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Investigation of Intestinal Protozoon Prevalence in Immunocompromised Patients at a University Hospital

Bir Üniversite Hastanesinde İmmünsüpresif Hastalardaki İntestinal Protozoon Prevalansının Araştırılması

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ABSTRACT

Objective: Immunocompromised patients are at a greater risk of developing intestinal parasite infections. In this study, we examined the presence of *Enterocytozoon bieneusi*, *Encaphalitozoon intestinalis* and other intestinal protozoa in stool samples of immunosuppressed patients.
Methods: A total of 100 stool samples were obtained from patients receiving chemotherapy because of solid organ tumour with haematological malignancies and those receiving immunosuppressive treatment because of rheumatic diseases, organ transplant patients and patients receiving treatment for HIV-related infections. Stool samples were examined by using the native-lugol method in which the stool concentration, modified Kinyoun acid-fast and trichrome staining methods and parasite presence were analysed. The stool samples were also examined for the presence of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* using an indirect fluorescent antibody method.
Results: Intestinal parasites were detected in 12% of all patients. The distribution of intestinal parasites in patients were 7% *Blastocystis* spp., 2% *Blastocystis* spp. + *Dientamoeba fragilis*, 1% *Blastocystis* spp. + *Entamoeba coli*, 1% *Blastocystis* spp. + *Giardia intestinalis* and 1% *G. intestinalis*. *Microsporidia* spp. were detected in 4% of all patients by the IFAT method and in 8% of all patients by calcoflour staining method.
Conclusion: In our study, the most prevalent parasite detected in the immunosuppressed patients was *Blastocystis* spp. The pathogenesis of *Blastocystis* spp. remains to be controversial, and their role in immunocompromised patients continues to remain unknown. Although these rates detected in our study are similar to the prevalence in the normal population, it is important to study these microorganisms in immunocompromised patients in terms of the associated decreasing morbidity and mortality rates.
Keywords: Immunosuppression, *Microsporidia* spp., parasites, *Blastocystis* spp.

ÖZ

Amaç: Bağışıklık sistemi baskılanmış hastalar, fırsatçı bağırsak parazit enfeksiyonları bakımından risk altındadır. Bu çalışmada, bağışıklık sistemi baskılanmış hastaların dışkı örneklerinde *Enterocytozoon bieneusi* ve *Encephalitozoon intestinalis* ile diğer bağırsak protozoonlarının varlığının incelenmesi amaçlanmıştır.
Yöntemler: Solid organ tümörü nedeniyle kemoterapi alan ya da hematolojik malignitesi olan, romatolojik hastalıklar nedeniyle immünsüpresif tedavi gören, organ nakli yapılan ve HIV enfeksiyonu nedeniyle tedavi almakta olan toplam 100 hastadan dışkı

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örnekleri alınmıştır. Dışkı örnekleri nativ-lugol yöntemi, dışkı konsantrasyon, modifiye Kinyoun asit-fast ve trikrom boyama yöntemleriyle incelenerek protozoon varlığı araştırılmıştır. Dışkı örnekleri, indirekt floresan antikor yöntemi kullanılarak *Enterocytozoon bieneusi* ve *Encephalitozoon intestinalis* varlığı açısından da incelenmiştir.

Bulgular: Hastaların %12'sinde, bir ya da daha fazla sayıda intestinal protozoon saptanmıştır. İntestinal protozoonların dağılımı ise %7 *Blastocystis* spp., %2 *Blastocystis* spp. + *Dientamoeba fragilis*, %1 *Blastocystis* spp. + *Entamoeba coli,* %1 *Blastocystis* spp. + *Giardia intestinalis* ve %1 *G. intestinalis* şeklindedir. *Microsporidia* spp. IFA yöntemi ile %4, kalkoflor boyama ile %8 oranında saptanmıştır.

Sonuç: Çalışmamızda, immünsüpresif hastalarda en fazla saptanan intestinal protozoon *Blastocystis* spp.'dir. *Blastocystis* türlerinin patojenitesi halen tartışmalı olup, immünsüpresif hastalardaki rolü tam olarak bilinmemektedir. Çalışmamızda bulunan bu oranlar normal toplumdaki prevalanslarla benzer olmakla birlikte; bağışıklık sistemi baskılanmış hastalarda bu mikroorganizmaların araştırılması, morbidite ve mortalitenin azaltılması açısından önem taşımaktadır.

Anahtar kelimeler: İmmünsüpresyon, *Microsporidia* spp., parazit*, Blastocystis* spp.

INTRODUCTION

Number of immunocompromised patients continues to increase as a consequence of immunosuppressive therapies for malignancies and immune-mediated disorders, infections with the human immunodeficiency virus (HIV), hematopoietic and solid organ transplants. These patients are at greater risk for developing more severe parasitic infections than are immunocompetent individuals. Although the pathogenesis of parasitic infections in immunosuppression is poorly understood, immunocompromised hosts are more likely to acquire infection, develop severe and disseminated disease and be unable to clear parasites (1,2). Intestinal protozoan infections are more frequently seen in the immunocompromised host. The most common intestinal protozoa in these patients are *Cryptosporidium, Cyclospora* and *Cystoisospora*. In recent years, *Blastocystis* spp. and *Microsporidia* spp. are reported to be emerging pathogens in the immunocompromised hosts (2).

Cryptosporidium is a coccidian protozoon parasite which may lead to diarrhea in both developed and developing countries. In immunocompetent patients cryptosporidiosis is generally asymptomatic or self-limited, however; in immunocompromised patients it can cause chronic and severe diarrhea and lead to life threatening disease because of dehydration. Extraintestinal manifestations like cholecystitis, pancreatitis and respiratory infection may also be seen in these patients (3-5). The other coccidian protozoa *Cyclospora* and *Cystoisospora* are more common in tropical and subtropical areas. Cyclosporiasis may vary from asymptomatic or moderate infection to severe intestinal disease and may present as traveler's diarrhea and as waterborne or foodborne outbreaks. *Cyclospora* and *Cystoisospora* infections are more severe, chronic and fatal in immunocompromised patients similar to cryptosporidiosis (6-8).

Microsporidia are obligate intracellular microorganisms which are closely related to fungi (9). *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common species that cause intestinal microsporidiosis. Although asymptomatic infections or watery diarrhea are common in immunocompetent patients, opportunistic infections including cholangitis, cholecyctitis, non-specific sinusitis and rhinitis may develop in case of immunosuppression (10,11).

In this study, we investigated the existence of intestinal protozoa in the stool samples obtained from immunocompromised patients.

METHODS

Patients

A total of 100 adult immunocompromised patients who were receiving immunosuppressive therapies for rheumatic diseases, transplantation or cancer and being treated for hematopoietic malignancies or HIV infection were included in the study. Data on demographic, clinical and laboratory parameters were recorded for each patient.

Protocol of the study was reviewed and approved by Non-interventional Ethics Board/Committee (decision number: G101493-09, date: 05.03.2014). Detailed information about the study was given to each participant in the study and an informed consent form was signed.

Sample Collection and Laboratory Analyses

One or more consecutive fresh stool samples were collected from the patients and transferred to the laboratory within 30 minutes after defecation. Stool concentration method (Parasep® Fecal Parasite Concentrators, Apacor, USA), direct microscopic examination (saline and iodine), Wheatley's trichrome staining and Kinyoun's acid fast staining methods were performed for identification of intestinal parasites (12-15). The presence of *E. bieneusi* and *E. intestinalis* antigens in the stool samples were investigated by a commercial indirect fluorescent antibody (IFA) kit in accordance with the manufacturer's instructions (Bordier Affinity Products, SA) and calcoflour staining method (16). All tests were performed in parasitology section of department of medical microbiology.

Statistical Analysis

Because of the low number of the patients, statistical analyses could not be made.

RESULTS

The age of the patients included in the study ranged from 19 to 76 years and 61% were male (Table 1). Forty-four patients were receiving immunosuppressive therapies for cancers such as lung cancer and breast cancer or being treated for hematopoietic malignancies such as lymphoma. Thirty-seven were receiving immunosuppressive drugs for rheumatic diseases such as ankylosing spondylitis and rheumatoid arthritis. Ten were receiving immunosuppressive therapies for solid organ transplantation. Five had HIV infection and four had hypogammaglobulinemia (Table 2).

Table 1. Age and gender distribution of the patients

Patients	n (%)	Mean age
Female	39 (39%)	49.3
Male	61 (61%)	46.4

Out of 100 patients, 26% had diarrhoea, in besides of 74% of the patients had no symptoms that point to a parasitic infection such as diarrhoea, abdominal pain, nausea or vomiting.

Intestinal parasites were detected in 12% of the stool samples examined with direct microscopic examination (saline and iodine), Wheatley's trichrome staining and stool concentration methods. The distribution of intestinal parasites in these patients were *Blastocystis* spp. in 7% (Figure 1A), *Blastocystis* spp. plus *Dientamoeba fragilis* (Figure 1B) in 2%, *Blastocystis* spp. plus *Entamoeba coli* in 1%, *Blastocystis* spp. plus *Giardia intestinalis* in 1%, *G. intestinalis* in 1%. Out of 12 patients whose stool samples revealed intestinal parasites, eight of them had diarrhea (Table 3).

Cryptosporidium, Cyclospora or *Cystoisospora* species were not detected with Kinyoun's acid fast staining method in any of the patients.

E. intestinalis was found in the 4% of the immunocompromised patients by using IFAT method (Figure 2A). *Microsporidia* spp. were detected in the 8% of the patients by calcoflour staining method (Figure 2B). None of these patients had gastrointestinal symptoms (Table 4).

According to these results, the most common parasite detected in immunosuppressed patients was *Blastocystis* spp. with a rate of 11%. Among these patients 63.6% had diarrhea.

One of the limitations of this study was the lack of control group. Due to the absence of a control group, statistical analysis could not be performed.

Table 2. Clinical distribution of the patients

Department	Diagnosis	n (%)
Rheumatology	Rheumatic diseases	37 (37%)
Hematology	Hematopoietic malignancies	26 (26%)
Medical oncology	Cancers	18 (18%)
General surgery	Transplantation	10 (10%)
Infectious diseases	HIV infection	5 (5%)
Infectious diseases	Hypogammaglobulinemia	4 (4%)

HIV: Human immunodeficiency virus

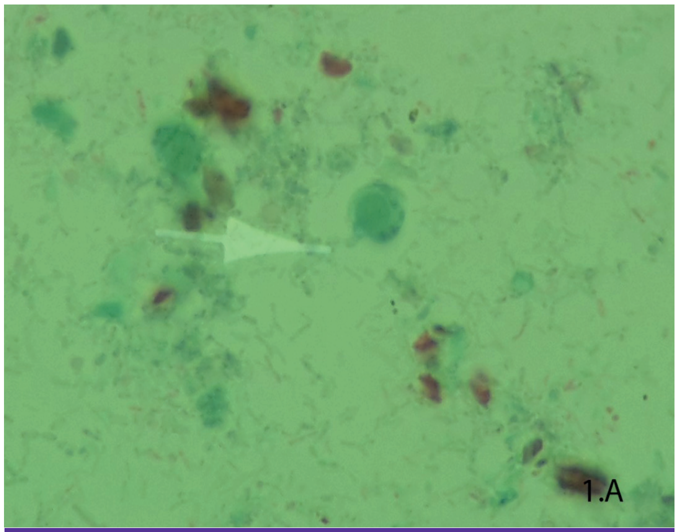

Figure 1A. *Blastocystis* spp. (Trichrome staining, x1000)

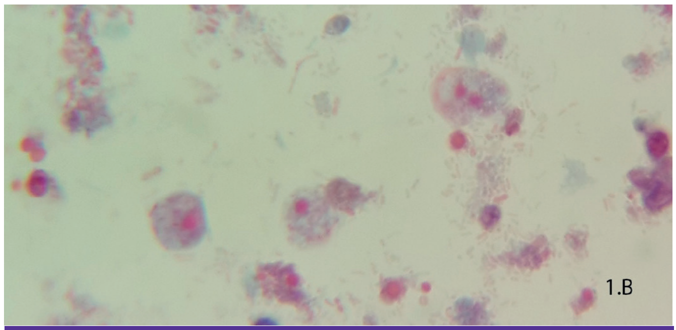

Figure 1B. *D. fragilis* (Trichrome staining, x1000)

DISCUSSION

Immunosuppression is an important risk factor for developing infections. There is a wide range of factors that causes immunosuppression in patients. Genetic deficiencies in functional compartment of the immune system such as common variable immunodeficiency (CVID), selective IgA deficiency and X-linked agammaglobulinemia is called primary immunodeficiency

Table 3. Distribution of intestinal parasites detected in immunocompromised patients

	Department	Patient's diagnosis	Diarrhea	Intestinal parasites
1	Infectious diseases	Hypogammaglobulinemia	+	*G. intestinalis* + *Blastocystis* spp.
2	Infectious diseases	Hypogammaglobulinemia	+	*G. intestinalis*
3	Infectious diseases	Hypogammaglobulinemia	+	*E. coli* + *Blastocystis* spp.
4	Medical oncology	Endometrial cancer	+	*D. fragilis* + *Blastocystis* spp.
5	Rheumatology	Ankylosing spondylitis	+	*D. fragilis* + *Blastocystis* spp.
6	Rheumatology	Ankylosing spondylitis	+	*Blastocystis* spp.
7	Rheumatology	Ankylosing spondylitis	+	*Blastocystis* spp.
8	Rheumatology	Ankylosing spondylitis	-	*Blastocystis* spp.
9	Rheumatology	Rheumatoid arthritis	+	*Blastocystis* spp.
10	Rheumatology	Rheumatoid arthritis	-	*Blastocystis* spp.
11	Hematology	Chronic lymphocytic leukemia	-	*Blastocystis* spp.
12	Hematology	Chronic lymphocytic leukemia	-	*Blastocystis* spp.

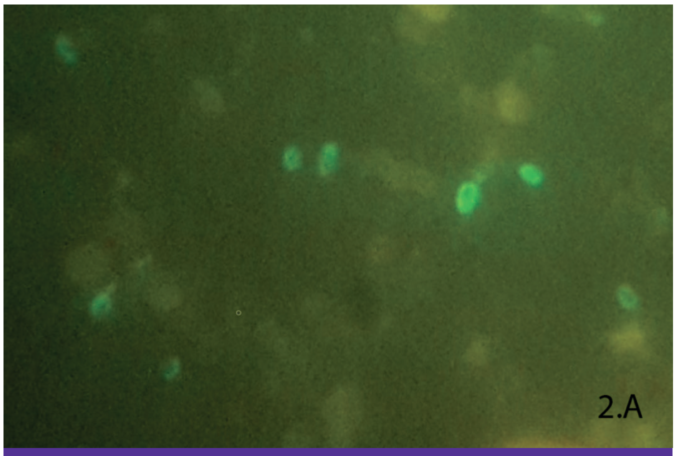

Figure 2A. *E. intestinalis* (IFAT, x1000)

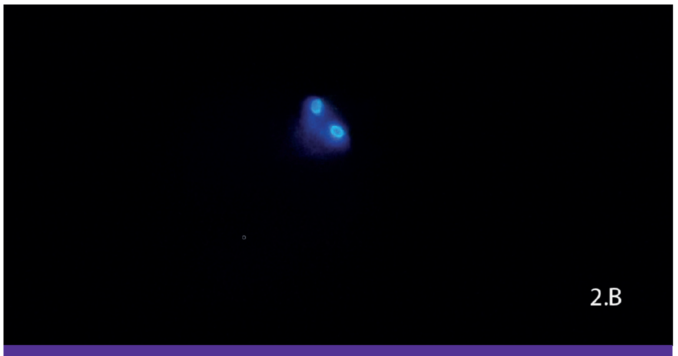

Figure 2B. *Microsporidia* spp. (calcoflour staining, x1000)

diseases. Secondary immunodeficiencies may develop due to another illness, condition or as a result of treatment. Metabolic diseases (e.g. diabetes mellitus, protein-losing enteropathy), treatments for malignancies, rheumatologic and autoimmune diseases, transplantation and HIV infection are among the causes of secondary immunodeficiencies (17).

Intestinal parasitic infections (IPI) are important causes of morbidity and mortality in patients with immunodeficiency. In this study, we aimed to investigate the prevalence of intestinal parasites and *Microsporidia* spp. in patients with immunocompromising conditions.

In a study conducted to investigate the prevalence of intestinal pathogens in patients with different immunological status, intestinal parasites were detected in both immunocompetent (6.5%) and immunocompromised patients (4.6%) (18). In another study held in Iran, the stool samples of 135 hemodialysis patients, 50 renal transplant recipients, 60 cancer patients and 20 HIV/AIDS patients were investigated for IPI. Intestinal parasites were found in 11.7% of the patients and *Blastocystis* spp. (4.2%) were the most prevalent parasite. In this study, the highest infection rate was detected among HIV/AIDS patients (25%). So indeed, HIV-infected patients are more susceptible to opportunistic intestinal infections (19). Accordingly, in a prospective study, out of 137 HIV-infected patients, 78.8% were diagnosed IPI and the most prevalent parasites were *Blastocystis* spp. (26.3%), *E. histolytica/dispar* (12.4%) and *G.intestinalis* (8.8%) (20). In a study conducted in Turkey, 65 HIV-positive patients were investigated for the presence of intestinal parasites and the distribution of the parasites was *Cryptosporidium* spp. (21.5%), *Cyclospora* spp. (3.1%) and *Blastocystis* spp. (10.8%) (21). In our study, five HIV-infected patients' stool samples did not reveal any intestinal parasite, that's probably due to the low number of patients.

Table 4. Distribution of intestinal parasites detected in immunocompromised patients, according to gender, age, presence of diarrhea and diagnosis

	***Blastocystis* spp.**	***G. intestinalis***	***D. fragilis***	***Microsporidia* spp. (Calcoflour staining)**	***E. intestinalis* (*Microsporidia* IFAT)**
Gender					
Female (n=39)	4	-	1	2	-
Male (n=61)	7	2	1	6	4
Age					
18-40 (n=32)	3	2	-	5	2
41-60 (n=49)	4	-	2	3	2
>61 (n=19)	4	-	-	-	-
Diarrhea					
Positive	7	2	2	-	-
Negative	4	-	-	8	4
Diagnosis					
Rheumatic diseases	6	-	1	4	3
Hematopoietic malignancies	2	-	-	1	-
Cancers	1	-	1	-	-
Transplantation	-	-	-	3	1
HIV infection	-	-	-	-	-
Hypogammaglobulinemia	2	2	-	-	-

HIV: Human immunodeficiency virus

Hypogammaglobulinemia is a disorder that is characterized by deficiency of immunoglobulins in the blood and CVID is the most common cause. Patients with hypogammaglobulinemia have an increased susceptibility to pathogens affecting mucous membranes of gastrointestinal tract (22). In a study conducted in Turkey, 26 of 37 CVID patients had diarrhea and intestinal parasites were detected in 13 of them. The most common parasites detected in this study were *Cryptosporidium* spp. (69.2%) and *G. intestinalis* (53.8%) (23). In the current study, four patients with hypogammaglobulinemia had diarrhea and three of them were diagnosed with IPI. *Blastocystis* spp. and *G. intestinalis* were detected in these patients' stool samples; however; *Cryptosporidium* spp. were not found in any of the patients. Essentially, intestinal coccidian parasites are common cause of diarrhea in immunocompromised individuals. In a study, out of 350 immunocompromised patients, *Cryptosporidium* spp. and *C. belli* oocysts were detected in three (0.9%) and four (1.1%), respectively (24). In another study, *Cryptosporidium* spp. were found in six (10.1%) and *C. belli* in four (6.7%) of 59 HIV-infected patients (25). Similarly, the higher prevalence of *Cryptosporidium* spp. in pediatric oncology patients with diarrhea compared to non-oncology patients is reported in a previous study (26). Taş Cengiz et al. (27) reported *C. belli* infection determined in both immunosuppressed and immunocompetent children. In our study, three (6.8%) of the 44 oncology/hematology patients' stool samples revealed *Blastocystis* spp.; although; none of them had intestinal coccidian parasites.

Rheumatologic and autoimmune diseases are the other causes of immunosuppression due to the use of immunomodulatory or immunosuppressive therapies (28). These patients have an increased risk of severe manifestations of IPI (29). Some intestinal cryptosporidiosis cases reported in patients using low-dose prednisone for rheumatoid arthritis and adalimumab for ankylosing spondylitis (30,31). In a study conducted in 36 patients with rheumatoid arthritis and ankylosing spondylitis, the frequencies of intestinal parasites were 25% and 33%, respectively. The difference between intestinal parasite detection rates in healthy and patient groups was not found statistically significant (32). In our study, six (16.2%) of the 37 patients receiving immunosuppressive therapies for rheumatic diseases had IPI, and *Blastocystis* were detected in all of these patients. *Blastocystis* is reported to be associated with a variety of gastrointestinal disorders; although; the pathogenic role of parasite is still controversial (33). In a study held in China, out of 381 stool samples collected from cancer patients, 7.1% and 1.3% were found positive for *Blastocystis* and *E. bieneusi,* respectively. In addition, significant association of *Blastocystis* with diarrhea was found in cancer patients (34). Similarly, *Blastocystis* were detected in 11% of the immunocompromised patients and 63.6% of them had diarrhea in our study.

Intestinal Microsporidia may cause severe and life-threatening infection in patients with immunodeficiency. In a previous study, *E. bieneusi* was detected in 14.5% (19/131) of the immunocompromised patients and 1.47% (1/68) of the immunocompetent individuals. A significant difference was detected between patients with immunodeficiency and healthy individuals in terms of prevalence of *E. bieneusi* (35). In another study, out of 310 samples collected from immunocompromised patients, 20% (62/310) were positive for *E. bieneusi* and 8.3% (26/310) were detected as other *Encephalitozoon* species by using molecular methods (36). In a study conducted with 98 rheumatology patients and 92 healthy individuals, *Microsporidia* spp. were identified significantly higher in patients in underlying immunosuppression; although; the prevalence of other intestinal parasites was similar in both patient and control groups (37). In another study, the prevalence of *E. intestinalis* and *E. bieneusi* in cancer patients under chemotherapy was detected very high (38). In our study, intestinal *Microsporidia* spp. were found mostly in rheumatology patients. Although it is reported that *E. bieneusi* is the most common *Microsporidia* species isolated from immunocompromised patients (39), in our study, *E. intestinalis* was identified more, but; none of the patients whose stool samples revealed *Microsporidia* had diarrhea. On the other hand, one or more intestinal parasites were detected in 30.8% of immunocompromised patients with diarrhea. Therefore, intestinal parasites should be investigated in these patients regardless of the presence of gastrointestinal symptoms.

Varying intestinal parasite prevalence rates in the previous studies as compared to that in the present study may be due to the patients' immunodeficiency level, number of patients enrolled the studies or methods used. The limitations of our study were the low number of patients and the inability to use molecular methods, but, our results may help to indicate the prevalence of *Microsporidia* spp. and intestinal parasites in our region. One of the other shortcomings of the study is that a healthy control group could not be included in the study. Another major limitation is the relatively low number of patients with diarrhea which is a further challenge in interpretation of the results in terms of existence of the opportunistic pathogen in relation to its pathogenicity on diarrhea.

CONCLUSION

When all results are evaluated, *Blastocystis* were the most prevalent intestinal parasite in 11% and *Microsporidia* spp. in 10% of the immunocompromised patients. Although these rates in our study are similar to those in the normal population, the investigation of these microorganisms in patients with immunodeficiency or individuals undergoing immunosuppressive drug treatment is important for reducing severe infections and mortality.

INFORMATION

Poster presentation in the 4. National Clinical Microbiology Congress. 08-12 Oct 2017, Ankara, Turkey.

* Ethics

Ethics Committee Approval: Protocol of the study was reviewed and approved by Non-interventional Ethics Board/Committee (decision number: G101493-09, date: 05.03.2014).

Informed Consent: Detailed information about the study was given to each participant in the study and an informed consent form was signed.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Surgical and Medical Practices: A.Ç.İ., S.A., O.A., A.İ.E., Y.B., Concept: F.K., A.Ç.İ., S.E., Design: F.K., S.E., Data Collection or Processing: F.K., A.Ç.İ., S.A., O.A., A.İ.E., Y.B., Analysis or

Interpretation: F.K., S.A.A., Y.A., S.E., Literature Search: F.K., Writing: F.K.İ, A.Ç.İ., S.A.A., Y.A., S.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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