

Serum trefoil factor 3 predicts disease activity in patients with ulcerative colitis

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Abstract. – OBJECTIVE: In this study, we aimed to evaluate the role of serum trefoil factor 3 (TFF3) as a biomarker of disease activity in patients with inflammatory bowel disease (IBD) and to compare TFF3 values with those of fecal calprotectin (FC).

PATIENTS AND METHODS: 128 patients with IBD were divided into four groups: 1) active ulcerative colitis (UC); 2) quiescent UC; 3) active Crohn's disease (CD); 4) quiescent CD. The serum levels of TFF3 and FC levels were assessed in all patients and 16 controls.

RESULTS: Patients with active UC had higher TFF3 levels than those with quiescent UC ($p < 0.001$), those with active ($p < 0.001$) or quiescent CD ($p < 0.001$) and controls ($p < 0.001$). We found a correlation between TFF3 and FC values in patients with active ($r = 0.478$, $p = 0.006$) and quiescent UC ($r = 0.528$, $p = 0.002$). TFF3 levels correlated with endoscopic activity in UC (evaluated by UC Endoscopic Index of Severity - UCEIS) ($r = 0.662$, $p < 0.001$).

CONCLUSIONS: Serum TFF3 is able to identify patients with active UC. It could be used as a marker to predict disease activity in patients with UC.

Key Words:

Crohn's disease, Inflammatory bowel disease, Biomarkers, Fecal calprotectin, UCEIS.

Introduction

Inflammatory bowel disease (IBD), which includes both Crohn's disease (CD) and ulcerative colitis (UC), is a chronic idiopathic inflammatory disorder affecting the gastrointestinal tract. The current understanding of the etiopathogenesis of IBD includes altered epithelial barrier function and a dysregulated immune response in genetically susceptible individuals as a consequence of a multi-

faceted interaction among environmental factors, the commensal gut microbiota and the colonic immune system or an impaired mucosal barrier¹. Alterations in the intestinal mucous components may impair the barrier function of the mucin layer and may be a contributing factor to IBD². In IBD, the mucin types and expression are affected by several factors, including the number of mucin-producing goblet cells, which are reduced in active disease and may alter the thickness and composition of the mucous gel layer³. An increasing body of evidence supports the involvement of trefoil peptides in mucosal surface protection and repair after injury. Trefoil factors (TFFs) include a family of three mucin-associated peptides (TFF1, TFF2 and TFF3) that are widely expressed in a tissue-specific manner in the gastrointestinal tract⁴. TFFs are expressed in several tissues that contain mucus-secreting cells, but they are most markedly expressed in the gastrointestinal tract. TFF3 was identified in 1991 as a rat cDNA sequence⁵ and as a human cDNA sequence in 1993⁶. TFF3 was initially known as the intestinal trefoil factor. TFF3 is predominantly secreted by goblet cells of the small and large intestine⁴ and protects the gastrointestinal mucosa from a variety of insults⁷. It is co-produced and secreted with mucin (MUC) 2⁸. Several studies^{9,10} have shown the protective function of all three TFFs in the gastrointestinal tract and their up-regulated expression at the site of mucosal damage. However, the role of TFF3 in IBD has not yet been clarified. To date, endoscopic evaluation is the most accurate way to assess disease activity in IBD¹¹. The location, extent and severity of IBD can be established with this procedure but its use is prevented by several drawbacks, as it is invasive, burdensome to patients, time-consuming, and expensive. Moreover, a reliable assessment of mucosal lesions can be hardly performed in clinical practice. So, the iden-

tification of alternative noninvasive biomarkers to accurately detect inflammation and monitor disease activity is advocated. Fecal calprotectin (FC) is a small calcium and zinc-binding protein which is found in abundance in neutrophilic granulocytes as well as in monocytes and macrophages¹². FC is a pioneer biomarker for intestinal inflammation frequently used for the assessment of mucosal activity in IBD patients¹³. Quantitative measurement of TFFs could be an important tool for elucidating the biological functions of the peptides and exploring their role as biomarkers for IBD. However, a few studies have investigated the clinical potential of TFF 3 in IBD. The aim of the current work was to evaluate the role of TFF3 as a biomarker of disease activity in patients with IBD and to compare TFF3 values with those of FC.

Patients and Methods

Study Design, Inclusion and Exclusion Criteria of Enrolled Subjects

This is a prospective observational study. We recruited patients referred to the IBD outpatient clinic of the “Tsaritsa Yoanna” University Hospital in Sofia between October 2015 and November 2016. We included patients who responded to all inclusion and exclusion criteria. Inclusion criteria were: (1) age: 18-85 years, (2) known UC or CD diagnosed according to the ECCO Guidelines^{14,15} (3) completion of written informed consent. Exclusion criteria were: (1) colorectal cancer or colon polyps, (2) indeterminate colitis, (3) history of colorectal surgery, (4) urinary incontinence (due to the risk of contamination of fecal samples), (5) pregnancy, (6) history of active non-steroidal anti-inflammatory drugs (NSAID) intake (2 tablets/week), (7) oral steroids or steroid enemas intake in the last 3 months, or initiation of azathioprine treatment within the last 3 months, (8) infectious colitis and (9) primary immunodeficiency. We defined clinical remission in UC as a Lichtiger Clinical Activity Index of 3 points or less¹⁶ and endoscopic remission - as Ulcerative Colitis Endoscopic Index of Severity (UCEIS) of 0 or 1 if the descriptor was limited to vascular pattern¹⁷. Clinical remission in CD was determined as Crohn’s disease activity index (CDAI) <150, and endoscopic remission – as lack of mucosal lesions (erosions, ulcers, aphthous lesions) on ileocolonoscopy. All the enrolled patients underwent a full medical assessment including a detailed medical history and physical examination. All therapies

taken by the patients before enrollment were recorded. All patients underwent colonoscopy within two weeks from the visit. Serum and fecal samples were collected within 1-2 days before colonoscopy. The serum levels of TFF3 and FC levels were assessed in all the patients and in 16 subjects, taken as a control group.

Enzyme Immunoassay

The serum levels of TFF3 were measured by ELISA (Quantikine ELISA, Human TFF3 Immunoassay, R&D Systems, Minneapolis, MN, USA). We strictly followed the manufacturer’s instructions.

Fecal Calprotectin

Calprotectin was analyzed in stool samples by means of point-of-care desk-top Quantum Blue Reader® (POC Reader) method. It is a lateral flow technology based on ELISA techniques. We performed the test according to the manufacturer’s instructions (Quantum Blue® Calprotectin, Bühlmann Laboratories AG, Switzerland). The POC device uses internal standards within a range of 30-300 µg/g and sensitivity of <10 µg/g, guaranteeing consistency in results. When we received the results > 300 µg/g, we performed additional 1:10 dilution with extraction buffer according to the manufacturer’s instructions, allowing us to receive FC levels up to 3000 µg/g. FC values above the upper limit of the measurement ranges were registered as 3000 µg/g and FC values below the lower limit were accordingly registered as 30 µg/g.

Statistical Analysis

The statistical analysis was performed using SPSS for Windows, Version 25.0. (SPSS Inc., Chicago, IL, USA). For data analysis the following statistical methods were used: descriptive statistics for tabular and graphical presentation of results, Kolmogorov-Smirnov test, Shapiro-Wilk test, Mann-Whitney test, Pearson correlation test and Spearman correlation test. The obtained results were assessed as statistically reliable in the threshold level of significance $p < 0.05$.

Ethics Approval

The study was approved by the Ethics Committee of the “Tsaritsa Yoanna” University Hospital in Sofia, Bulgaria. Before initiating, written informed consent was obtained from all patients and healthy controls included in the study. The study protocol conforms to the Ethical guidelines of the 1975 Declaration of Helsinki (6th revision,

Table I. Patients' demographics.

	Crohn's disease	Ulcerative colitis
No. of patients	64	64
Females	30 (46.9%)	26 (40.6%)
Age	40.2 ± 9 (19-60)	39.4 ± 9 (18-63)
Disease duration, mean ± SD (years)	4.78 ± 3.8	4.2 ± 3.5
Current smoking	18 (28.12%)	11 (17.19%)
Location of disease	L1 (ileal) – 7 (10.9%) L2 (colonic) – 24 (37.5%) L3 (ileocolonic) – 33 (51.6%)	E1 (proctitis) – 9 (14%) E2 (left-sided colitis) – 30 (46.9%) E3 (extensive colitis) – 25 (39.1%)
Disease phenotype	B1 (inflammatory) – 54 (84.4%) B2 (stricturing) – 6 (9.4%) B3 (penetrating) – 4 (6.2%)	-

2008) as reflected in a priori approval by the Institution's Human Research Committee.

Results

72 males and 56 females of 40 ± 9 (18-63) years were enrolled in this study. An overview of the demographic patient characteristics at baseline is provided in Table I. Sixty-four patients presented active IBD (32 with active UC and 32 with active CD) and 64 were with IBD in both clinical and endoscopic remission (32 with quiescent UC, 32 with quiescent CD).

Control Group

The mean level of TFF3 in the control group was 5.85 ng/ml (4.69-7.04), with a standard deviation

(SD) of 0.83 and median 5.82 (Table II). In the present study, we accepted an increased level above 1,96xSD + mean value (5.85), e.g. levels above 7.48 ng/ml. All of the controls were below this level.

TFF3 Levels in UC Patients

The levels of TFF3 in patients with UC are presented in Table II. The mean level of TFF3 in the group of patients with active UC (n=32) was 10.12 ng/ml which was markedly higher than the levels of TFF3 in the controls ($p < 0.0001$). UC patients in remission had mean values of TFF3 of 6.48 ng/ml (5.15-8.51), with a SD 0.93 and median 6.52 (Table II), with a trend toward significant difference from controls ($p = 0.059$). Patients with active UC had remarkably higher TFF3 levels than quiescent UC patients ($p < 0.001$) (Figure 1).

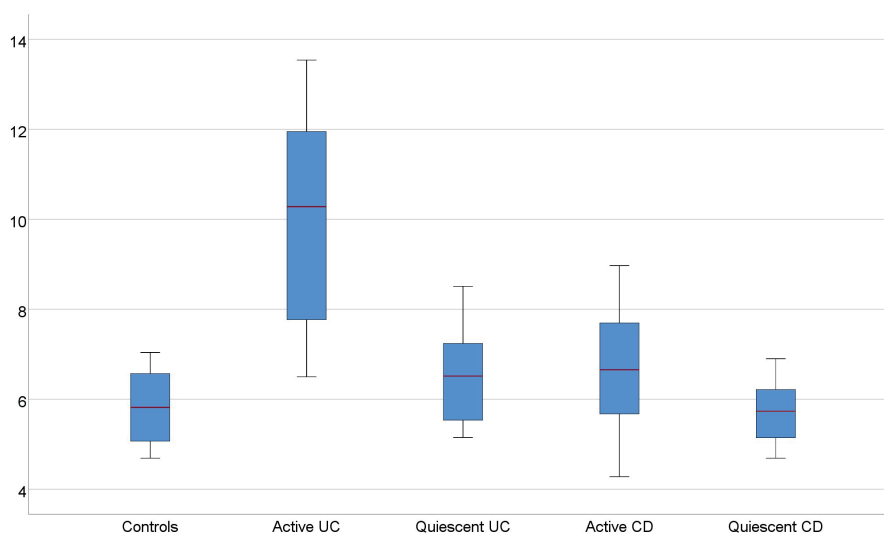


Figure 1. Boxplot of serum trefoil factor 3 concentrations in controls, patients with active ulcerative colitis (UC), patients with UC in remission, patients with active Crohn's disease (CD) and patients with CD in remission. Data are presented as box and whisker plots showing median (horizontal line), interquartile range (box) and range of measurements (whisker).

Table II. Serum levels of trefoil factor 3 (ng/ml) in patients with active ulcerative colitis (UC), quiescent UC, active Crohn’s disease (CD) and quiescent CD.

	No.	Mean	Median	SD	Min	Max
Control group	16	5.85	5.82	0.83	4.69	7.04
Patients with active UC	32	10.12	10.28	2.41	6.50	13.54
Patients with quiescent UC	32	6.48	6.52	0.93	5.15	8.51
Patients with active CD	32	6.67	6.66	1.38	4.28	8.97
Patients with quiescent CD	32	5.76	5.74	0.72	4.69	6.90

TFF3 Levels in CD Patients

Mean levels of TFF3 in patients with active CD (6.67 ng/ml) and quiescent CD (5.76 ng/ml) did not markedly differ from those of controls ($p=0.093$ and $p=0.720$, respectively - Figure 1). There was no significant difference in TFF3 levels between patients with active CD and those with quiescent CD ($p=0.522$).

Comparison of TFF3 Levels Between UC and CD Patients

Patients with active UC had significantly higher values of TFF3 compared with those with active CD ($p<0.001$) and patients with quiescent CD ($p<0.001$). The mean level of TFF3 in patients with UC in remission was remarkably hi-

gher compared to patients with CD in remission ($p=0.021$) (Figure 1).

Correlation Between TFF3, FC and Endoscopic Activity

We found a significant correlation between TFF3 and FC levels in patients with UC overall (Pearson correlation coefficient $r=0.473$, $p<0.001$) (Figure 2) and, separately, in both active UC ($r=0.478$, $p=0.006$) and quiescent UC patients ($r=0.528$, $p=0.002$) (Figure 3). We did not observe any marked correlation between the two parameters neither in patients with CD nor in controls. There was a significant correlation between TFF3 and UCEIS in UC patients (Spearman correlation coefficient $r=0.662$, $p<0.001$) (Figure 4). There

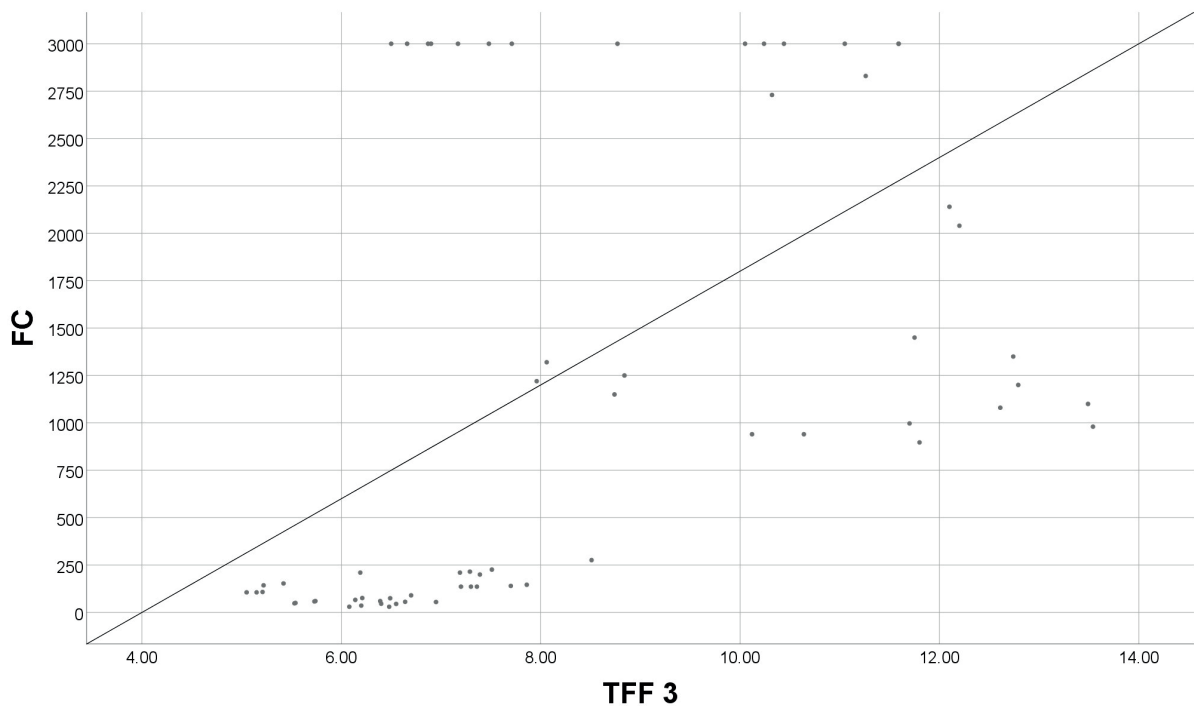


Figure 2. Correlation between trefoil factor 3 (TFF3) and fecal calprotectin (FC) values in patients with ulcerative colitis ($r = 0.473$, $p < 0.001$).

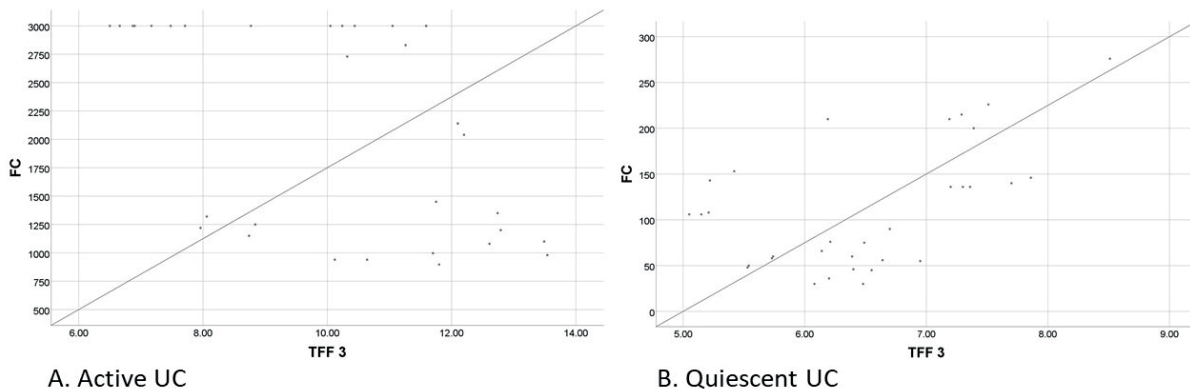


Figure 3. Correlation between trefoil factor 3 (TFF3) and fecal calprotectin (FC) values in patients with active ulcerative colitis (UC) ($r = 0.478, p=0.006$) **A**, and in patients with quiescent ulcerative colitis ($r = 0.528, p=0.002$) **B**.

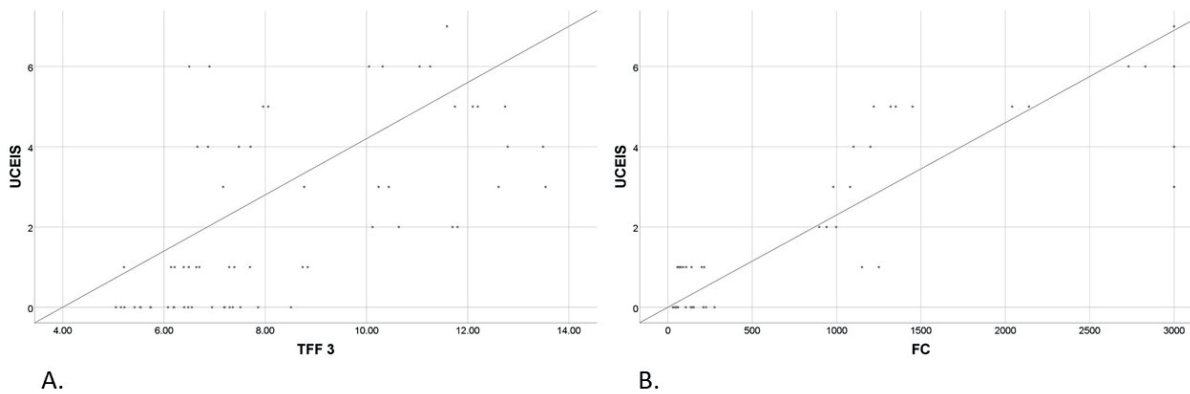


Figure 4. Correlation between Ulcerative Colitis Endoscopic Index of Severity (UCEIS) and trefoil factor 3 (TFF3) concentrations ($r = 0.662, p < 0.001$) **A**, and correlation between UCEIS and fecal calprotectin (FC) concentrations in ulcerative colitis patients ($r = 0.836, p < 0.001$) **B**.

was no statistical significance between the mucosal lesions seen during endoscopy in CD patients and TFF3 levels ($p=0,215$). FC concentrations correlated remarkably both with UCEIS ($r=0.836, p<0.001$) (Figure 4) in UC patients and with endoscopic activity in CD patients ($p<0.001$).

Discussion

Currently, endoscopy with intestinal biopsies is the most accurate way to evaluate the disease activity in IBD¹⁸. However, repetitive endoscopic examinations are invasive, expensive, time-consuming, and hardly accepted by the patients. Many non-invasive biomarkers to monitor disease activity have been investigated over the years, but the ideal one is still needed. As a factor that

plays a key role in maintaining the integrity of intestinal mucosa, TFF-3 could be considered as a potential candidate to fill this gap.

According to our best knowledge, our work is the first that aimed to investigate the clinical value of TFF3 as a biomarker of disease activity in patients with IBD. Overall, we found that TFF3 levels were able to predict disease activity in UC but not in CD, as suggested by several findings arisen from the present study. We noticed that the mean levels of TFF3 in active UC were markedly higher than those identified in patients with quiescent UC, which were similar to those of the control group. Furthermore, we found a significant correspondence between TFF3 levels and UCEIS. This finding suggests that TFF3 could be used to identify patients with quiescent UC and potentially those with mucosal healing. Srivastava et al¹⁹ showed that se-

rum TFF3 could point out patients with mucosal healing in a group of UC patients in clinical remission or with mild activity with reasonable sensitivity and specificity. Patients with active UC had significantly higher TFF3 levels than other groups, including those with active CD. This finding is not unexpected, because in active UC the mucous epithelium is always affected, the mucus barrier is impaired and the inflammation affects the mucosa and superficial submucosa²⁰. On the other hand in CD the mucus defects are segmental, with skip areas in the midst of diseased intestine and transmural inflammation is present²⁰. Probably for these reasons, although there are CD patients with increased TFF3 levels, the mean levels of TFF3 in patients with active CD was not significantly higher than in controls. All these data confirm the hypothesis that TFF3 levels are related to mucosal inflammation of the intestine and increase with the presence of mucosal damage. Although multiple cellular and animal studies have documented the crucial role of TFFs in the epithelial restitution of the gut, a few studies have been performed in patients with IBD to investigate the clinical potential of TFF in IBD. Vestergaard et al²¹ did not find any significant fluctuations of TFF3 in three patients with UC who underwent treatment with prednisolone with clinical improvement. Grønbaek et al²² found that serum TFF3 levels correlated with disease activity indices in patients with UC and they noticed a trend towards reduction in TFF3 levels with clinical improvement after therapy with steroids. Many non-invasive biomarkers have been investigated over the years, but still an ideal biomarker to detect and monitor disease activity in IBD is needed. To date, FC is the most sensitive and most extensively investigated non-invasive biomarker in IBD. Many studies described that FC concentrations correlate well with endoscopic activity in UC²³⁻²⁵. In the current work, FC showed excellent correspondence to mucosal changes assessed by UCEIS in UC patients which was better compared to the correlation observed between TFF3 and UCEIS.

In our study, a significant correlation between FC and TFF3 levels was observed in both patients with active and quiescent UC. On the contrary, there was no correlation observed in CD patients and controls. These results are not surprising because TFF3 should be considered a marker for mucosal damage that could occur as a result of an inflammatory process rather than a marker for inflammation itself. We showed that the mean TFF3 levels in patients with active UC are markedly higher than those of quiescent UC

and correlate well with FC in both patients with active and quiescent UC. Hence, TFF3 could be used to predict disease activity and differentiate between active and quiescent UC. Furthermore, TFF3 has the potential to be used as a marker for mucosal healing in patients with UC. Serial measurement of TFF3 would be more useful for monitoring clinical and endoscopic activity in these patients. The lack of correlation between TFF3 and FC values in CD patients, the lack of correspondence between TFF3 and mucosal changes in CD and the lack of statistical significance of TFF3 values between CD patients and controls put into question the role of TFF3 in CD. Up to the best of our knowledge, this is the first work that correlates TFF3 concentrations with those of FC in UC patients and among the first ones investigating TFF3 in CD patients. Recently, Eder et al²⁶ showed lack of correlation between FC and TFF3 in 10 CD patients and no correlation between TFF3 levels and Simple Endoscopic Score for Crohn's disease.

Conclusions

We found that serum human TFF3 is able to predict disease activity in patients with UC, and that it correlates well with FC levels and endoscopic activity, so it could be used as a non-invasive marker to predict disease activity in these patients. Furthermore, larger studies are needed to confirm the role of TFF3 as a non-invasive biomarker of disease activity in patients with UC.

Conflict of Interest

The authors received no financial support for the research, authorship, and/or publication of this article. The Authors declare that they have no conflict of interests.

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