タイトル	Evaluation of Changes in the Neutrophil-Lymphocyte Ratio after Helicobacter pylori Eradication
作成者（著者）	Ishii, Takamasa / Sasaki, Yosuke / Komatsu, Fumiya / Kijima, Sho / Maeda, Tadashi / Urita, Yoshihisa
公開者	The Medical Society of Toho University
発行日	2019.06.01
ISSN	21891990
掲載情報	Toho Journal of Medicine. 5(2). p.62-68.
資料種別	学術雑誌論文
内容記述	Original Article
著者版フラグ	publisher
JaLCDOI	info:doi/10.14994/tohojmed.2018-032
メタデータのＵＲＬ	https://mylibrary.toho-u.ac.jp/webopac/TD21931333

Original Article

Evaluation of Changes in the Neutrophil-Lymphocyte Ratio after *Helicobacter pylori* Eradication

Takamasa Ishii Yosuke Sasaki* Fumiya Komatsu
Sho Kijima Tadashi Maeda and Yoshihisa Urita

Department of General Medicine and Emergency Care, Toho University, Faculty of Medicine, Tokyo, Japan

ABSTRACT

Introduction: Low-grade inflammation is associated with important chronic diseases, such as diabetes, cardiovascular disease, and cancer. The neutrophil-lymphocyte ratio (NLR) has been widely used as a biomarker for systemic inflammation. *Helicobacter pylori* (*H. pylori*) eradication is widely recommended because *H. pylori* causes chronic inflammation and increases the risk of developing peptic ulcers and gastric cancer. The aim of this study was to determine the association between *H. pylori* infection and NLR.

Methods: Forty-four patients undergoing upper endoscopy, rapid urease test, and routine blood examination were recruited, and 23 of them were evaluated after eradication therapy.

Results: NLR significantly decreased after eradication ($p < 0.01$). The NLR values were 2.16 ± 0.97 and 1.72 ± 0.65 at baseline and 2 months after eradication, respectively. There was no significant difference in the grade of atrophic gastritis, presence of peptic ulcers, hyperplastic polyps, sex, and age between the patient groups. NLR significantly reduced after *H. pylori* eradication, and an increase in NLR might depend on systemic inflammation induced by *H. pylori* infection to a certain degree.

Conclusions: Changes in NLR might provide additional information to confirm *H. pylori* eradication.

Toho J Med 5 (2): 62–68, 2019

KEYWORDS: neutrophil-lymphocyte ratio, *Helicobacter pylori*, chronic inflammation, eradication therapy

Introduction

Systemic inflammation can be measured using inflammatory markers, such as neutrophil-lymphocyte ratio (NLR). Since NLR can be measured using simple blood tests in a cost-effective manner, this ratio has been widely used as a biomarker for systemic inflammation, which is associated with the prognosis of cancer treatments, coronary interventions, coronary artery bypass grafting, and Alzheimer's disease.[1–5] Low-grade inflammation is associated with important chronic diseases, such as diabetes, cardiovascular disease, and cancer.[6–12] Chronic inflammation is considered one of the basic pathogenic processes in the development of cancers,[9, 10] and this process plays a role in the formation and activation of atherosclerosis and insulin resistance.[7, 12]

Infection with *Helicobacter pylori* (*H. pylori*) causes chronic inflammation and significantly increases the risk of developing duodenal and gastric ulcer disease and gastric cancer.[13] Preoperative NLR has been found to be associ-

*Corresponding Author: Yosuke Sasaki, 5-21-16, Omorinishi, Ota, Tokyo 143-8541, Japan, tel: +81–3–3762–4151
e-mail: yousuke.sasaki@med.toho-u.ac.jp
DOI: 10.14994/tohojmed.2018-032

Received Dec. 8, 2018: Accepted Dec. 27, 2018
Toho Journal of Medicine 5 (2), June 1, 2019.
ISSN 2189–1990, CODEN: TJMOA2

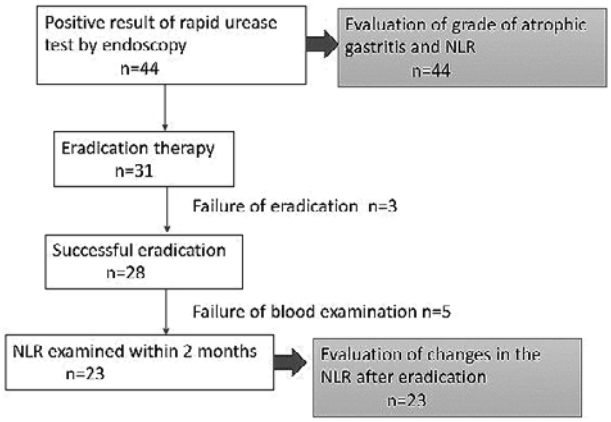

Fig. 1 Study flow diagram

ated with the outcomes for various malignancies, including gastric cancer.[14, 15] Although there are many reports about an association between *H. pylori* infection and the risk of gastric cancer, the literature on the relationship between NLR and *H. pylori* infection is very scant. In the American College of Gastroenterology Clinical Guideline 2017, there is insufficient evidence to support routine testing for and treatment of *H. pylori* in asymptomatic individuals with a family history of gastric cancer or patients with lymphocytic gastritis, hyperplastic gastric polyps, and hyperemesis gravidarum.[16] However, a recent meta-analysis of 24 studies, 22 of which were conducted in Asia, showed that the treatment of *H. pylori* infection in asymptomatic and that infected adults led to a decreased incidence of gastric cancer.[17] Since it is possible that the eradication of *H. pylori* infection could prevent the development of gastric cancer through changes in gastric atrophy and intestinal metaplasia, endoscopy is needed to confirm the reduction of inflammation in gastric mucosa after treatment. An alternative noninvasive method is also needed to evaluate post-treatment change in the inflammation of the gastric mucosa in clinical practice. In this study, we examined the association between *H. pylori* infection and NLR before and after *H. pylori* eradication to evaluate the potential and usefulness of NLR as a prognostic biomarker for *H. pylori* eradication therapy.

Material and Methods

This retrospective study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of Toho University Omori Hospital approved this study (approval number M17306). The study flow diagram is shown in Fig. 1.

Patients

The subjects of the present study were 44 patients undergoing upper endoscopy, rapid urease test, and routine blood examination on the same day at our department between June 2015 and November 2016. We excluded patients with any history of cancer and medical history because of the potential of anti-inflammatory effects stemming from the use of some medications, including proton pump inhibitors, histamine-2 receptor blockers, and/or antibiotics, in the past 2 months. Patients with an acute inflammatory or infectious disease, severe tissue damage, acute massive hemorrhage, and malignancies or hematological diseases that affect neutrophils and lymphocytes were also excluded.

All included patients had a positive result for rapid urease test. There were 20 men and 24 women with an average age of 62.0 years (range: 30-87 years). Of them, 31 patients received eradication therapy consisting of vonoprazan (20 mg), clarithromycin (400 mg), and amoxicillin (1500 mg) per day for 7 days.

Blood examination

Peripheral blood samples were taken at the time of endoscopy or the ^{13}C-urea breath test after an overnight fast for the measurement of blood counts, including neutrophils and lymphocytes. The NLR values were calculated as the ratio of the absolute neutrophil and lymphocyte counts, and white blood cell count was determined by a hemocytometer.

^{13}C-urea breath test

The ^{13}C-urea breath test was performed 2 months after the end of eradication therapy to evaluate whether *H. pylori* was successfully treated. After overnight fasting, 100 ml of tap water and 100 mg of ^{13}C-urea solution were used. Breath samples were taken at baseline and 20 minutes after administration. These breath samples were analyzed using an infrared spectral analyzer (POC One, Otsuka Electronics Co., Ltd.) that measures the change in the carbon isotope ratio (^{13}carbon dioxide [CO_2]/$^{12}CO_2$) in breath gas trapped in the air. The $^{13}C/^{12}C$ ratio was calculated and is expressed as delta over baseline (‰). A value of <2.5‰ was considered negative, resulting from a successful eradication.

Grading of *H. pylori*-infected gastritis

Atrophic gastritis was classified into two stages by observing the location of the atrophic border in the stomach[18]: closed type and open type. In the closed type, the atrophic borderline is located at the lesser curvature. In

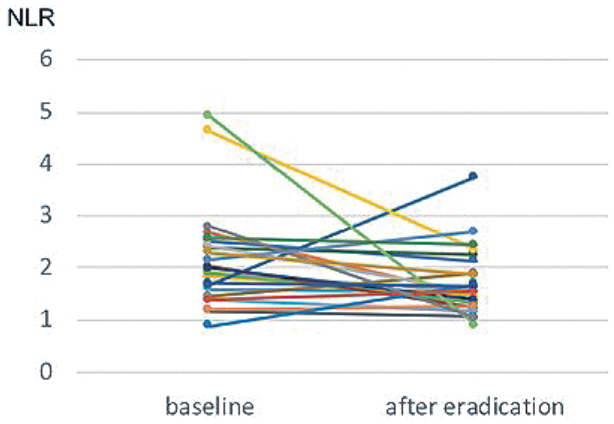

Fig. 2 Changes in the neutrophil-lymphocyte ratio (NLR) after eradication in all patients (n = 23)

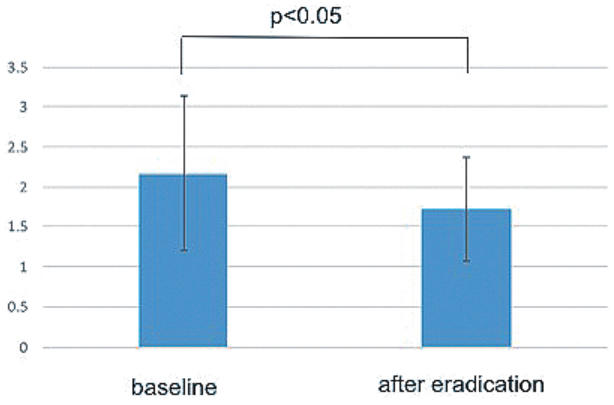

Fig. 3 Changes in the neutrophil-lymphocyte ratio after eradication in all patients

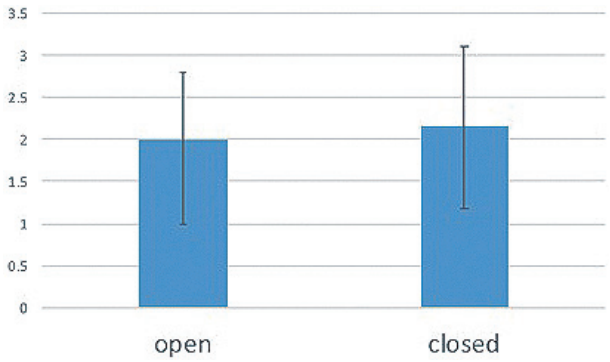

Fig. 4 The neutrophil-lymphocyte ratio in patients with atrophic gastritis (open type) and non-atrophic gastritis (closed) evaluated by endoscopy

the open type, the atrophic borderline lies between the lesser curvature and anterior wall of the body or the atrophic region spreads throughout the entire stomach, resulting in hypochlorhydria due to a reduction of parietal cells.

Statistical analysis

Differences in NLR before and after eradication were evaluated using Wilcoxon signed-rank test. Mann-Whitney test was used to analyze the differences in inter-group means. Mean comparisons of NLR among the three groups were performed with the Kruskal-Wallis H statistic.

Statistical analyses were performed using JMP version 13.0 (SAS Institute Inc.). A p-value < 0.05 was considered statistically significant.

Results

Changes in NLR after *H. pylori* eradication

Eradication therapy was successful in 28 patients, but 5 patients failed a follow-up blood examination. Finally, 23 patients were examined at baseline and after treatment. They included 9 men and 14 women who were between the ages of 30 and 60 years (average 49.7 ± 9.9 years). Endoscopic findings revealed closed-type gastritis in 16 cases and open-type gastritis in 7. Duodenal and gastric ulcers were found in five and two cases, respectively. Four of five patients who failed in eradication therapy had open-type gastritis, and their mean age was 70.0 ± 0.8 years.

Changes in NLR at baseline and after eradication are shown in Fig. 2 and Fig. 3. NLR significantly decreased after eradication ($p < 0.05$). The NLR values were 2.16 ± 0.97 and 1.72 ± 0.65 at baseline and 2 months after eradication, respectively. Four patients (17.4%) had an increased NLR value after eradication.

NLR and atrophic gastritis

Atrophic gastritis (open type) and non-atrophic gastritis (closed type) were found in 24 and 20 patients, respectively. The open-type group included 8 men and 16 women aged 52-97 years (average: 69.2 years), whereas the closed-type group included 10 men and women aged 36-79 years (average: 56.0 years). The patients with closed-type gastritis were significantly younger than those with open-type gastritis ($p < 0.01$, Mann-Whitney test). As shown in Fig. 4, NLR in the open-type group was not significantly different from that in the closed-type group ($p = 0.326$).

NLR and endoscopic findings other than gastritis

Hyperplastic polyps were found by endoscopy in four patients (9.1%), and peptic ulcers were found in nine patients (20.5%), including four with gastric ulcers, four with duodenal ulcers (three with an ulcer scar), and one with both. The NLR values were 1.99 ± 0.30 in those with polyps, 2.43 ± 0.29 in those with ulcers, and 2.01±0.80 in those with gastritis alone (Fig. 5). Although NLR tended to be

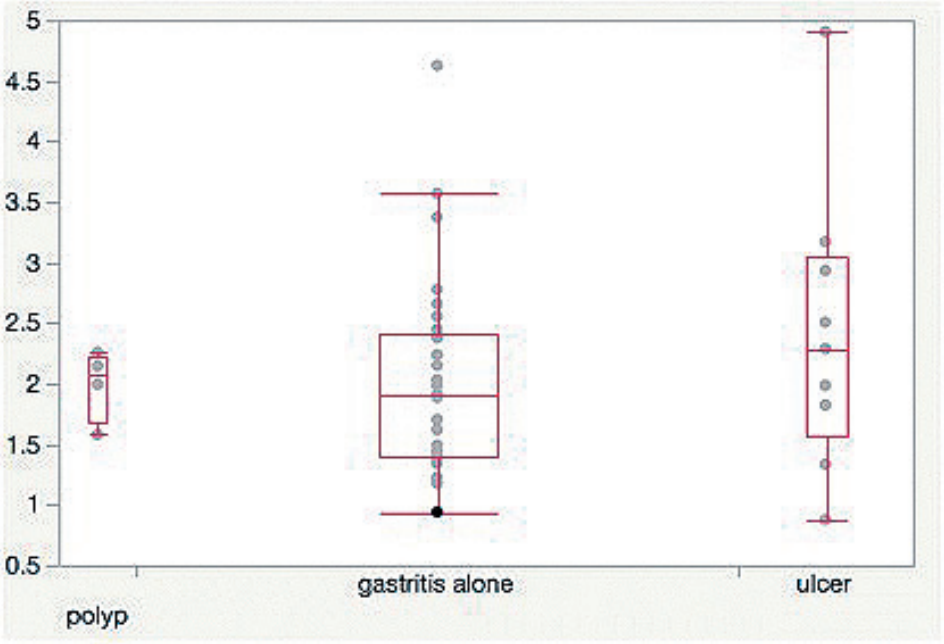

Fig. 5 The neutrophil-lymphocyte ratio in patients with and without local endoscopic findings such as peptic ulcers and hyperplastic polyps

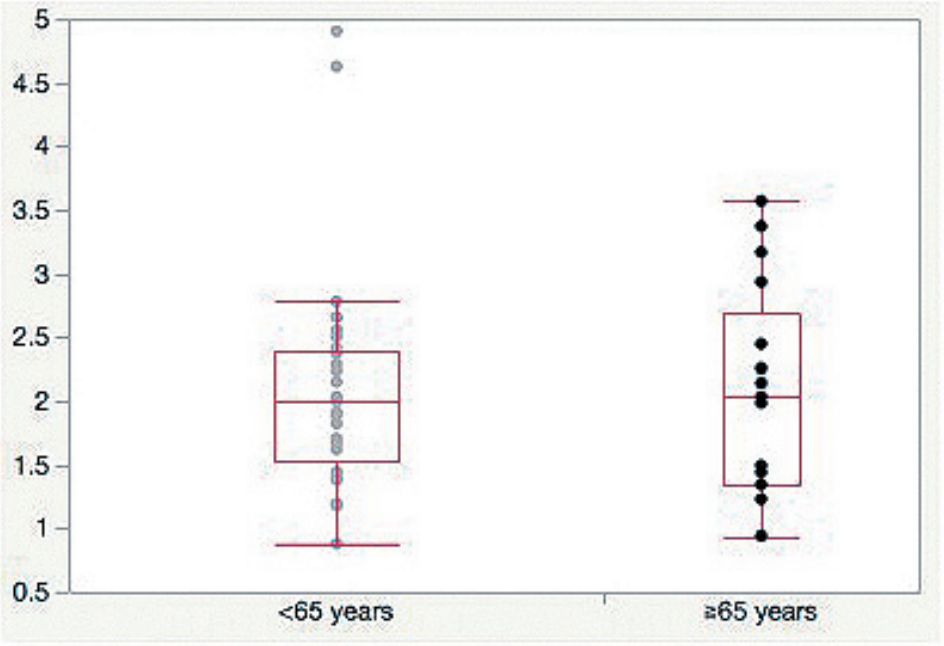

Fig. 6 Comparison of the neutrophil-lymphocyte ratio between patients aged 65 years or older and those aged less than 65 years

highest in the ulcer group, there was no significant difference among the three groups ($p = 0.443$).

Of the nine patients with peptic ulcers, NLR was examined in six patients after eradication therapy. The NLR values decreased from 1.80 ± 0.50 to 1.61 ± 0.48 after eradication. The decline of NLR was smaller in patients with peptic ulcers among all patients.

NLR and age

As shown in Fig. 6, the patients were classified into two groups to analyze the effect of age on NLR. There was no

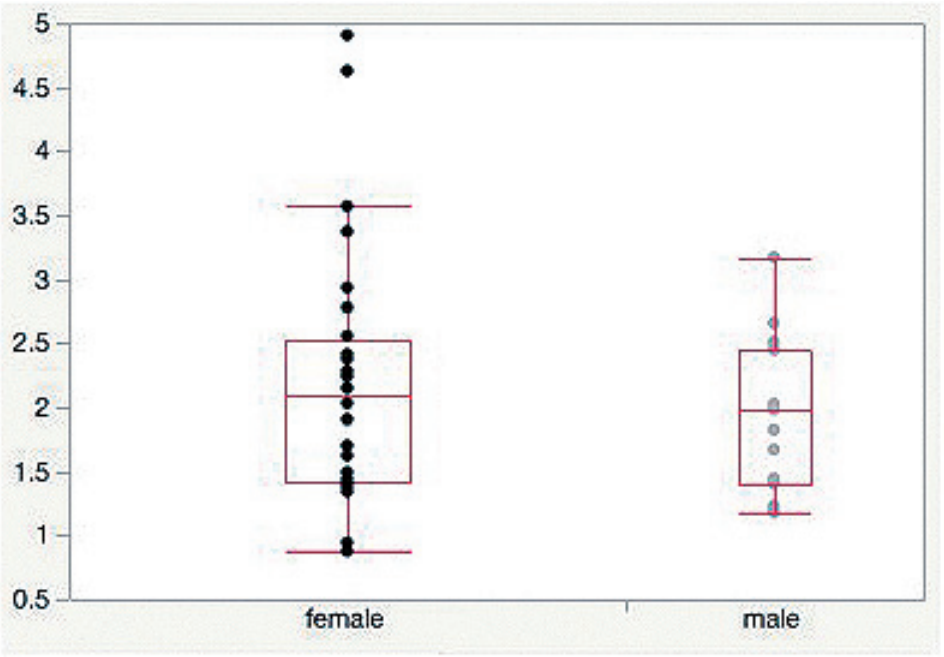

Fig. 7 Comparison of the neutrophil-lymphocyte ratio in men and women

significant difference between the younger and older groups ($p = 0.894$).

NLR and sex

A comparison of NLR between men and women is demonstrated in Fig. 7. There was no significant difference between men and women ($p = 0.328$).

Discussion

NLR, a simple, widely available, and low-cost index obtained from complete blood counts, has recently been proposed as a predictor of the onset, progression, and prognosis of several chronic inflammatory diseases and cancers.[1–10] This makes interindividual comparison of NLR very difficult because numerous factors affect it. Therefore, there is no consensus regarding the NLR cut-off value as yet. Remarkably, despite the well-known close association between *H. pylori* infection and chronic inflammation, there is very limited information on the relationship between NLR and *H. pylori* gastritis. Considering that the neoplastic process is mediated by different inflammatory cells, physicians should not forget that *H. pylori* interacts with host cells to initiate an immune response when analyzing NLR in patients with cancer, especially in those with gastric cancer. Actually, a high NLR is associated with a worse prognosis in patients with gastric cancer.[14, 19]

The present study is the first report about the decrease in NLR after *H. pylori* eradication therapy, although there were a few studies on the relationship between NLR and *H. pylori* infection.[20–22] Contrary to our results, a Korean study on 17,028 adults who completed routine health check-ups revealed that chronic infection with *H. pylori* was not associated with various inflammatory markers including NLR.[20] Guclu et al.[21] also reported a lower NLR in patients with *H. pylori* infection. Farah et al.[22] demonstrated a higher NLR in patients with the symptoms of *H. pylori* infection compared with in those without these symptoms and controls. Although four patients (17.4%) had an increased NLR after eradication in the present study, the mechanism has not been clarified. The various results suggest that NLR is influenced by various factors.

From the results of this study, NLR was found to be significantly decreased after eradication, suggesting that an increase in NLR depends on systemic inflammation induced by *H. pylori* infection to a certain degree. This has a great weightage in the analysis of the clinical significance of NLR in any medical field, especially in Asia because of the high prevalence of *H. pylori* infection.

Reference values for NLR have not been determined as yet. It has been reported that the NLR cut-off values for prognosis vary from 2.5 to 5, and the Western population has a higher cut-off value than the Asian or African populations.[23–25] Lee[26] demonstrated that NLR is generally lower in the Asian population than in other races, which is consistent with the findings of previous studies. In South

Korea, NLR was found to have sex-specific differentiation within the same age: NLR was higher in <50-year-old women than in <50-year-old men, whereas the reverse was true in >51-year-old women and men. Therefore, the NLR of patients with *H. pylori* infection was also compared by sex and age in this study. As shown in Fig. 5 and Fig. 6, a significant difference was not found between the sex and age groups. This finding is because the average age of the patients recruited herein was 62.0 years, although estrogen and progesterone increase neutrophil recruitment from the bone marrow.[27]

A predominant *H. pylori*-specific T-cell response characterized by high interferon-γ, tumor necrosis factor-α, and interleukin-12 production is, to some extent, associated with a peptic ulcer, whereas the secretion of Th1 and Th2 cytokines appears in uncomplicated gastritis.[28] This information suggests that immune responses to *H. pylori* differ between patients with and without peptic ulcers. As shown in Fig. 4, NLR tended to be highest in the ulcer group, but the difference did not reach a statistical significance. Recently, Tanrikulu[29] reported that NLR did not differ between the peptic ulcer group without perforation and control group. Although the result is incomparable with our results because the *H. pylori* status was not examined, the fact that immune response to *H. pylori* is not affected by the development of peptic ulcers is extremely fascinating.

There are some limitations to this study. First, this was a retrospective study; therefore, other inflammatory mediators such as the high sensitivity C-reactive protein level could not be evaluated. Thus, we could not determine whether NLR was a better inflammatory marker in this study. Second, our small sample size might have led to limitations in the statistical analysis. Third, our data may have underestimated the degree of systemic inflammation regardless of the exclusion criteria because only a physical examination and medical interview can be used to screen for other inflammatory diseases. Finally, control subjects without *H. pylori* infection were not recruited in this study. It is very difficult to recruit normal subjects without chronic inflammation even if they do not complain of any symptom. Therefore, a control group was not set, but NLR was evaluated before and after eradication to analyze the association between NLR and *H. pylori* infection. Further study is needed to evaluate the application of NLR in the management of *H. pylori*-related diseases.

In summary, we investigated NLR of patients with *H. pylori* infection. This is the first report to show that NLR significantly decreases after *H. pylori* eradication. Indeed, there were some patients with an intermediate result of the ^{13}C-urea breath test, although their endoscopic findings improved and abdominal symptoms disappeared. Changes in NLR might provide additional information to confirm eradication of *H. pylori*. Furthermore, an increase in NLR might depend on systemic inflammation induced by *H. pylori* infection to a certain degree. This finding may contribute to further studies aimed at analyzing the relationship between NLR and other inflammatory diseases.

Acknowledgements: This study did not receive any specific grants from funding agencies. The Excel file data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest: None declared.

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