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Original Article

A histopathological view at the long-term effects of COVID-19 on the gastrointestinal system in children: a single center experience

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ABSTRACT

Background. Subacute and chronic long-term effects of coronavirus disease 2019 (COVID-19) in different organ systems have been studied in post-COVID patients recently. COVID-19 may cause gastrointestinal (GI) system findings due to the presence of its receptor, angiotensin converting enzyme type 2 (ACE2), which is extensively expressed in the GI tract. In this study, we aimed to evaluate the post-infectious histopathological alterations of COVID-19 in pediatric patients who had GI symptoms.

Methods. Fifty-six specimens of upper endoscopic biopsies (including esophagus, stomach, bulbus and duodenum) obtained from seven patients and 12 specimens of lower endoscopic biopsies obtained from one patient who had GI symptoms after having COVID-19 (proven by polymerase chain reaction [PCR]) were evaluated as the study group. Forty specimens from five patients presenting with similar complaints but without COVID-19 were selected as the control group. All biopsy materials were immunohistochemically stained with the anti-SARS-CoV-2S1 antibody.

Results. In all biopsies of the study group, anti-SARS-CoV-2S1 antibody was detected with moderate cytoplasmic positivity in epithelial cells and inflammatory cells in the lamina propria. No staining was observed in the control group. Epithelial damage, thrombus, or no other specific findings were detected in the GI tract biopsies of any of the patients.

Conclusions. The virus antigen was detected immunohistochemically in the stomach and duodenum, but not in the esophagus, even months after infection and causes gastritis and duodenitis. No specific histopathological finding was observed from non-COVID-19 gastritis/duodenitis. Therefore, the post-COVID-19 GI system involvement should be kept in mind in patients presenting with dyspeptic symptoms even if several months have passed.

Key words: COVID-19, gastrointestinal, child, endoscopy, immunohistochemistry, biopsy.

COVID-19, caused by the novel coronavirus named SARS-CoV-2, was announced as a pandemic by the World Health Organization (WHO) in March 2020. The first case in Türkiye was seen on March 11, 2020. According to WHO data, more than 750 million cases and more than 6.5 million deaths have been reported worldwide, to date.[1]

Although it is seen in all age groups, it differs between adults and children. While the most common clinical symptoms are fever and cough, the majority of the pediatric age group is asymptomatic and has mild to moderate disease. Moreover, leukocyte counts in children are generally normal compared to adults. The incidence of critical illness and vomiting

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symptoms in children under 1 year of age was found to be higher than in the normal population. Although COVID-19 is typically an airborne infection, its receptor, angiotensin converting enzyme type 2 (ACE2) is extensively expressed along the GI tract. Diarrhea was reported in 8% of COVID-19 (+) patients, and vomiting in 7%.[2] Atypical clinical symptoms associated with the GI system and prolonged shedding of virus or viral fragments in the feces of infected individuals raised concerns about whether the virus can be transmitted by the oral-fecal route and the GI tract serves as a reservoir for reinfection.[2-7]

In this study, we aimed to evaluate the symptoms, histopathological alterations and endoscopic findings caused by COVID-19 in the GI tract of pediatric patients.

Material and Methods

In this retrospective study, fifty-six specimens of upper endoscopic biopsies (including esophagus, stomach, bulbous and duodenum) obtained from seven patients and 12 specimens of lower endoscopic biopsies obtained from one patient who had alarm GI symptoms after having COVID-19 infection (proven by PCR) were evaluated as the study group.

SARS-CoV-2 PCR positivity was checked from the Turkish Ministry of Health information system. Patients who had one or more positive results on the system were included as the study group.

Endoscopic evaluation decision was made according to the presence of alarm symptoms, according to American Academy of Pediatrics Subcommittee on Chronic Abdominal Pain as involuntary weight loss, difficulty swallowing (dysphagia) or painful swallowing (odynophagia), significant vomiting (bilious, protracted, projectile, or otherwise worrisome), chronic severe diarrhea (≥3 loose or watery stools per day for more than two weeks) or nocturnal diarrhea, unexplained fever, urinary symptoms (change in bladder function, dysuria, hematuria, flank pain), back pain, positive family history (inflammatory bowel disease (IBD), celiac disease, peptic ulcer disease, familial Mediterranean fever), bloody diarrhea or melena, skin changes (rash, eczema, hives), deceleration in linear growth (i.e., height gain <5 cm/year in a prepubertal child) and/or delayed puberty, oral aphthous ulcerations, localized right upper quadrant tenderness, localized pain, organomegaly, perianal abnormalities (i.e., skin tags, fissures, fistulae), and a stool sample positive for occult blood.[8]

None of the patients had a known chronic disease and their complaints emerged after the COVID-19 infection. The control group was selected as eight patients of the same age and gender, who presented with similar complaints and alarm symptoms and no positive PCR for SARS-CoV-2 at any time before or during onset.

All PCR results were negative during the endoscopy in both the study and control group.

Upper endoscopic biopsies were taken from the esophagus, stomach, bulbous and duodenum and lower endoscopic biopsies were taken from the ileum, colon and rectum and then were evaluated with hematoxylin and eosin (H&E) staining in both groups. Biopsies of seven patients in the study group and five patients in the control group were immunohistochemically stained with the anti-SARS-CoV-2 Spike Glycoprotein S1 (SARS-CoV-2S1) antibody.

Biopsy samples were fixed in %4 formaldehyde solution and embedded in paraffin with a routine procedure. Three millimeter-thick sections were cut from tissue blocks and stained with H&E. The SARS-CoV-2 Spike Glycoprotein S1 (rabbit polyclonal antibody, Abcam, Cambridge, UK, Cat# ab275759, gr3366181-3: 1:200 dilution) was used for the immunohistochemical staining and the procedure was held according to the manufacturer's instructions.

Verbal and written consent was taken from the patients and parents.

Ethics approval

The ethics committee of Ankara Training and Research Hospital approved the study protocol with the number E-21/724.

Results

Five girls and three boys were included and the median age was 16 (7-17) years in both groups included in the study. The mean time between the COVID-19 PCR positivity and biopsy was 5.8 months in the study group.

In the study group, the esophagus, stomach, bulbous and duodenum biopsies of seven patients and the ileum, colon and rectum biopsies of one patient were evaluated. There were no findings other than congestion in the esophageal biopsies and rare lymphocytes in the squamous epithelium in four biopsies (Fig. 1A). In gastric biopsies, two samples of each corpus and antrum were evaluated. All patients had active gastritis (Fig. 1B). In duodenum and bulbous biopsies, mild active inflammation was observed in six patients and chronic non-specific inflammation was observed in one patient (Fig. 1C). Biopsies of the ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum were examined from one patient. Flattening of the villi was noted in ileum biopsy. Edema was present in the colon biopsies. In the GI tract biopsies of eight patients, no epithelial damage, thrombus, or specific finding was found.

In the control group, the esophagus, stomach, bulbous and duodenum biopsies of seven patients and colon and rectal biopsies of one patient were evaluated. There were no findings other than congestion in esophageal biopsies and rare lymphocytes in the squamous epithelium in two biopsies. In gastric biopsies, two samples of each corpus and antrum were evaluated. All patients had active gastritis. In the duodenum and bulbous biopsies, mild active inflammation

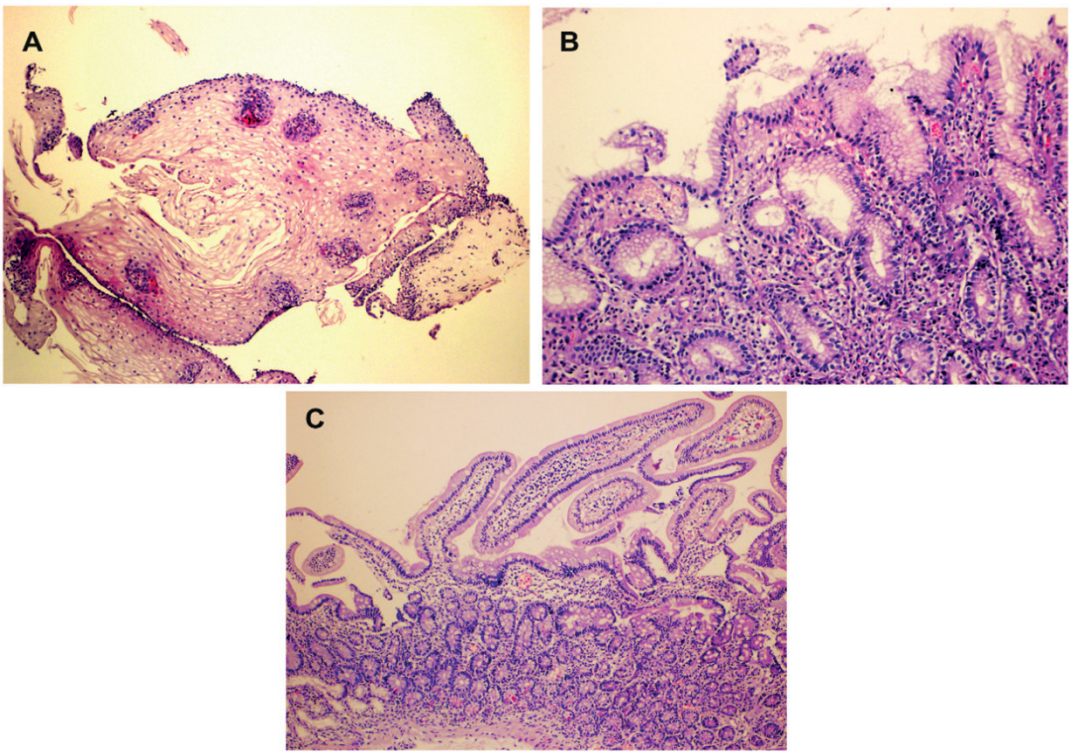

Fig. 1. Hematoxylin and eosin (H&E) staining.
Congestion in esophagus **(A)**, active chronic inflammation in gastric mucosa **(B)**, chronic inflammation in duodenum **(C)** of study group. (Magnification: X100 for **A** and **C**; X200 for **B**)

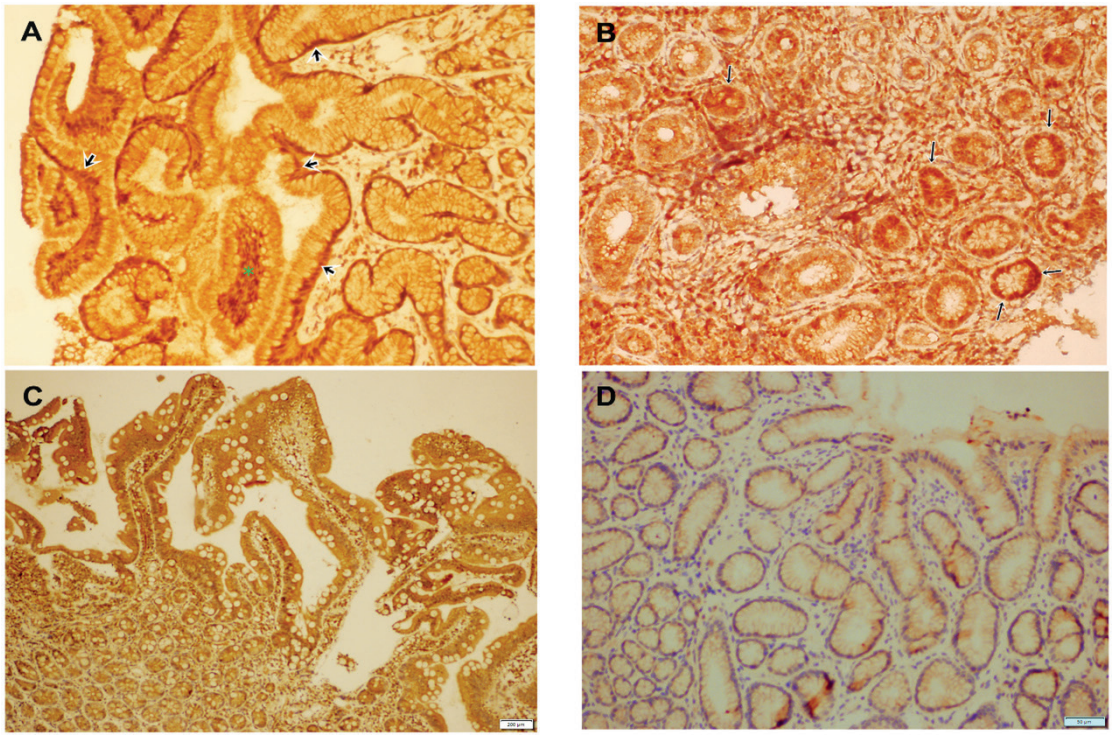

Fig. 2. Anti-SARS-CoV-2S1 antibody immunohistochemical staining.
Positive staining of anti-SARS-CoV-2S1 antibody in gastric mucosa **(A, B)**, duodenal mucosa **(C)** of study group. Negative staining of anti-SARS-CoV-2S1 antibody in gastric mucosa **(D)** of control group.
(Magnification: X200 for **A** and **D**; X100 for **B** and **C**)

was observed in two patients and chronic non-specific inflammation was observed in five patients. In one patient, the biopsies of the cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum were examined. Minimal active inflammation was observed in cecum and transverse colon biopsies, and non-specific chronic inflammation was observed in other biopsies.

Gastric (Fig. 2A, 2B) and duodenal biopsies (Fig. 2C) in seven cases of the study group, gastric and duodenal biopsies in five cases of the control group were evaluated by immunohistochemical staining with the anti-SARS-CoV-2S1 antibody. In all biopsies of the patient group, moderate cytoplasmic positivity was detected in epithelial cells and inflammatory cells in the lamina propria. No staining was observed in four cases in the control group (Fig. 2D), and weak cytoplasmic staining was observed in gastric epithelium and inflammatory cells in only one case.

Discussion

In the present study, moderate cytoplasmic positivity was detected in all biopsies with immunohistochemical staining with the anti-SARS-CoV-2S1 antibody in epithelial cells and inflammatory cells in the lamina propria in the study group, whereas no staining was observed in the control group. In addition, this finding may be evidence that the virus stays longer in the digestive system of the patients than the respiratory system and the inflammatory response lasts longer. To the best of our knowledge, this was one of the first studies evaluating the histopathological effects of COVID-19 on the GI tract in pediatric patients.

Although COVID-19 is typically an airborne infection, its receptor, ACE2 is extensively expressed along the GI tract.[3]

SARS-CoV-2 infection is manifested by respiratory symptoms but GI symptoms such

as nausea, vomiting, and diarrhea are also reported and common.[9,10]

SARS-CoV-2 consists of a spike (S), membrane (M), and envelope proteins that are embedded in the lipid bilayers and nucleic capsid (N) proteins covering single-stranded RNA. The S proteins are the key structures that attach to host cell receptor proteins ACE2 which is also present on the small intestinal epithelial cells in the GI tract in the duodenum, jejunum, and ileum but not the colon.[10,11]

ACE2 is considered as a homolog of ACE, but they have opposite actions in the body. ACE2 converts angiotensin 2 (Ang 2) to angiotensin 1-7 (Ang (1-7)). Ang (1-7) shows its effects via activation of its cognate G protein-coupled Mas receptor (MasR). ACE2/Ang (1e7)/ MasR pathway is considered anti-inflammatory and antifibrotic whereas ACE/AngII/AT1R pathway is proinflammatory and pro-fibrotic.[10,12]

Physiologically, ACE2/Ang (1e7) mediates neutral amino acid transport and facilitates the release of antimicrobial peptides to decrease gut dysbiosis and prevent inflammation in the GI tract. ACE2 functions decrease during SARS-Cov-2 infection and it may lead to an increase in Ang 2 and Ang (1-7). This may result in gut dysbiosis and inflammation in the GI tract.[10]

On the other hand, leaky gut can be mitigated or exacerbated with either the gain or loss of ACE2 expression as shown in animal models. Therefore, interaction of SARS-CoV-2 with ACE2 in the GI tract may lead to damage of the barrier function via disrupting barrier proteins ZO-1, occludin, and claudins, and increase in inflammatory cytokine production, which in turn may lead to dysbiosis and exacerbation of intestinal inflammation.[10,13,14]

It has been reported that 20% of children with COVID-19 were found to be asymptomatic, on the other hand, 7-8% of symptomatic cases presented with vomiting and diarrhea.[2] However, drug-induced GI symptoms such as nausea, abdominal pain, and diarrhea cannot be ignored, as many patients develop diarrhea after hospitalization or medication administration.[9] In the study group, two (28.5%) patients had vomiting, four (57.1%) patients had epigastric pain, four (57.1%) patients had regurgitation and five (%71.4) had anorexia. Three patients had received a proton pump inhibitor and/or antacid therapy before their admission. One patient had been hospitalized in the intensive care unit for 13 days with the diagnosis of multi-system inflammatory syndrome in children and adolescents (MIS-C) and received anakinra therapy four months before their admission.

A recent review estimated the prevalence of GI symptoms to be 17.6% in patients with COVID-19 [14]. The exact incidence of digestive symptoms is a matter of debate, with one study reporting anorexia as the most common symptom (39.9-50.2%), while other studies report diarrhea most commonly in both adult and pediatric populations (2-49.5%).[9,15] Fang et al. found that more than 50% of the cases with diarrhea presented after admission and initiation of antiviral therapy, and approximately 22.2% of them had loose stools before diagnosis of COVID-19.[16] There are reports of GI bleeding in addition to acute hemorrhagic colitis in the literature.[17] In our study, only one patient had diarrhea in the acute period of COVID-19, but there was no diarrhea at the time of admission to our clinic. Except for one patient, who received anakinra treatment, none of seven patients in study group received non-steroidal anti-inflammatory drugs (NSAID), steroids or antibiotics. No patient received specific antiviral treatment.

Except for nonspecific anorexia, approximately 20% of the patients develop GI symptoms. In our study, five (71.4%) patients had anorexia, but none of the patients had any complaints of weight loss leading to percentile loss. GI symptoms generally worsen with the progression of the disease and show a more insidious onset of the disease. In our study, all patients stated that dyspeptic complaints started after respiratory tract complaints. None of our patients had any complaints of GI bleeding.

SARS-CoV-2 binds to the ACE2 receptor and enters cells and multiplies. Although COVID-19 is typically an airborne infection, its receptor, ACE2, is present throughout the GI tract and virus has been identified in the surface epithelial cells of the stomach, small intestine and stomach, and in the colon. Atypical clinical symptoms associated with the GI system and evidence of prolonged shedding of virus or viral fragments in the feces of infected individuals have raised concerns over whether the virus can be transmitted by the oral-fecal route and whether the GI tract acts as a reservoir for reinfection.[18] According to Wang et al.[5], live virus from stool samples raised the possibility of fecal transmission of SARS-CoV-2, which might be resistant to the acidic environment in the human intestine and could be transmitted via the fecal route, as it was detected in the stools of approximately 32.8% of positive cases.[3,5]

However, while reverse transcription-polymerase chain reaction (RT-PCR) can detect viral fragments, but not the entire virus, stool cultures for SARS-CoV-2 are still incomplete or have low specificity.[19] Therefore, further studies are needed to understand the mechanisms of transmission and incubation times, along with contagiousness and clinical course duration.

The presence of viral nucleocapsid protein in COVID-19(+) patients has been confirmed in nearly the entire GI lumen, such as the stomach, duodenal, and rectal glandular epithelial cells, except for the esophagus. In our study, from a histological point of view, the GI epithelium shows plasmacytic and lymphocytic infiltration with interstitial edema mainly in the stomach, duodenum, and rectum, and patchy lymphocytic infiltration in the esophagus.

Therefore, COVID-19 can cause digestive symptoms through direct viral invasion in target cells and/or immune-mediated tissue and end-organ damage.[7] Consistent with the literature in our study, H&E examinations in the COVID-19 positive group revealed congestion in esophageal biopsies and rare lymphocytic infiltration in the squamous epithelium. Active inflammation with lymphocytic and plasmacytic infiltration was observed in all gastric biopsies, and in all duodenum and bulbous biopsies except one, and edema was observed in colon biopsies. However, interestingly, flattening of the villi was detected in the ileum biopsy, and as far as we can observe in the literature, such a finding has not been detected before. Congestion was detected in esophageal biopsies similar to colon biopsies.

It has been reported in the literature that extensive alveolar damage consisting of permanent and intracapillary thrombosis in alveolar epithelial cells and capillary endothelial cells was detected in lung biopsy of COVID-19(+) patients.[20] In contrast to the lung, no epithelial damage, thrombus, or specific finding was detected in any of the eight patients' GI tract biopsies in our study.

In the control group, gastric biopsies had similar findings, while chronic inflammation was found in all patients except two, in duodenal and bulbous biopsies. This supports the fact that the patients started to complain of diarrhea and abdominal pain after COVID-19, and that chronic inflammation had not yet developed. However, the fact that the current histopathological findings were also in our control group shows that it does not cause gastritis and/or duodenitis specific to SARS-CoV-2.

In our study, moderate cytoplasmic positivity was detected in all biopsies with immunohistochemical staining with anti-SARS-CoV-2S1 antibody in epithelial cells and inflammatory cells in the lamina propria in the study group, whereas, no staining was observed in the control group. In addition, this finding may be evidence that the SARS-CoV-2 stays longer in the digestive system of the patients than the respiratory system and that the inflammatory response lasts longer.

A higher incidence of critical illness and vomiting symptoms was found in children under one year of age, which may be a finding that the

virus replicates less in acidic environments. The acute character of the inflammation detected in our study revealed the reason why the patients presented with vomiting and it may also explain the higher incidence of vomiting in patients under 1 year of age, whose gastric pH value is more alkaline compared to older ages.

Entry of viruses into the cells is an important part of interspecies transmission. All coronaviruses encode a surface glycoprotein and spike protein that bind to host cell receptors and mediate virus entry.[21,22] A protein that binds to Spike1-glycoprotein was used in the immunohistochemical staining used in our study.

The distribution of ACE2 staining positivity is mainly in the cytoplasm of gastric and intestinal epithelial cells and in the cilia of glandular epithelial cells. This indicates that SARS-CoV-2 can invade target organs of the digestive tract via ACE2 receptors and cause primary damage.[23-25] In all biopsies of the patient group, moderate cytoplasmic positivity was detected in epithelial cells and inflammatory cells in the lamina propria.

ACE2 is a key enzyme in the renin-angiotensin system and is thought to play an important role in intestinal inflammation and regulating diarrhea.[26,27]

However, He et al.[28], pathological results of autopsies performed on patients who died from COVID-19 by ACE2, proinflammatory cytokines including interleukin (IL)-1β and IL-6 were highly expressed so it was reported that proinflammatory cytokine expression did not occur in cells that did not express ACE2.[15,28]

In our study, only one patient with the diagnosis of MIS-C received anakinra (IL-1 blocker) treatment, but no different findings were found in the pathology preparation examined, compared to other patients in the study group.

The strength of the present study is the long term histopathological evaluation of the GI tract after COVID-19. We are aware of the small number of patients, but 108 pathology specimens were evaluated. Each patient who underwent upper GI endoscopy had biopsies taken from the esophagus, stomach, and duodenum, and one patient had 12 colon biopsies taken from various segments of the colon.

The study combines both clinical and pathological evaluation and offers some recommendations for treatment. All preparations were examined using a special stain for SARS-CoV-2 with a special technique.

The limitations of our study were the low number of patients in the study and control groups, as it would be difficult to conduct such a study with a large number of patients in the pediatric population, and the failure to detect asymptomatic carriers prior to their admission.

In this study, we detected COVID-19 in the stomach and duodenum of pediatric patients even months after the infection which resulted in gastritis and duodenitis. For this reason, the history of COVID-19 should be questioned in patients who are admitted to pediatric outpatient clinics with dyspeptic complaints, and it should be kept in mind that SARS-CoV-2 may cause GI system complaints even several months after the infection.

The results obtained in our study need to be supported by more comprehensive studies in order to gain certainty. At this point, we think that our study will shed light on more comprehensive studies in the future.

Ethical approval

The ethics committee of Ankara Training and Research Hospital approved the study protocol with the number E-21/724. Verbal and written consent was taken from the patients and parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SS; data collection: SS, ÖT, ŞK; analysis and

interpretation of results: SS, MÇ, SH; draft manuscript preparation: SS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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