Original Research Article

Recent Fecal Biomarker for Inflammatory Bowel Disease, Neutrophil Gelatinase Associated Lipocalin

Abstract

Background: In the field of IBD many biomarkers have been studied to find the ideal noninvasive biomarker that best correlates with disease severity and prognosis.

NGAL is a biomarker that have been studied and measured either in serum or fecal in different pathological conditions such as AKI, IBD and autoimmune disorders.

In our study we aim to evaluate the accuracy of fecal NGAL levels in correlation with endoscopic scoring, and common serum inflammatory markers in patients with IBD.

Methods: In this randomized prospective controlled study Fecal NGAL levels were measured using the Elisa technique in 30 patients with ulcerative colitis 10 patients with Crohn's disease and 20 healthy controls. The results were correlated to colonoscopic severity index and commonly used serum inflammatory markers (highly sensitive CRP, ESR, white blood cell count).

Results: Fecal NGAL levels (median and interquartile range) were significantly elevated in patients with active ulcerative colitis (UC) 6.05 (3.6–15.1) mg/kg and Crohn's disease (CD) 4.9 (1.5– 7.7) mg/kg, compared to healthy controls (HC) 0.3 (0.1–0.4) mg/kg. Sensitivity and specificity were 94.7% and 95.7%, respectively, for distinguishing between active IBD and HC.

Conclusions: Fecal NGAL is a noninvasive biomarker that strongly correlate to different parameters of the disease activity in IBD patients. Because most biomarkers are only found in granulocytes, NGAL's epithelial localization could provide additional diagnostic information.

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Keywords: Fecal Biomarker, Inflammatory Bowel Disease, Neutrophil Gelatinase Associated Lipocalin.

Introduction

Inflammatory bowel disease (IBD) is a group of autoimmune disease with gastrointestinal and extra gastrointestinal manifestation with different disease activity and clinical presentations [1].

Colonoscopy remains the golden standard for diagnosis and follow up but in some conditions like Crohn's Disease of small intestine, endoscopic follow up remains difficult. Additionally, colonoscopy is an invasive procedure, which is not always well tolerated by the patients [2].

The non-invasive and cost-effective serologic tests are usually used to assess the degree of inflammation and to track the activity of the disease. Because no single marker proved to be effective in measuring the activity of the disease, rather the combination of biomarkers may improve the accuracy in diagnosing and monitoring IBD [3].

Fecal calprotectin is a well-established IBD biomarker, proven to have a good correlation with inflammation activity in IBD and is well-known in clinical practice. However, the variability of the biomarker sensitivity and specificity has been reported in several meta-analyses.

The NGAL protein is a tiny (25 kDa) protein that is expressed in a variety of cells, including neutrophilic granulocytes and the epithelium of the gastrointestinal, respiratory, and urogenital tracts, and is particularly abundant in the intestinal epithelial cell layer during inflammation. When compared to healthy persons, LCN2, the coding gene for NGAL, is one of the most over-expressed genes in the colonic mucosa in ulcerative colitis (UC) and CD [4, 5]. This protein differs from the widely known faecal IBD indicators [4] due to the mucosal distribution of inflammation.

Patients and Methods:

This randomized prospective controlled study was carried out at the endoscopy unit of the internal medicine department at Tanta University Hospital after approval from Ethical

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Committee and obtaining informed written consent from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

Forty adult cases with definitive diagnosis of UC or Crohn's disease (confirmed by clinical, endoscopic, and histological workup) were included.

Patients with comorbid disease that may affect laboratory data and inflammatory mediator such as liver cirrhosis, renal failure, pregnancy, diabetes mellitus were excluded.

Subjects were divided into 3 groups as the following: group A (20 healthy individuals who had normal colonoscopy serving as control group), group B (10 patients who were previously diagnosed with CD) and group C (30 patients who were previously diagnosed with UC).

All Cases Were Subjected to the Following:

history taking, complete physical examination, Laboratory investigations including Complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels and liver and kidney function tests.

Fecal NGAL was estimated using ELISA technique. All fecal samples were collected in a sterile container and were analyzed within 2hours after collection.

Colonoscopy with biopsy: Endoscopy was performed by experienced gastroenterologists blinded to the results of the NGAL High-definition video scope using cope (epk©i.scan 5000) was used in all examinations (Pentax medical. Japan). Biopsies were obtained from inflamed or healed colonic mucosa or from random sites if inflamed area endoscopically detectable. The endoscopic disease activity was assessed according to the Montreal classification of severity of IBD (0: Inactive disease and normal mucosa, 1: Mild, 2: Moderate, 3: Severe disease).

Statistical analysis:

Statistical analysis of the present study was conducted, using SPSS version 26 (IBM®, USA).

The F test was used to compare quantitative data that were expressed as mean and standard

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deviation (SD). The median and interquartile range(IQR) were used to present quantitative non-parametric data (e.g., NGAL). Categorial variables were expressed as frequency and percentage and analyzed by Chi-square test. The overall diagnostic performance was assessed by ROC curve analysis. P value ≤ 0.05 was considered statistically significant.

Results

The incidence was higher in the third decade of life and incidence decreased with advanced aging. There were 3 female patients and 7 male patients in the CD group and in the UC group 14 patients were female and 16 patients were male with male predominance was high in the studied groups. **Table 1**

Table 1: Comparison between the three studied groups according to demographic data.

		Control (n=20)		CD (n=10)		UC (n=30)		Test of sig.	P
		No.	%	No.	%	No.	%		
Sex	Female	8	40.0	3	30.0	14	46.7	X^2=0.853	0.471
	Male	12	60.0	7	70.0	16	53.3		
Age (years)	Min. – Max.	18.0 – 57.0		25.0 – 45.0		18.0 – 50.0		F=1.046	0.358
	Mean ± SD.	34.65 ± 11.69		31.10 ± 5.43		30.90 ± 8.55			

Abdominal pain and diarrhea were the main presentations of CD, one patient was presented by fever or bleeding per rectum. In the UC group the main presentations were diarrhea and fresh bleeding per rectum, 2 patients presented with fever and 8 with abdominal pain. **Table 2**

Table 2: The main clinical presentation of the two studied groups.

	CD (n=10)		UC (n=30)	
	No.	%	No.	%
Bleeding per rectum	1	10.0	20	66.7
Diarrhea	4	40.0	30	100.0
Fever	1	10.0	2	6.7
Abdominal pain	10	100.0	8	26.7

Levels of ESR was markedly elevated in patient with IBD in comparison to the control group (p-value <0.001). **Table 3**

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CRP level was important inflammatory marker that was significantly elevated in the diseased group in comparison to the control group suggesting that CRP is an important marker in diagnosis and monitoring of disease severity (p-value < 0.001). **Table 3**

Hemoglobin level was severely decreased in the affected groups in comparison to the control group suggesting that iron deficiency anemia is an important predictor of IBD (p-value < 0.001). **Table 3**

Leukocytosis was found in patient with IBD and WBCs were significantly elevated in the affected group in comparison to the control group suggesting that elevation of WBCs count act as important biomarker that correlates with severity of inflammation (p-value<0.001). **Table 3**

NGAL level was significantly elevated in IBD in comparison to the control group suggesting the important role of NGAL as inflammatory biomarker in diagnosis and monitoring of the disease severity of IBD (p-value <0.001). **Table 3**

Table 3: Comparison between the three studied groups

	Control (n=20)	CD (n=10)	UC (n=30)	F	P
ESR 1st hour (mm/hour)	10.0 – 30.0	40.0 – 90.0	40.0 – 100.0	**45.499***	**<0.001* p1<0.001* p2<0.001***
	17.0 ± 6.16	54.50 ± 19.64	52.27 ± 15.03		
ESR 2nd hour (mm/hour)	20.0 – 40.0	50.0 – 120.0	70.0 – 120.0	**114.434***	**<0.001* p_1<0.001* p_2<0.001***
	27.80 ± 7.64	70.80 ± 21.34	80.57 ± 10.80		
CRP (mg/L)	1.0 – 6.0	10.0 – 96.0	16.0 – 106.0	**13.955***	**<0.001* p_1=0.001* p_2<0.001***
	3.40 ± 1.57	40.50 ± 29.23	31.40 ± 25.25		
Hemoglobin (g/dl)	11.0 – 16.0	10.0 – 12.50	7.0 – 11.0	**25.711***	**<0.001* p_1=0.002* p_2<0.001***
	12.02 ± 1.14	10.69 ± 0.74	10.03 ± 0.89		
WBCs	4.0 – 10.0	7.0 – 13.0	8.0 – 16.0	**23.415***	**<0.001* p_1<0.001* p_2<0.001***
	5.74 ± 1.37	9.67 ± 2.52	10.04 ± 2.61		
NGAL (mg/kg)	0.34 – 0.89	0.77 – 2.32	0.47 – 3.92	**51.319***	**<0.001* p_1=0.005* p_2<0.001***
	0.43 ± 0.15	1.34 ± 0.52	2.49 ± 0.95		

p_1: p value for comparing between Control and CD
p_2: p value for comparing between Control and UC

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Colonoscopy had been done to all patient and colonoscopic findings regarding the distribution of the lesions and the affected areas of colon were studied and illustrated. In the CD group, 3 patients with mild disease,6 patients with moderate disease and one patient with sever disease were included. In UC group, 8 patients were found with mild disease, 20 patients with moderate disease and 2 patients with severe disease. **Table 4**

Table 4: Colonoscopic finding using Montreal classification in the studied groups

	CD (n=10)		UC (n=30)	
	No.	**%**	**No.**	**%**
Mild (E1)	3	30.0	8	26.7
Moderate (E2)	6	60.0	20	66.7
Severe (E3)	1	10.0	2	6.7

The inflammatory marker ESR&CRP were elevated with elevation of NGAL level suggesting significant correlation between NGAL level and inflammatory mediator. There was a significant positive correlation between WBCs count and NGAL level in the two patients' groups (CD and UC). The severity of the disease was increased with elevation of NGAL level suggesting that a significant correlation is present between NGAL level and colonoscopic severity. **Table 5**

Table 5: Correlation between NGAL level and different parameters in each group.

	NGAL			
	CD (n=10)		UC (n=30)	
	r	**P**	**r**	**p**
ESR 1st hour	0.951	**<0.001***	0.460	**0.010***
ESR 2nd hour	0.894	**<0.001***	0.366	**0.047***
CRP	0.757	**0.011***	0.544	**0.002***
WBCs	0.740	**0.014***	0.580	**0.001***
Colonoscopy	0.658	**0.038***	0.473	**0.008***

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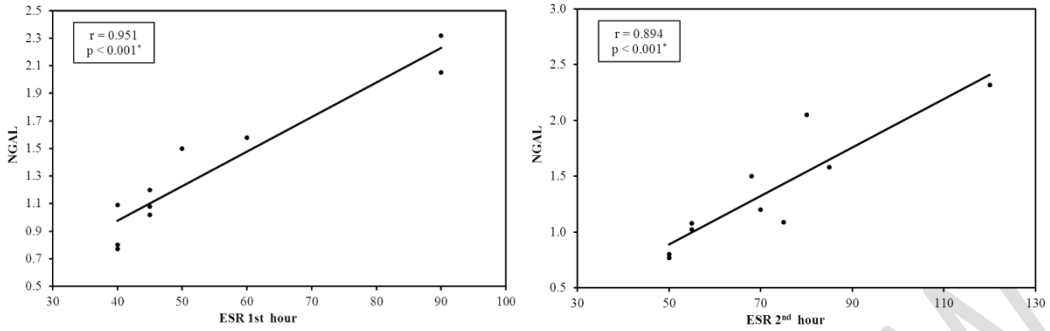

Figure 1: Correlation between NGAL level and ESR first and second hour level in CD group.

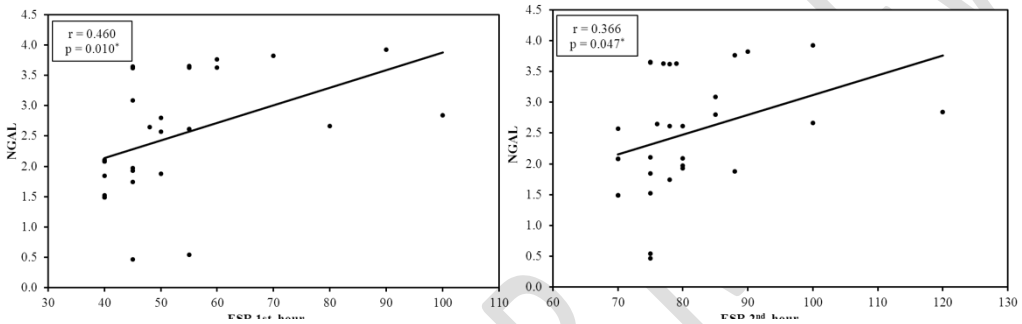

Figure 2: Correlation between NGAL level and ESR first and second hour level in UC group.

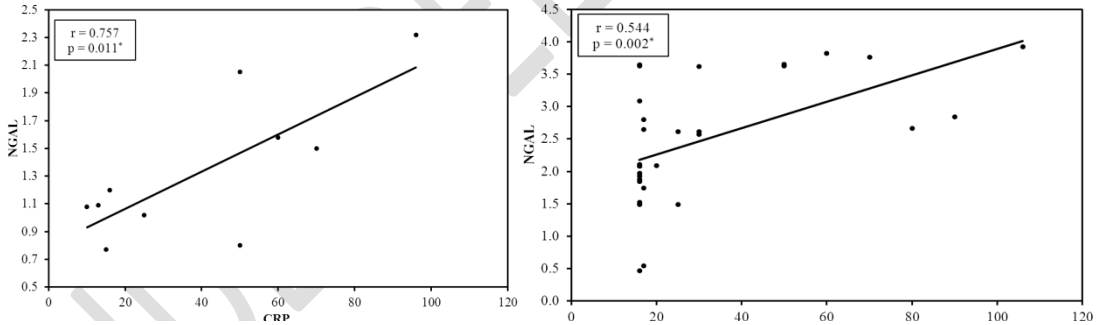

Figure 3: Correlation between NGAL level and CRP level in UC AND CROHNS group.

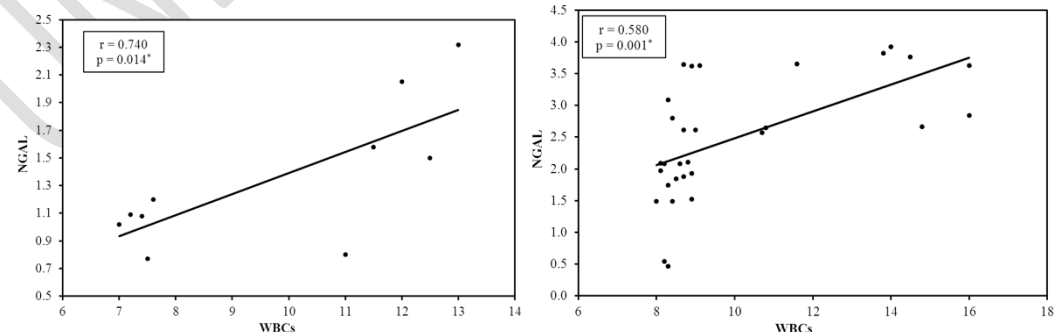

Figure 4: Correlation between NGAL level and WBCs count in uc and CD group

Table 6: Correlation between NGAL level and colonoscopic severity.

	NGAL			
	CD (n=10)		UC (n=30)	
	r	p	r	p
Colonoscopy	0.658	0.038*	0.473	0.008*

The Cut off value of NGAL for diagnosis of CD was >0.767 mg/kg with sensitivity 90 % and specificity 90 %. Area under the curve (AUC) was 0.983 in the ROC curve analysis with 81.8% positive predictive value (PPV) and 94.7% negative predictive value (NPV). The accuracy of NGAL measurement for diagnosis of CD was 90 %. **Table 7 Figure 5**

The Cut off value of NGAL for diagnosis of UC was >0.54 mg/kg with sensitivity 93.33 % and specificity 90 %. Area under the curve (AUC) was 0.992 in the ROC curve analysis with 93.3 % positive predictive value (PPV) and 90% negative predictive value (NPV) The accuracy of NGAL measurement for diagnosis of UC was 92%. **Table 7 Figure 6**

Table 7: Agreement (sensitivity, specificity) for NGAL to diagnose CD and UC patients (n = 10) from control (n = 20)

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
CD	0.983	<0.001*	0.947 – 1.018	>0.767	90.0	90.0	81.8	94.7	90
UC	0.992	<0.001*	0.976 – 1.008	>0.54	93.33	90.0	93.3	90.0	92.0

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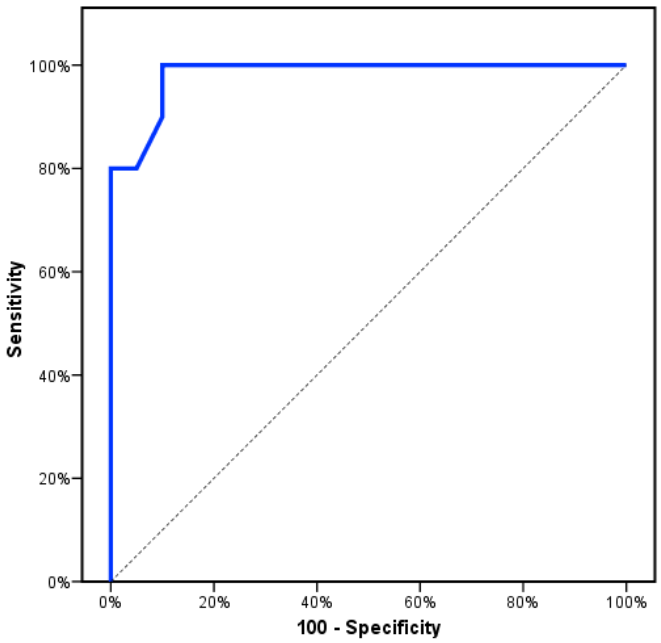

Figure 5: ROC curve for NGAL to diagnose CD patients (n = 10) from control (n = 20)

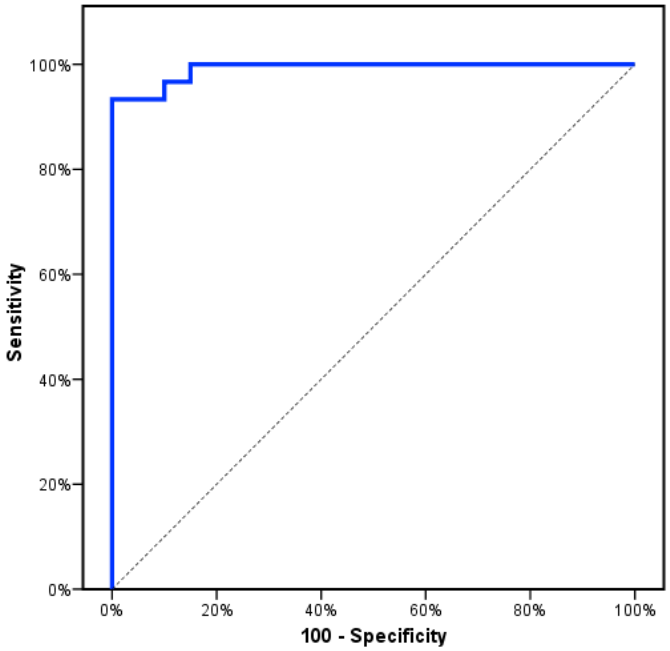

Figure 6: ROC curve for NGAL to diagnose UC patients (n = 30) from control (n = 20)

Discussion

There is a global increase in the screening and diagnosis of inflammatory bowel disease, IBD is a group of disease characterized by remission and relapse. Patients with IBD requires frequent

monitoring and screening by different serological, fecal biomarkers and colonoscopy. fecal and serological biomarkers are easily obtained, reproducible, objective and less invasive methods. Some studies found that NGAL is present in the colonic epithelium with high expression, suggesting that NGAL evaluation may help in the assessment of the inflammatory bowel disease activity [6].

Our study aimed to correlate the frequently used serological biomarkers with disease activity and to determine specificity and sensitivity of Fecal NGAL in correlation with the frequently used serological biomarkers and colonoscopic disease activity.

Calprotectin and lactoferrin, two established faecal indicators, are granulocyte-specific compounds [6]. We propose that a new biomarker be developed that reflects distinct features of the inflammatory process than the current ones. According to earlier findings from our group [1,] fe-NGAL could be a viable potential marker in this regard. In addition to being expressed in granulocytes, NGAL expression is regulated in the colonic epithelium during inflammation. In a more chronic inflammatory state with low numbers of infiltrating granulocytes, fe-NGAL may be a more sensitive test than calprotectin. Furthermore, fe-NGAL should be investigated in children with IBD, where meta-analyses suggest that calprotectin has a limited specificity. In our study we measured the level of Fecal NGAL and serum inflammatory markers (ESR, CRP, WBCs) of 40 patients with IBD with variable degree of disease severity and compared that to 20 healthy controls. We found a correlation between the NGAL level and another inflammatory biomarker and colonoscopic findings.

WBCs count was statistically significantly increased in patients with IBD than in control group. In agreement to our study, a study performed by Ashraf M. Okba1 et al. [7] who, showed that

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significantly higher WBC in an active UC group compared to both inactive UC patients and controls group (P = 0.000).

Regarding the ESR level and CRP, in our study it was statistically significantly increased in patients than in control group and in agreement with our study, a study performed by Sheng-Qiang Gao, et al. [8] showed that ESR and CRP level was significantly increased in CD patients than in control group (P ≤0.001).

Correlation between Fecal NGAL level and colonoscopic findings was found in our study and showed that Fecal NGAL has a strong correlation to colonoscopic findings, and this comes in agreement with the results of Silje Thorsvik, et al. [9] who similarly showed that Fecal NGAL has an excellent correlation with colonoscopic findings.

Fecal NGAL was measured in our study and was found to be significantly increased in patients with IBD than in the control group. In the ROC analysis of our material, we found AUC was 0.983 with sensitivity and specificity for Fecal NGAL of 90 % and 90 %, respectively, for distinguishing between CD and heathy control.

In agreement with our study, a study per formed by Silje Thorsvik, et al. [9] who showed that fecal NGAL is markedly raised in active UC and CD compared with irritable bowel syndrome (IBS) and healthy controls with sensitivity and specificity for Fecal NGAL of 95.7 % and 94.7 %, respectively.

Also, in agreement with our study, a study performed by Dilyara Mukhametova, et al. [10] showed that fecal NGAL is markedly raised in active IBD compared to the healthy controls.

The difference in NGAL cut-off values between studies could be related to differences in populations and the number of cases and controls. Overall, our results indicate that fe-NGAL is a

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viable biomarker in the field of IBD. Nielsen et al. proposed the use of fe-NGAL as a biomarker for IBD. [4].

The limitations of our study were the small sample size and the single center study. Further studies based on large number of patients with lower GIT symptoms are required to determine the potential role of serum NGAL in organic colonic diseases other than UC including CRC in non-UC patients. Also, further studies are required to evaluate the role of NGAL in assessing response to therapy for UC.

Conclusions:

In conclusion, faecal NGAL is a useful non-invasive diagnostic for assessing UC activity, and it has a positive correlation with inflammatory indicators (CRP and ESR) and disease activity markers (Mayo and UC histopathology grading).

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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