Original Article

Human Epidermal Growth Factor Receptor-2 Assessment in Cancerous and Precancerous Lesions of the Stomach in Presence of *Helicobacter pylori*

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Abstract

Aims: Importance of human epidermal growth factor receptor-2 (HER-2) testing in cancer is increasing, and in the other hand, there is a hypothesis about roles of *Helicobacter pylori* (*H. pylori*) in gastric cancer (GC) development. We seek to investigate the HER-2 expression in the presence of *H. pylori* infection in patients with gastric GC and precancerous lesions. **Subjects and Methods:** From 224 archived blocks, 58 samples with chronic gastritis, 58 intestinal metaplasia (IM), 58 gastric dysplasias, and 50 gastric adenocarcinoma cases were selected as our principal groups. Each group was subdivided into *H. pylori*-positive and *H. pylori*-negative subgroups. Immunohistochemical method was performed for detection of HER-2 expression in gastric biopsies using a polyclonal antibody. The staining intensity was quantified and differences among groups were compared using Mann–Whitney U and Kruskal–Wallis tests. $P < 0.05$ was set as statistically significant. **Results:** Results showed that HER-2 expression among the four principal groups was statistically significant ($P < 0.05$). There was a significant increase of immunohistochemical expression of HER-2 in *H. pylori*-positive chronic gastritis in comparison with *H. pylori*-negative subgroup ($P < 0.05$). Difference of HER-2 expression between *H. pylori*-positive and *H. pylori*-negative subgroups in dysplasia, IM, and GC groups was not statistically significant ($P > 0.05$). **Conclusion:** The present study proposed that HER-2 overexpression in gastric tissues probably had important roles in the progression of cancer and above-mentioned process was probably associated with *H. pylori* infection in the early stages of precancerous lesions.

Keywords: *Adenocarcinoma, gastric cancer, gastritis, Helicobacter pylori, human epidermal growth factor receptor-2*

Introduction

In the present century, gastric cancer (GC) still is a second cancer-related cause of death in the world. Limitations in diagnostic programs of GC lead to the late detection of disease in advanced stages that result in low rate of only 5-year survival.[1-3] There is not a homogenous pattern for GC incidence in different countries, and it has been noted that the survival rate of GC patients varies based on the geographical and ethnical differences.[1,4,5]

Gastric carcinogenesis is a multifactorial process that can be mediated by various factors, including *Helicobacter pylori (H. pylori)* infection (as a major reason), genetic background, and effects of the environment contains; smoking, salty intake, and low-vegetable diet.[6-8] Discovering of GC in early stages could be possible by a survey on underlying lesions.[4] Correa model indicated that chronic gastritis, intestinal metaplasia (IM), gastric dysplasia, and invasive adenocarcinoma are a sequence of precancerous conditions lead to cancer development in the normal mucosa.[9] Chronic gastritis could develop to atrophic type that relatively progresses to IM, low-grade dysplasia, and high-grade dysplasia.[7] Eventually, GC is the terminal stage in this deteriorating process.[4,9]

Previous reports indicated that infection with *H. pylori* in patients with gastritis could trigger this tumorigenesis cascade.[5,10] These findings make this bacterium as an interesting factor for investigation of GC incidence. It has been shown that susceptibility of patients to the infection with *H. pylori* and also the frequency of GC varied among different

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geographical regions. Thus, the study on *H. pylori* infection and its association with GC in different populations are increasing.[11]

Since 1986, that overexpression of human epidermal growth factor receptor-2 (HER-2) reported in the patients with GC, many studies have focused on determining of HER-2 status worldwide.[1,12] There are increasing evidence that HER-2 overexpression in GC patients is probably associated with the advanced stage of the disease and decreased overall survival.[13,14]

Kanayama *et al*.[14] proposed that mutations in the HER-2 gene that result in its overexpression probably occurs in early steps of gastric carcinogenesis.[14]

There are limited studies on the expression of HER-2 in precancerous conditions of the stomach, and the association between HER-2 expression and *H. pylori* infection in these conditions have not explored till now.[1,10] On the other hand, data on the HER-2 expression in Iranian patients with GC are scarce and there is a wide variation in the published results.[15,16] The authors were not able to find any research on the association of *H. pylori* infection and HER-2 expression in the patients with gastric precancerous lesions in literature.[10,17]

We proposed that infection with *H. pylori* could be a possible reason for the progression of gastritis to GC and increase in HER-2 expression in GC patients. We hypothesized that this overexpression began from early stages of precancerous conditions. Therefore, we aimed to investigate immunohistochemical expression of HER-2 in GC and its precancerous lesions in the presence of *H. pylori* infection.

Subjects and Methods

Selection criteria

Endoscopic biopsy specimens of chronic gastritis, IM, gastric dysplasia, and gastric adenocarcinoma were selected as our four principal groups. Specimens were obtained from 224 archived blocks in the Department of Clinical Pathology and Cytology, Imam Ali Hospital, Zahedan, Iran, from 2010 July to 2015 December. The exclusion criteria for this case–control study were a history of immune disorders, recent recipient of steroids, and anti-*H. pylori* drugs. Original biopsies were obtained as formalin-fixed, paraffin-embedded archived blocks. Finally, 58 blocks with chronic gastritis, 58 IM blocks, 58 gastric dysplasias, and 50 gastric adenocarcinoma cases were selected (Convenience sample selection). The present project was conducted in accordance with the Declaration of Helsinki for conducting research involving humans and approved by the Ethics Committee of Research in the Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.RES.1394.285). All patients had signed a written consent for using their tissue specimens for researching purposes.

Helicobacter pylori status

In each principal group, two subgroups have been identified according to the presence or absence of *H. pylori* infection. *H. pylori* statuses of patients were extracted from patient information forms that were diagnosed by the rapid urease test. It was confirmed with Giemsa staining in tissue samples.

Immunohistochemistry

Immunohistochemistry (IHC) was used for localization of HER-2 within gastric biopsies from all groups. All IHC procedures were performed using the following staining protocol by Novolink polymer detection kit (RE7140-K, The United Kingdom). All steps were done at room temperature (25°C) in a humidified chamber.

Tissue samples were cut to 4-μm thickness and were mounted on slides coated with a suitable tissue adhesive (Histogrip CL00-8050, Cedarlane, Canada). Sections were allowed to air dry at room temperature overnight. Then, the sections were deparaffinized, rehydrated, and finally, were washed in distilled water. All sections for immunostaining were processed for heat-induced antigen retrieval. Slide-mounted sections were immersed in boiling sodium citrate buffer (0.01 M, pH 6.0) for 10 min (in 120°C).

To neutralize endogenous peroxidase activity, sections were blocked with a peroxidase-blocking reagent (Novocastra, United Kingdom) for 10 min and were washed in a bath of Tris buffer (0.05 M Tris-HCl, pH 7.0–7.6). Then, the slides were incubated with Protein Block (Novocastra, United Kingdom) for 5 min, followed by overnight incubation with the anti-HER-2 primary antibody (mouse polyclonal; MS-730-R7, Thermo, UK) at 4°C. Sections were incubated with Novolink Post Primary for 30 min and then with Novolink Polymer for 30 min at room temperature.

Peroxidase activity was developed with the DAB (3,3'-diaminobenzidine) working solution (100 μl of DAB Chromogen added to 1 ml of Novolink DAB Substrate Buffer) for 30 min. Sections were counterstained with Mayer's hematoxylin for 5 min and were rinsed in fresh distilled water. Sections were dehydrated and then were mounted with Entellan (Merck).

Positive controls (breast cancer biopsy) and negative controls (omission of the primary antibody) were run for each batch of slides.

Evaluation of immunostaining

Two expert histologists performed immunohistochemical analysis on masked-label slides.

HER-2 IHC was scored using the scoring scheme proposed by Hofmann *et al*.[18] and employed in the trastuzumab for GC (ToGA) cohort of the gastroesophageal junction and

gastric carcinomas as follows (ToGA score): 0, no staining or membranous reactivity in <10% of cells; 1+, weak, barely perceptible membranous reactivity in >10% of cells; 2+, complete or basolateral membranous reactivity that is either nonuniform or weak in intensity in the at least 10% of cells; and 3+, complete or basolateral membranous reactivity of strong intensity in ≥10% of cells. Cases with strong and moderate (2+/3+) intensity of immunohistochemical reactivity were considered positive and cases with low intensity (+1) and without reactivity as negative.[2]

Statistical analyses

Statistical calculations were performed by employing SPSS 16.0 software (SPSS Inc., Chicago, USA). Values were presented as mean ± standard error of the mean, and differences were compared using Mann–Whitney U and Kruskal–Wallis tests. In all evaluations, $P < 0.05$ were regarded as statistically significant.

Results

Demographic data of the participants were presented in Table 1. Age and sex were matched among groups. Average age was 53.97 ± 18.84, 100 cases (44.60%) were female and 124 (55.40%) were male.

There is no positive reaction of HER-2 in normal gastric mucosa in any of the participants. However, there was a weak cytoplasmic staining in the specialized glands and membranous staining in the foveolae in the tissue sections. The clear membranous positive reaction was observed in HER-2 cases with immunohistochemical 3+ score. Cell-to-cell junctions showed a lateral- or U-shaped staining pattern in the cellular membranes. Membranous staining in immunohistochemical 2+ score cases was found too. In the cases with immunohistochemical 1+ score, a membranous reaction was visible only at a high ×40 magnification using light microscopy.

The results of the present study showed that the difference in HER-2 expression among patients with chronic gastritis, metaplasia, dysplasia, and GC was statistically significant ($P < 0.05$) [Table 2 and Figure 1].

Table 1: Demographic data of patients with chronic gastritis, gastric dysplasia, intestinal metaplasia, and gastric adenocarcinoma

Groups	Parameters		
	Age (mean±SD)	Gender, *n* (%)	
		Female	Male
Chronic gastritis	43.89±13.62	34 (58.60)	24 (41.40)
Gastric dysplasia	53.43±22.20	8 (13.80)	50 (86.20)
IM	58.58±14.35	32 (55.20)	26 (44.80)
Gastric adenocarcinoma	63.33±18.42	26 (52.00)	24 (48.00)
Total	53.97±18.84	100 (44.60)	124 (55.40)

SD: Standard deviation, IM: Intestinal metaplasia

There was a significant difference in immunohistochemical expression of HER-2 in chronic gastritis *H. pylori*-positive and *H. pylori*-negative subgroups ($P < 0.05$) [Table 2].

The difference of HER-2 expression between *H. pylori*-positive and *H. pylori*-negative subgroups in dysplasia, IM, and GC groups was not statistically significant ($P > 0.05$) [Table 2]. Positive expression of HER-2 in dysplasia samples was more than gastritis cases. However, HER-2 positive expression in IM and GC groups decreased.

Although when the HER-2 expression was compared between all *H. pylori*-positive (106 [47.30%] cases) and *H. pylori*-negative (118 [52.70%] cases) patients (regardless of the type of gastric lesion), the expression increased in the *H. pylori*-positive subgroup and the difference between two subgroups was statistically significant ($P = 0.005$).

The difference of HER-2 expression between eight subgroups of patients (regard to gastric lesion type and *H. pylori* infection) was statistically significant ($P = 0.001$) [Table 2].

In addition, the results of Fisher's exact test showed that HER-2 expression is dependent on *H. pylori* infection ($P < 0.05$).

Discussion

The present study showed that there was a statistically significant difference in HER-2 expression among chronic gastritis, IM, dysplasia, and GC samples. HER-2-positive expression in dysplasia was more than gastritis cases. However, positive expression of HER-2 in IM and GC cases decreased.

We did not find similar study on the comparison of HER-2 expression among these four groups in literature. He *et al*.[12] reported positive expression of HER-2 in 22.33% of patients with GC. Raziee *et al*.[15] showed that there was a positive expression of HER-2 in 26% of Iranian patients with GC. In the present study, 32% of patients with GC were HER-2 positive. Jin *et al*.[19] found that 72.90% of patients with early GCs were HER-2 positive.

Ecker *et al*.[20] showed that the regulation of HER-2 protein increased in dysplasia and had a strong association with elevated susceptibility to cancer invasion.[20] It has been reported that primary antibody used for immunohistochemical detection of HER-2 could affect its expression widely.[1] Another reason for variation in the rate of HER-2 expression among different studies is determining methods that used for HER-2 detection, including IHC, FISH, and DISH and scoring criteria. However, the differences in tumor type and genetic backgrounds of patients could also impress obtained results.[2,13]

We found that there was a significant difference in immunohistochemical expression of HER-2 in chronic

Table 2: Immunohistochemical expression of human epidermal growth factor receptor-2 in patients with chronic gastritis, gastric dysplasia, intestinal metaplasia, and gastric adenocarcinoma

	n (%)	HER-2		*P*	
		Positive	Negative		
Chronic gastritis	58 (25.90)	20 (34.50)	38 (65.50)	0.017†,*	0.016‡,*
H. pylori positive	28 (48.30)	14 (50.00)	14 (50.00)		
H. pylori negative	30 (51.70)	6 (20.00)	24 (80.00)		
Gastric dysplasia	58 (25.90)	26 (44.80)	32 (55.20)		
H. pylori positive	28 (48.30)	16 (57.10)	12 (42.90)	0.071†	
H. pylori negative	30 (51.70)	10 (33.30)	20 (66.70)		
IM	58 (25.90)	10 (17.20)	48 (82.80)		
H. pylori positive	28 (48.30)	4 (14.30)	24 (85.70)	0.568†	
H. pylori negative	30 (51.70)	6 (20.00)	24 (80.00)		
Gastric adenocarcinoma	50 (22.30)	16 (32.00)	34 (68.00)	0.074†	
H. pylori positive	22 (44.00)	10 (45.50)	12 (54.50)		
H. pylori negative	28 (56.00)	6 (21.40)	22 (78.60)		
Total	224 (100.00)	72 (32.10)	152 (67.90)	0.001‡,*	

Data were presented as n (%). †Mann–Whitney U-test, ‡Kruskal–Wallis test, *Significant differences between groups.
H. pylori: Helicobacter pylori, HER-2: Human epidermal growth factor receptor-2, IM: Intestinal metaplasia

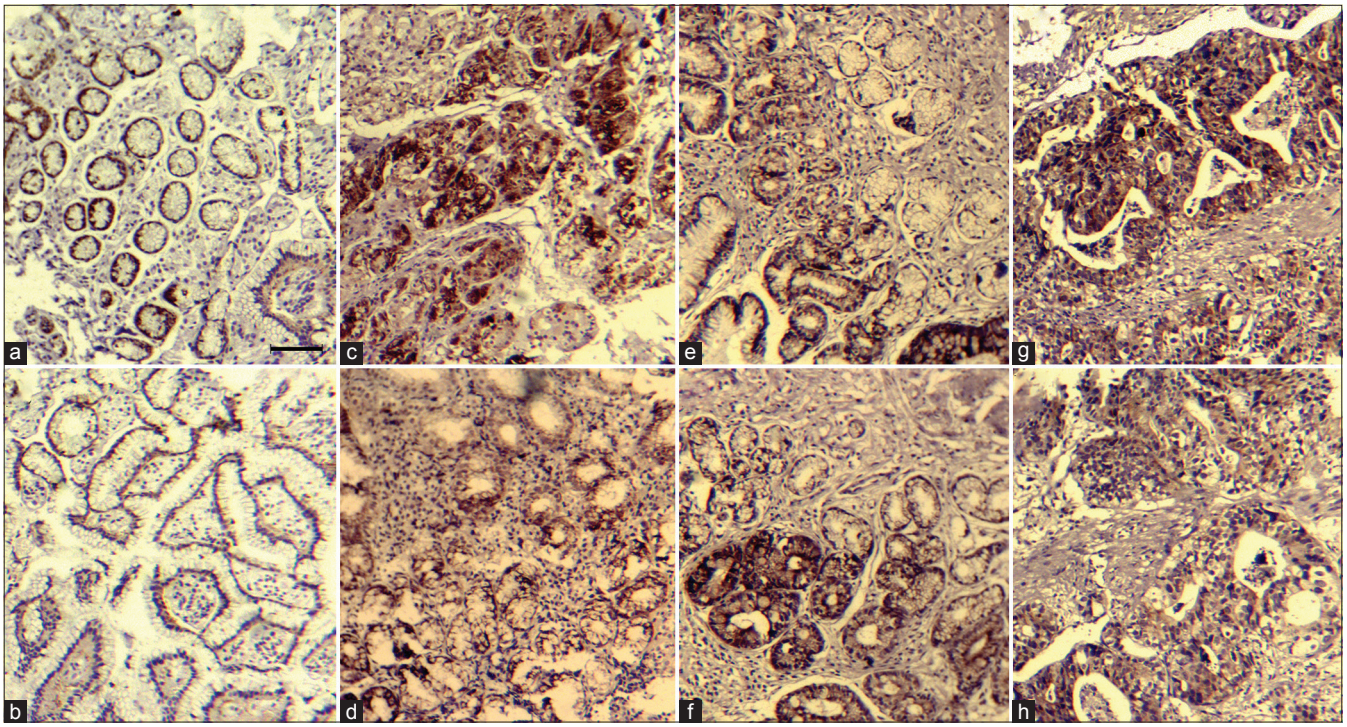

Figure 1: Immunohistochemical human epidermal growth factor receptor-2 overexpression in gastric cancer and precancerous lesions of gastric biopsies, ×100 magnification, scale bar = 40 μm. (a) Chronic gastritis, *H. pylori* positive, (b) Chronic gastritis, *H. pylori* negative, (c) Gastric dysplasia, *H. pylori* positive, (d) Gastric dysplasia, *H. pylori* negative, (e) Intestinal metaplasia, *H. pylori* positive, (f) Intestinal metaplasia, *H. pylori* negative, (g) Gastric adenocarcinoma, *H. pylori* positive, and (h) Gastric adenocarcinoma, *H. pylori* negative

gastritis *H. pylori*-positive and *H. pylori*-negative subgroups. However, most of the chronic gastritis cases (41.37%) were HER-2 negative and *H. pylori* negative. To the best of our knowledge, this is the first time that HER-2 expression in line with *H. pylori* infection is reported in a wide series of gastric precancerous and invasive lesions.

It has been shown that patients with chronic gastritis that associated with *H. pylori* infection might have higher risks for gastric carcinogenesis.[10,21] In most patients with *H. pylori* infection, inflammation develops to gastritis. *H. pylori* colonization in the gastric mucosal layer is considered the first event in series of gastric precancerous lesions. Overall, based on the performed evaluations 60% of GC cases and up to 5% of all cancers were induced by *H. pylori* infection.[21,22]

Due to a strong possible link between *H. pylori*-induced gastritis and gastric carcinogenesis, *H. pylori* monitoring

in these patients probably is a suitable tool for recognition of patients and in the larger view detection of areas with higher risk of GC.[8] *In vitro* studies established that infection with P1 and 26695 strains of *H. pylori* are able to activate HER-2 signaling in the cells.[23]

Our results showed that the HER-2 expression in the gastric dysplasia *H. pylori*-positive and *H. pylori*-negative subgroups did not differ significantly. Positive expression of HER-2 in gastric dysplasia cases with *H. pylori* positive were more than *H. pylori*-negative cases. However, most of the dysplasia cases (34.48%) in the present study were HER-2 negative and *H. pylori* negative.

The gastric dysplasia is an intermediate phase between atrophic gastritis and GC.[4,7] Therefore, assessment of a key factor involved in the progression of precancerous lesions to invasive malignancies is critical.[7] Published studies on the HER-2 expression in the gastric dysplasia are scanty.[4,24] Detection of HER-2 amplification in the gastric dysplasia showed that it could involve in primary steps of gastric carcinogenesis.[25] Jin *et al*.[7] showed that HER-2 overexpression could be a prognostic factor in the prognosis of gastric dysplasia in the route of carcinogenesis.[7] Lee *et al*.[26] suggested that overexpression of HER-2 could be a high-specificity indicator for diagnosis of high-grade dysplasia. However, this key factor was not sensitive enough.[26] Overall, primary stages of GC can be identified by HER-2 overexpression in patients with gastric dysplasia.[4,24,26]

The role of *H. pylori* infection in HER-2 expression has not been investigated previously in patients with the gastric dysplasia.[26] According to our findings, difference of HER-2 expression in *H. pylori*-positive and *H. pylori*-negative cases with the metaplasia was not statistically significant. Positive expressions of HER-2 in *H. pylori*-positive patients with the metaplasia were lesser than *H. pylori* negative. Increased evaluation of gastric IM that is mostly caused by chronic *H. pylori* infection has been suggested to be a precancerous lesion with elevated susceptibility to gastric carcinogenesis.[7,27] In the previous study that performed by Chong *et al*.,[13] the difference in the presence of IM in samples between HER-2 positive and HER-2 negative groups was not significant. Jin *et al*.[7] also found that there was no association between HER-2 overexpression and presence of IM in gastric samples.

Our findings showed that the positive expression of HER-2 in GC cases with H. pylori positive were more than *H. pylori*-negative cases. However, the difference of HER-2 expression between two subgroups of *H. pylori*-positive and *H. pylori*-negative GC cases was not statistically significant. In addition, most of GC cases (37.93%) in the present study were HER-2 negative and *H. pylori* negative.

Wang *et al*.[11] reported that *H. pylori*-positive status was more frequent in nonneoplastic samples in comparison with malignant lesions. Decrease in acid secretion that results from cancer-related injury in the gastric mucosal layer lead to change in pH of the environment into the unsuitable range for *H. pylori* surviving. It has been established as a possible reason for the increased prevalence of advanced stages of cancer in GC patients with a negative status of *H. pylori* infection.[11] Selcukbiricik *et al*.[17] studied the role of HER-2 expression in *H. pylori* infection and its subsequent relation with disease outcome in early GC and lymph node metastasis. They showed that 86% of HER-2-positive samples were *H. pylori* positive and 94% of HER-2-negative cases were negative for *H. pylori* infection.

Chong *et al*.[13] showed that 28.2% of patients with GC were *H. pylori* positive and 57.10% of HER-2-positive cases had an IM. However, difference in the presence of *H. pylori* infection between HER-2-positive and HER-2-negative groups was not significant and 78.60% of HER-2-positive patients were *H. pylori* negative. Although in their study, most of HER-2-negative GC patients were *H. pylori* negative. In the other study that had been performed by Lee *et al*.,[28] it was shown that 65.5% of multiple early GC patients and 64.20% solitary early GC cases were *H. pylori* positive. Unfortunately, in most studies on GC, infection with *H. pylori* considered as a side factor in demographic data and not investigated as a pivotal agent in HER-2 expression in statistical analysis.[13,28]

Different rates of *H. pylori*-positive status among above-mentioned studies probably results from differences in *H. pylori* detection methods.[11] Routine methods that used for *H. pylori* assessment is able to detect only active infection. Therefore, selection of GC patients with treated *H. pylori* infection and undetected infection could affect obtained results.[13]

According to the ToGA reports, frequency of HER-2 overexpression varies among different mainlands. Therefore, all of the effective factors in cancer prevalence, such as race, genetic basis, diet, and incidence of *H. pylori* infection may affect HER-2 expression and result in different rates of it in various populations.[1] Potential of *H. pylori* infection in gastric carcinogenesis influenced by the interaction of many factors, including host immune response, host genetic susceptibility, genetic factors of the bacterium, virulence determinants of bacteria, bacterial strains, and environmental agents.[8,23]

We compared the HER-2 expression between all *H. pylori*-positive and *H. pylori*-negative subjects (regardless of the type of gastric lesion) and found that the difference between them was statistically significant. Positive expression of HER-2 in *H. pylori*-positive cases was more than *H. pylori*-negative subgroup.

The rates of HER-2 expression varied in GC patients from different studies, it could be due to the difference in

methods of protein and gene detection and other differences that were mentioned above.[1]

There are some evidence suggest that *H. pylori* should be eradicated to an early prevention of cancer development in patients with precancerous gastric lesions.[8,17] As, it has been reported that only *H. pylori* eradication in nonatrophic gastritis was able to prevent GC progression.[27]

Our study has some limitations, including the lack of family history, lifestyle, and patient follow-up in the archived data forms. The combination of two or more methods for determination of HER-2 positivity and *H. pylori* status could increase the sensitivity and specificity of diagnosis. However, because of the limitation on costs and time, performing all of those techniques is impractical in clinical practice.

Conclusion

The present study proposed that HER-2 overexpression in the early lesions of gastric tissues probably had important roles in the progression of cancer and above-mentioned process was probably associated with the presence of *H. pylori* infection in the early stages of precancerous lesions. The underlying molecular mechanisms that result in different expression of HER-2 in the presence of *H. pylori* infection is unclear and requires further investigation.

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Conflicts of interest

There are no conflicts of interest.

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