-TNF-α agents approved for UC are infliximab (2) (i.v use), adalimumab (3) and golimumab (4) (s.c use, both). Improving knowledge concerning the pathogenesis of IBD had lead to the development in the last years of a great panel of new molecules for the treatment of both, CD and UC. According to different mechanism of action, others biologics approved for UC are the α4β7 anti-integrin (vedolizumab) and, very recently, a small molecule Janus-kinase inhibitor (tofacitinib). (5-6)

Open questions of biological treatment

The approval of anti-TNF-α agents, and in general of biologics, has changed our concept of therapeutic goals, thanks to the achievement of deep remission and mucosal healing (MH) rates far superior to conventional therapies. Mucosal healing is associated with an improved long-term prognosis for UC and CD. (7-10) A prolonged use of biologics improves outcome but also increases potential patients' risks in terms of safety, i.e. infections. Studies report relapse rates of 40 - 50% over a 2-year period following discontinuation of anti-TNF-α. (11) So far, there is no general agreement on when to discontinue anti-TNF-α therapy. Another unsolved question is, in case of combination therapy, which treatment is more appropriate to stop and which to continue (biologic or

immunomodulator) in order to guarantee long-lasting deep remission together with an acceptable safety.

Among the predictive factors of relapse following anti-TNF-α discontinuation in CD are elevated C-reactive protein (CRP), leukocyte or neutrophil count, elevated faecal calprotectin (FC) levels, lower haemoglobin levels, absence of mucosal healing, and wall thickening at Entero-MRI. (12-14) However, nowadays a stratification of patients that guide therapeutic management is still lacking and de-escalation strategy remains a case-by-case decision. (15-16)

A significant percentage of patients will primarily not respond to anti-TNF treatment, will loose response to anti-TNF-α or develop adverse reactions most likely due to the development of anti-drug antibodies (ADAs). Hence, biomarkers as predictors of disease outcome and response to therapy are still to be determined.

Endoscopic scores and definition of mucosal healing.

For patients with UC, the most used score to assess clinical disease activity is the Mayo score. It consists of four sub-scores (stool frequency, rectal bleeding, endoscopic findings and physician's global assessment) that ranges from 0 to 3 points for the achievement of a final total score of 0 to 12 points. According to this score, UC is classified as in remission (≤ 2 points), mild disease (3-5 points), moderate disease (6-10 points) and severe disease (11-12 points). (17)

The full Mayo score mentioned above is used also to assess separately, clinical and endoscopic activity. Regarding the latter, the endoscopic sub-score is divided into four degrees of severity according to endoscopic findings: remission (0 points, no mucosal lesions); mild disease (1 points, erythema and mild friability), moderate disease (2 points, marked erythema, friability, erosions and disappearance of the vascular pattern), severe disease (3 points with spontaneous bleeding and ulcerations). Others endoscopic scores have been proposed in the last years, e.g. the UC Endoscopic Index of Severity (UCEIS) that, compared with the endoscopic Mayo score, divided ulcers into superficial and deep and ranges from 0 to 4 points. The Mayo score remains nowadays the most

frequently used scoring system. Although in many studies mucosal healing included also an endoscopic Mayo score of 1, MH is defined in most of the clinical trials for UC as a complete absence of lesions (Mayo score: 0). In the present study, in the method section, we used this latter definition.

Overview on biomarkers in Inflammatory Bowel Diseases.

Research for a predictive and prognostic biomarker available in clinical practice and able to predict a poor outcome in IBD is an ongoing challenge. According to US National Institute of Health a biomarker is "*a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*." (18)

An ideal biomarker for the management of IBD patients should be inexpensive, non-invasive and able to indicate a severe clinical outcome (in order to stratify patients for early treatment or top-down approach) and to predict the response to therapy (or giving information on when to stop treatment). Several biomarkers have been studied in the last years with the aim to detect inflammation during patients' follow-up. The best known and most frequently used markers of inflammation are C-reactive protein (CRP) and faecal calprotectin (FC). CRP is an acute-phase protein produced by the liver, its serum level is increased in response to inflammation. It is the most frequently used serum biomarker for diagnosis and follow-up of IBD because of its low cost and its non-invasiveness. However CRP is not the ideal biomarker because of the following limitations: its poor specificity (it increases in several clinical situations such as infections, cancer, sepsis and inflammatory conditions in general), its scarce correlation with endoscopic grade of inflammation and the fact that in some situations, in CD or UC, it may not increase. Increasing diagnostic power and accuracy of CRP is when combined with the biomarker faecal calprotectin. FC is a calcium-binding protein, member of the S100 family of zinc-binding proteins, being a heterodimer of S100A8/A9 and is present mainly in neutrophils. (19) It increases during active gut inflammation as a result of its release by neutrophils migrated into bowel lumen and its utility in the diagnosis (especially for early diagnosis and screening) and follow-

up of IBD is well established. (20) It is now the most useful biomarker used in clinical practice driving therapeutic decisions and recent studies showed also a good correlation with endoscopic and histological findings. (21-22)

Another surrogate biomarker of endoscopic inflammation is the Neutrophil gelatinase-associated lipocalin - matrix metalloproteinase 9 (NGAL-MMP-9) complex; NGAL is a protein released by injured tissue while MMP-9- is a zinc-dependent peptidase from the family of MMPs involved in tissue damage. Serum levels from both markers are increased in patients with active IBD and in a recent study it has been related to MH in UC patients. (23)

Regarding tissue biomarkers, several pro-inflammatory cytokines have been studied as predictor of long-term outcome and response to therapy (i.e. Interleukin (IL)-8 and IL-17 as predictor of anti-TNF treatment response in CD and UC, respectively or interferon (IFN)-γ in perianal fistulizing disease in CD as predictor of infliximab response, while other cytokines such as IL-12, and IL-6 are increased with mucosal inflammation but their role as predictor of treatment response is unknown. (24)

Mucosal cytokines profile in Ulcerative Colitis.

Cytokines play a pivotal role in inflammatory bowel diseases and their expression depends upon the specific setting and disease phase. The mucosal cytokine profile in patients with active UC is characterized by an increased mRNA expression of several cytokines as interferon gamma (IFN-g), IL-13, IL-17A, IL-1b, IL-6, TNF and IL-8. (25) Thus, UC seems to be associated with an increased expression of cytokines reflecting innate immune response such as TNF, IL-1b, IL-6 and IL-8.

In the last years an increasing number of transcriptomic studies improved the characterization of the cytokine microenvironment inside the UC and CD inflamed mucosa. Some of these cytokines are involved in IBD pathogenesis but they are not regulated locally in inflamed tissue. (26).

Detailed cytokine pathways in inflamed and not inflamed mucosa were assessed in a recent Italian study together with their association with biochemical, endoscopic and histologic activity, and their correlation with pharmacologic therapy. IL-1Ra, IL-6, IL-8, IL-17, induced Protein (IP)-10,

monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1a, MIP-1b resulted increased in UC inflamed vs not inflamed intestinal mucosa. No differences were found between treatment groups (conventional vs anti-TNF-a regimens) and according to CRP levels. Moreover IL-1Ra resulted increased in the group with the highest histological level of inflammation. (27)

Other studies on cytokine pathway focused on differences of expression in mucosal heling vs active UC. Another work from Norway research group, focused on healing mediators in the mucosa of UC in clinical remission, showed as among the mucosal transcripts examined, the were differences of regulation in genes in MH patients versus active patients and normal controls. Patients with clinical remission compared with normal mucosa had differences in the expression of 10 genes: 8 genes upregulated pro-inflammatory transcripts (IL1B, IL33, TNF, TRAF1, CLDN2, STAT1, STAT3 and IL13Ra2) and 2 downregulated (pro-inflammatory TBX21 and anti-inflammatory TGFB1). Differences were found also in patients with Mayo endoscopic score of 1 in comparison to 0. Moreover, up-regulation of JAK-STAT pathway was present not only in active inflammation but also in patients in clinical remission (its role in non-inflamed UC mucosa is partially known). (28) These differences between clinical and endoscopic remission and between endoscopic Mayo score 1 vs 0 suggested that the panel of cytokine expressed could be used as a predictor of long-term outcome in particular in the setting of deep remission.

A better knowledge of cytokine expression profile could be helpful to better understand treatment response mechanism. One of the most investigated settings is that of patients treated with anti-TNF-α.

TNF-α is a pro-inflammatory cytokine produced mainly by Th1-pathway cells and is known to activate pro-inflammatory cytokines including IL-1b and IL-6. Its role as mediator of inflammation in IBD has been extensively studied. It has been demonstrated a down-regulation of mucosal TNF and IFN-g mRNA expression in UC patients treated with infliximab and that genes expression differs in patients that are responders or not responders to treatment. (29-30) It is also demonstrated that

patients with an anti-TNF treatment failure showed a more severe pro-inflammatory cytokine profile before the start of anti-TNF treatment (i.e IL-1b, IL-17A, IL-6 and IFN-g are less expressed in responders compared to non-responders as well as IFN-g and IL-12p70 were increased in non-responders). (31) These observations suggested a potentially use of mucosal cytokine profile as biomarkers predicting treatment response.

The target of mucosal-TNF-α as biomarker of clinical outcome and response to therapy has been poorly investigated. Studies from a Norwegian research group showed in a cohort of 59 UC patients with moderate to severe disease that TNF-α expression was an independent predictive factor of clinical and endoscopic remission after treatment with infliximab ($p = 0.01$ and $p = 0.003$, OR: 2.5 and 4.8, respectively) (32). Most recently they showed also that the normalization of expression levels of m-TNF-α in patients who stopped treatment (infliximab) after endoscopic remission, predicted long term (>12 months) remission. (33)

Aim of the present study.

- To assess whether mucosal TNF-alpha levels are able to predict treatment response with anti TNF-alpha.
- To create a threshold level to detect inflammation according to endoscopic disease activity.

MATHERIALS AND METHODS.

The project.

This prospective study started in September 2019 as part of a longitudinal project "*Advanced study in IBD"* the ASIB study, carried out since 2014 by the research group of Gastroenterology and Nutrition from University Hospital of North-Norway (Tromsø, Norway). The project started in 2014 in Norway with the aim to determine TNF-α levels in colonic mucosa of patients with Inflammatory Bowel Disease in well-defined clinical situations (e.g.: untreated at debut of disease, in clinical and endoscopic remission following treatment, resistant to biologic treatment) in order to assess if m-

TNF-α values are related to treatment response and/or maintenance of remission after treatment withdrawal. In September 2019 the project started in Messina (AOU Policlinico – Gastroenterology Unit) with the evaluation of m-TNF-α levels in a local cohort of patients to assess its validity as biomarker of inflammation and predictor of treatment response.

Patients.

From June 2019 to June 2021 we started to prospectively collect samples from our IBD patients cohort (Gastroenterology Unit – AOU Policlinico G. Martino, Messina). All patients followed in our Unit with an endoscopic and histological diagnosis of Ulcerative Colitis who underwent endoscopic evaluation were included in the study. Together with the patients' cohort we collected also data from a control cohort with expected absence of colonic inflammation, in order to find a threshold level for detecting inflammation.

Inclusion criteria for IBD patients.

- All patients with suspected UC or recent diagnosis of UC naïve to biological / IMM therapy.
- All patients with control colonoscopy at one year from the start of biological therapy and during follow-up for re-evaluation / exacerbation.
- Signed consent before enrollment.
- Outpatient or inpatient, men and women aged> 18 years

Inclusion criteria for controls.

- Patients who underwent colonoscopy for screening purposes with proven absence of inflammation both at endoscopy and histology.
- Signed consent before enrollment.
- Outpatient or inpatient, men and women aged> 18 years

Exclusion criteria for IBD patients.

- Colectomized or patients with ileal pouch-anal anastomosis (IPAA)
- Any condition that prevents the patient from giving consent

Exclusion criteria for controls.

- Any endoscopic or histological finding of inflammation
- Irritable bowel syndrome (IBS) with diarrhoea as main symptom or chronic diarrhea or abdominal pain
- Any condition that prevents the patient from giving consent

Data collection and outcomes measures.

The following data were collected in a structured database for each patient: gender, age at diagnosis and age at start of therapy, smoking status (never, ex, or active smoker), disease duration from diagnosis to endoscopy, previous biological treatment and treatment for IBD at the time of evaluation. Regarding disease extent, patients were classified in accordance with the Montreal classification. Duration of biological treatment was also recorded.

Patients was evaluated at the enrolment (at the time of colonoscopy) and after 3 and 6 months after endoscopy. Treatment response was evaluated according to current clinical practice with clinical and biochemical evaluation.

Primary outcome: to correlate tissue level of TNF-α to treatment response with anti-TNF-alpha and to maintenance of remission in those patients with mucosal healing.

Secondary outcomes: to correlate tissue level of TNF-α to clinical and endoscopic inflammation and to find a threshold level for detecting inflammation in tissue biopsies.

Clinical evaluation of disease activity was performed with the 9-point partial Mayo score.

We defined as clinical response a reduction of pMS ≥ 3 points compared with baseline.

Concerning treatment failure, we defined primary failure (PF) as persistence of symptoms and serologic evidence of inflammation at the end of the induction period, and loss of response (LOR) as worsening of symptoms and serologic evidence of inflammation after an initial response.

Clinical remission was defined as partial Mayo score ≤ 1 without additional steroids and normalized biochemistry and MH defined as Mayo endoscopic subscore < 1.

The presence of inflammation was evaluated with endoscopic findings (according to endoscopic Mayo score), with clinical disease activity together with biochemical markers (i.e CRP) and with histological evaluation. For histological evaluation, samples will be fixed and embedded in paraffin and will be evaluated by expert pathologists.

Endoscopic biopsy sampling and storage.

All samples were taken in UC patients as a part of routine examinations and part of our diagnostic workup. Colonoscopy was performed up to the terminal ileum, in all the explored sections, at least two biopsy samples were taken for each segment of the colon according to guidelines. In patients with new UC diagnosis or with endoscopic disease activity, biopsies for dosage of m-TNF-α were taken from the most inflamed site. In those patients with mucosal healing, biopsies for dosage of m-TNF-α were taken from the previously most inflamed site.

All endoscopic diagnoses were confirmed by histological examination at our Pathology Unit. For control patients a single biopsy was taken from normal mucosa of sigmoid colon.

Biopsies were immediately collected in RNA-later (Thermofisher Scientific, Waltham, Massachusetts, USA) and stored at 4 °C overnight before they were stored at -70 °C until analysis.

RNA extraction and cDNA synthesis.

Biopsies were homogenized using a Tissue Ruptor Disposable Probe, (Qiagen, Hilden, Germany). We performed RNA extraction with RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA concentration was measured at 260 nm using a Nanodrop RNA quantification (Thermofisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions. Then 1 μg of total RNA was reverse transcribed using QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) in 20 μL reaction mix, according to the manufacturer's instructions.

Mucosal TNF determination with real-time PCR.

Realtime PCR was carried out on BioRad CFX 96 (BioRad, Hercules, California, USA) using the NovaPrima TNF qPCR kit (NovaTec Immunodiagnostica, Dietzenbach, Germany) under the following conditions: 95˚C for 3 min, 95˚C for 0:10 min and then treated at 60˚C for 0:30 min. The reaction procedure was repeated for 44 cycles. For each reaction 2 μL of cDNA were used and the samples were run in duplicate. Absolute quantification of the samples was performed by the supplied standard curve with correction for housekeeping gene expression RNA polymerase subunit II A (POLR2A). Housekeeping gene correction was made at cycle threshold (CT) level based on plate geometric mean for POLR2A.

Mucosal TNF-α concentrations were expressed as copies/μg total RNA.

Statistical analysis.

Statistical analysis was carried out using SSPS version 22.0 software for Windows. Categorical variables were summarized using absolute frequencies and percentages. Descriptive statistics included the calculation of mean values with standard deviation (SD) or median with their range or IQR, for all continuous variables. Mann-Whitney test and Kruskal-Wallis test were used to compare patients and controls.

When comparing m-TNF-α values of the three groups (active disease, inactive disease and controls), the Bonferroni correction was applied and a p value <0.017 was considered statistically significant.

Spearman's correlation was used to correlate m-TNF-α values and outcome during follow-up.

P-values < 0.05 were considered statistically significant.

Ethical consideration.

The study was approved by the local Ethics Committee with protocol n. 81/2018

RESULTS.

Patients and descriptive data.

Sample from 130 patients were prospectively collected from June 2019 to June 2021. Seventy-five samples were excluded from analysis during laboratory procedures. Features of patients excluded for technical reasons were similar to those of patients included [respectively: male 45% vs 50% (p=0.77); MH 33% vs 28% (p=0.66); active disease 67% vs 73% (p=0.52), patients on anti-TNFs 17% vs 28% (p=0.29)] and the final sample was representative of the population initially enrolled.

A detailed flow-chart of the enrolled patients is showed in **Figure 1**.

Forty patients with ulcerative colitis and 15 normal controls were included in the final analysis. Patients' baseline characteristics are summarized in **Table 1**. UC patients' mean age (SD) at the time of endoscopy was 44 (14.6) years; 20 (50%) of them were males. Mean (SD) duration of disease from diagnosis to the endoscopy was 11.5 (9.6) years. Sixteen patients were on biologic at baseline (11 patients with anti-TNF-α and 5 patients with Vedolizumab). Eleven patients were treatment naïve and out of these, 5 patients started after endoscopy an anti-TNF-α treatment.

At endoscopy 29 patients (73%) had an active disease with an endoscopic Mayo score ≥ 1 while 11 patients showed mucosal healing. Most patients with active disease had a moderate disease activity (endoscopic Mayo score 2) as shown in **Table 1**.

Concerning controls, only demographic data were collected since no bowel diseases were present at the time of endoscopy. The mean age (SD) was 59 (12.9) years and 8 (53%) patients were male.

Enrolled patients were followed after endoscopy for a mean time (SD) of 7 (4) months.

A detailed flow-chart of the patients according with treatment group, endoscopic finding and clinical outcome at 6 months is showed in **Figure 2.** All patients with mucosal healing at endoscopy, maintained remission during follow-up and treatment was continued.

M-TNF-α measurement: biomarker of inflammation.

Patients with UC had a significantly higher m-TNF-α value (mean ± SD) than controls (3373 copies/µg ± 4362 vs 1593 copies/µg ± 956; p= 0.033).

Based on endoscopic disease activity we divided UC patients into "inactive" if they showed a mucosal healing or "active" that included an endoscopic Mayo score ≥ 1. We showed that patients with active disease had higher m-TNF-α values compared with both inactive patients and controls (**Figure 3**): m-TNF-α levels mean (± SD) of inactive, active and controls were 1199 copies/µg ± 938 copies/µg ± 4861 and 1113 copies/µg ± 638 respectively (p= 0.006). No difference was found between inactive patients and controls (p=0.815). Moreover m-TNF-α was significantly related also to endoscopic Mayo score (p=0.007, rs=0.3) (**Figure 4**), while no correlation was found with partial Mayo score (p= 0.662).

According to conventional treatment at baseline we found that mean m-TNF-α values were similar among patients with or without steroids, IMMs, mesalamine and biologics (**Table 2**). Although not statistically significant we observed that the lowest m-TNF-α values were those of patients on biological treatment.

M-TNF-α measurement in patients with MH and outcome.

We found that patients in clinical remission at baseline and with an endoscopic Mayo score ≤1, that maintained remission during the first 6 months of follow-up were 15. As shown in **Figure 5** the maintenance of remission correlates with lower m-TNF-α levels (p=0.026).

We analysed also the 7 patients undergoing anti-TNF treatment: median m-TNF-α value was higher in those patients with a treatment failure after 6 months [3791 (1101 – 3795) copies/µg vs 2482 (1401,75 – 3962,25) copies/µg; p=0.85]. However due to the small sample size these results are not statistically significant.

Finally, although the small sample size we found that according to a threshold of 2084 copies/µg, m-TNF-α achieved for detecting inflammation a sensitivity of 47.5% (95%CI: 31.50 – 63.87%) and a specificity of 100% (95%CI: 78,20 – 100,00%) and an overall diagnostic accuracy of 61.82% (95%CI: 47.73 - 54.79%). The AUROC value is 0.688 (95% CI: 0.551-0.826; p=0.033) **(Figure 6)**. The threshold of 2084 copies/µg of m-TNF-α achieved a positive predictive value (PPV) of 100%.

DISCUSSION.

In the present study we assessed the reliability and feasibility of m-TNF-α measurement in patients with UC. This project was aimed to optimize biological treatment by guiding clinical decision, in particular in those patients who reached MH and in which the decision to stop or maintain treatment is a challenge. We measured, for the first time in an Italian population, expression levels of mucosal TNF-α with a new diagnostic kit, the NovaPrime TNF kit. A Norwegian research group has been conducting studies on the measurement of m-TNF-α for several years based on an in-house m-TNF-α PCR mucosal method (34) both in CD (35) and UC. The study by Olsen et al (34) aimed to measure m-TNF-α levels in a cohort of untreated UC patients. They found that tissue m-RNA levels of TNF-α in UC patients were 3.4 times higher than that in healthy controls (44 UC patients vs 28 controls).

We showed in the present study a similar difference, although less pronounced, with doubled m-TNF-α levels in UC (active and inactive) patients compared with controls (3373 copies/µg ± 4362 vs 1593 copies/µg ± 956; p= 0.033). This difference was more pronounced when considering patients with active disease (inflamed) versus patients with no inflammation (inactive disease and controls). When comparing severity of endoscopic inflammation in UC patients, we found a significant positive correlation between m-TNF-α levels and severity of inflammation (p=0.007), similar results were achieved by the Norwegian study with the difference that endoscopic disease activity was assessed with UCDAI. Conversely, they found a positive correlation also with clinical score of inflammation. In the present study, we showed much lower mean levels of m-TNF-α than in the Norwegian study. This difference could be explained by differences in enrolled patients (untreated patients vs treated patients in our study) or may be due to genetic differences.

In order to explore a possible role of m-TNF-α as biomarkers predicting outcome, patients in remission at baseline or with mild endoscopic disease (Mayo score =1) but in clinical remission were followed for 6 months (due to the small sample size we could not stratify the patients by drug class). We found that maintenance of remission was related to lower m-TNF-α levels (p=0.026). A more recent paper by Olsen et al (2016) (33) aimed to assess expression levels of m-TNF-α in a group of UC patients treated with an intensified induction therapy with infliximab (0,2,6 week of induction followed by maintenance treatment every 4 weeks until endoscopic remission). After 2-6 weeks from the last infusion a colonoscopy was performed and, in patients who achieved MH, biological therapy was discontinued. They showed with an in-house method for TNF measurement (<7500 copies/µg RNA as cut-off for normal level) that normalization of m-TNF-α gene expression predicted a median relapse-free survival of 20 months after withdrawal of IFX compared to a median relapse-free survival of 5 months in the group with elevated m-TNF-α expression. However, it is difficult to compare our results with those of this latter paper due to the different treatment populations and to the different m-TNF-α cut-off values. It is interesting to note as our cut-off level for inflamed patients is much lower compared with those of the Norwegian cohort (2084 copies/µg RNA vs 7500 copies/µg

RNA). This could be partially explained by differences in methodology and laboratory procedure (different kit for TNF quantification). Another possible explanation is a geographic difference in gene expression that should be further investigated.

There are also other limitations of this study. First the small sample size that limited our analysis without a treatment stratification. A stratification according different treatments (in particular anti-TNF-α patients) should be investigated and TNF-α values should be evaluated before and after treatment with a sufficient follow-up period. Furthermore, an increase in the sample of the control group will allow to get a more precise estimate of the local reference cut-off. Correlation of m-TNF-α with inflammation was performed only on the basis of endoscopic activity, histological correlation was not performed due to a non-homogeneous reporting of the grade of inflammation. Finally, the high number of patients excluded from analysis due to laboratory procedures suggests the need to improve the technique.

Conclusions.

Levels of m-TNFα were increased in UC patients with active disease compared to normal controls and patients in remission. Furthermore, in patients with inactive disease, lower m-TNF-α values were associated with maintenance of remission over the following 6 months. Similar results, although not statistically significant was reached in patients on anti-TNF treatment. These results suggested a possible use of this biomarker in predicting treatment response. Studies with a longer follow-up and with a larger sample size are needed to confirm this hypothesis and to better define a threshold value for detecting inflammation.

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TABLES

Baseline characteristics	**Cases** N= 40	**Controls** N = 15
Age at endoscopy; *Mean(SD)*	44 (14.6)	59 (12.9)
Gender-male; *n(%)*	20 (50)	8 (53)
Time from diagnosis to endoscopy; years *Mean(SD)*	11.5(9.6)	
Endoscopic activity; n (%) *Active* *MH*	29 (73) 11(28)	
Disease location*; n (%) E1 E2 E3	4 (9) 23 (58) 13 (33)	
Clinical activity; n(%) *Remission* *Active*	23 (58) 17 (43)	
Endoscopic Mayo score; n(%) *Mayo 0;* *Mayo 1 ;* *Mayo 2;* *Mayo 3;*	10(26) 7(18) 18(46) 4(10)	
Biologics; n (%) *Anti-TNFα* *Vedolizumab*	11(28) 5(13)	
Conventional Treatment; n(%) 5-ASA Steroids IMM	33(82) 10(25) 6(15)	
Treatment naïve; n (%)	11(27.5)	

Table 1. Baseline patient's characteristics. SD= standard deviation; IMM= immunomodulators. Data are expressed as numbers (percentages) or mean with SD. *Disease location is given according to Montreal Classification

Treatment	N (%)	m-TNF-α median (Q1-Q3)	P
IMMs			
Yes	6 (15)	2191,5 (436-4538)	0,936
No	32 (85)	1891 (1080-4214,7)	
Steroids			
Yes	10 (26)	1662,5 (918,5- 6926)	0.8995
No	28 (74)	1891 (1006,25- 3385,5)	
5-ASA			
Yes	31 (82)	2084 (984 - 6926)	0.332
No	7 (18)	2084 (1073-2781)	
All biologics			
Yes	16 (41)	1242,5 (322,25 - 4509,75)	0,778
No	23 (59)	2370 (1101- 3791)	
Anti-TNFs			
Yes	11 (28)	1222 (306 - 4973)	0.574
No	28 (72)	2277 (1080 - 3711,75)	

Table 2. m-TNF-α values according to treatments. Data are expressed as median with their IQR.

IMMs= immunomodulators.

FIGURES

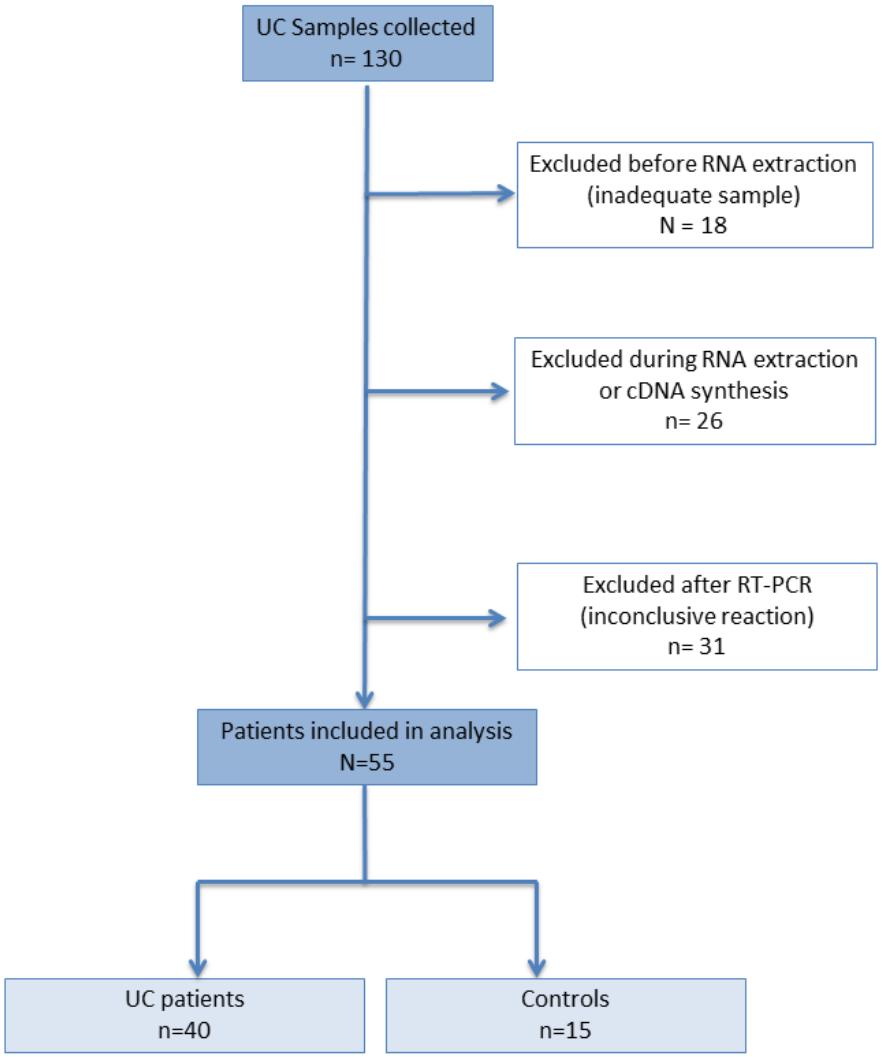

Figure 1. Flow-chart of the enrolled patients.

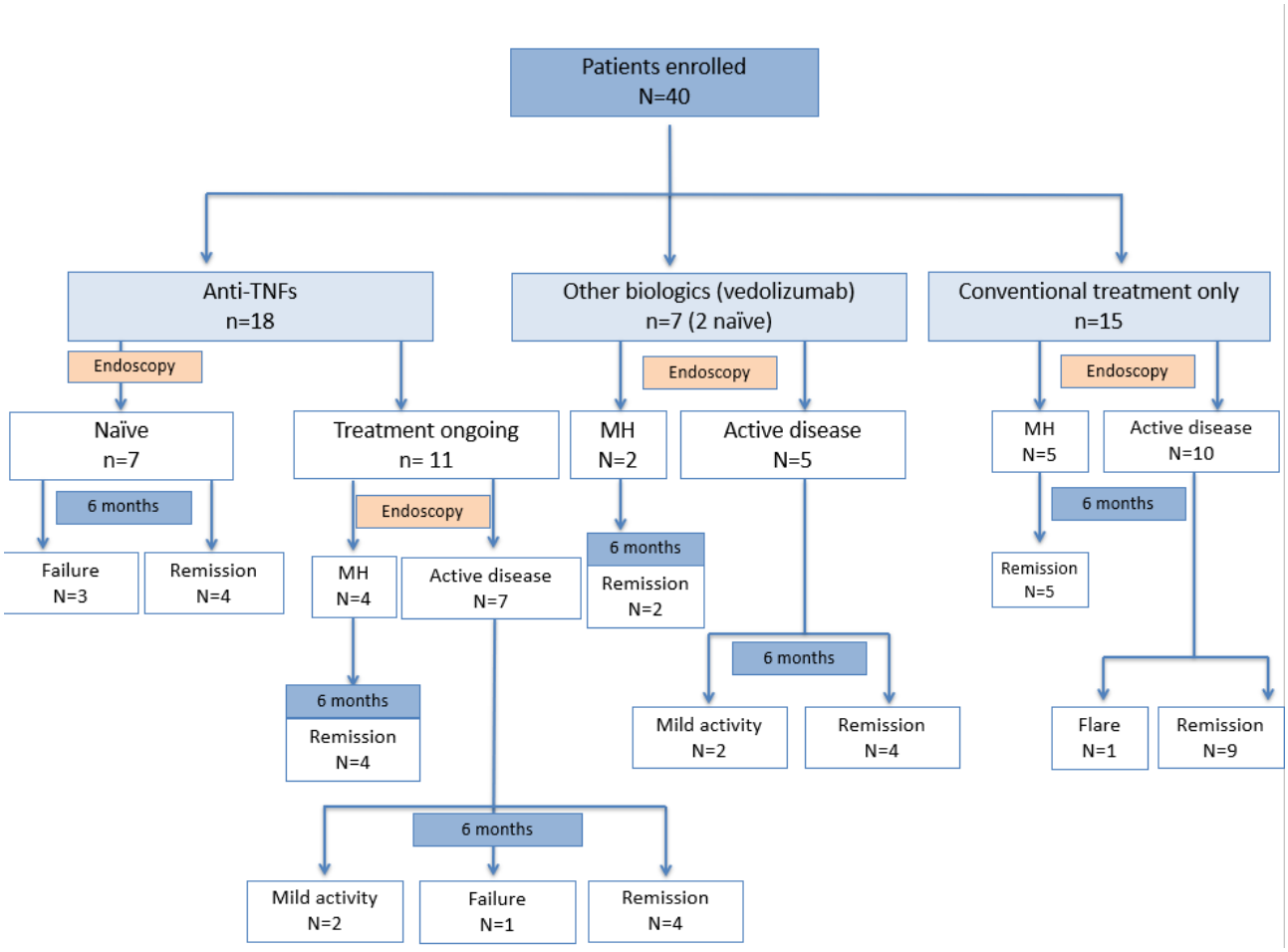

Figure 2. Flow-chart of the enrolled patients according with treatment group, endoscopic finding and clinical outcome at 6 months. MH= mucosal healing.

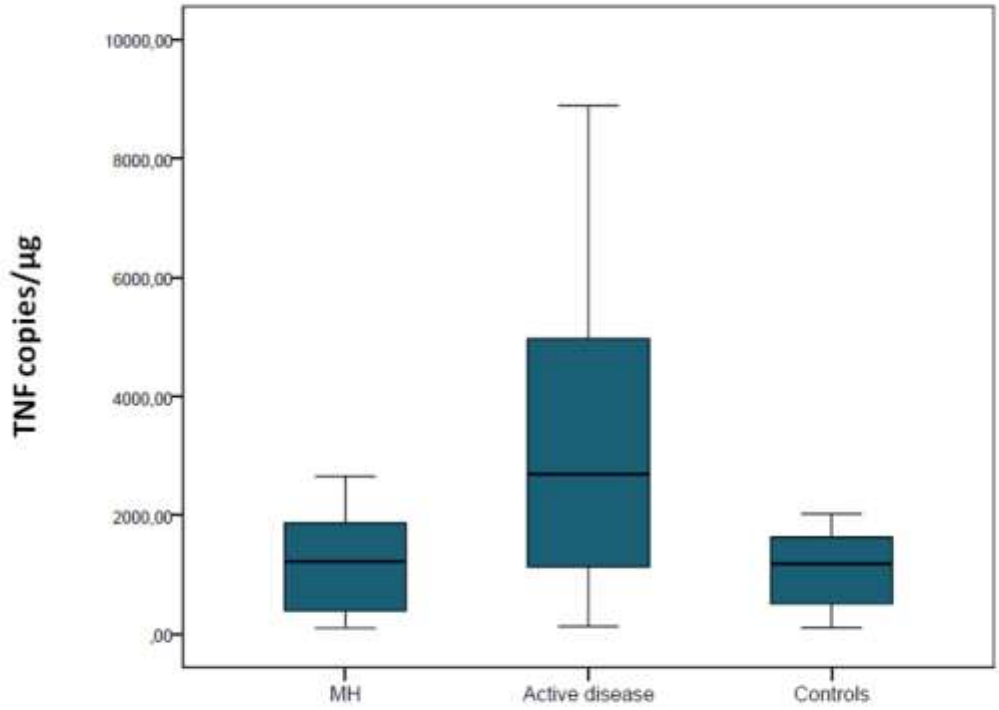

Figure 3. Comparison between the 3 patient groups according to endoscopic activity. Data are expressed as copies/µg of RNA and mean values with their standard deviation (SD) are represented in figure.

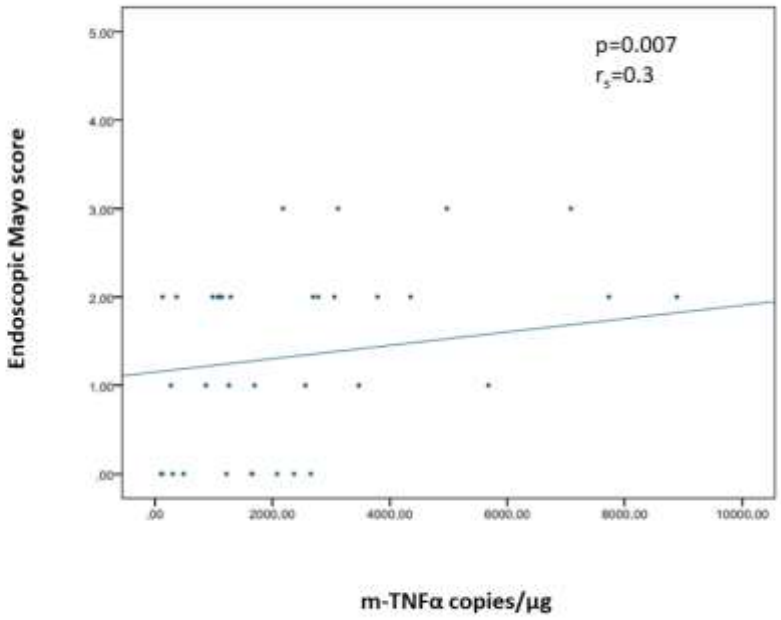

Figure 4. Relationship between m-TNF-a levels and endoscopic score of inflammation (Mayo score) in ulcerative colitis (UC). Correlation coefficient was calculated with the Spearman rank correlation test.

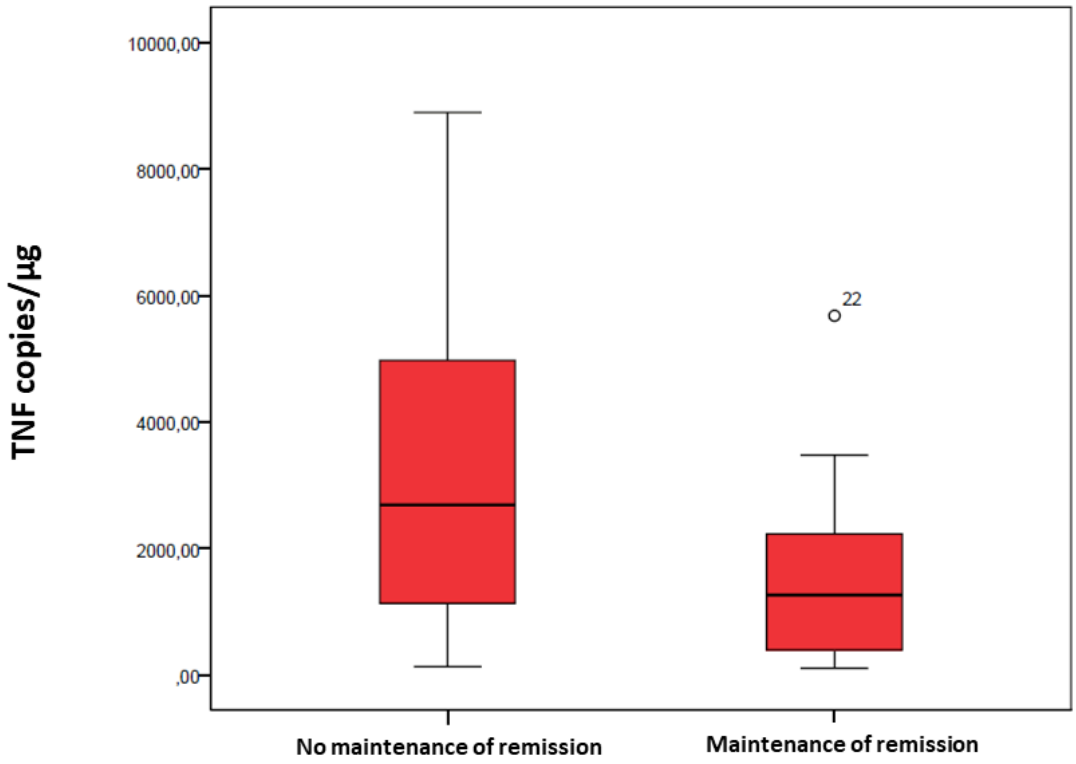

Figure 5. Maintenance of remission during follow-up related to m-TNF-α values. Correlation coefficient was calculated with the Spearman rank correlation test.

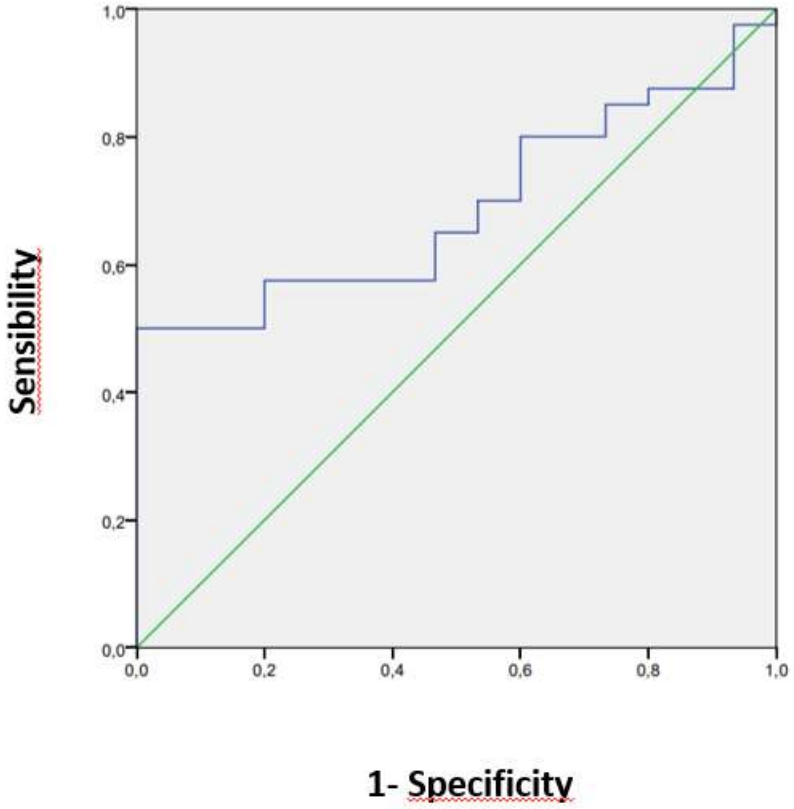

Figure 6. Receiver operating characteristic (ROC) curve for the assessment of m-TNF-α threshold for detecting inflammation. The AUROC value is 0.688 (95% CI: 0.551-0.826; p=0.033)