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Original article

Evaluation The Significance of Anti-streptolysin O and Anti-deoxyribonucleases B in The Diagnosis of Streptococcus pyogenes in Sore Throat Patients

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Abstract:

- ***Background*:** Sore throat is a commonly observed condition in pediatric clinics and emergency departments, with viral infections being the most frequent cause. This study aimed to assess the impact of serum levels of ASO (anti-streptolysin O) and ADNB (anti-DNase B) in patients with sore throat infected with streptococcus pyogenes.
- ***Methods:*** A cross-sectional study was conducted between December 15, 2022, and March 15, 2023. A total of 317 sore throat patients aged 5 to 17 years were recruited from Samarra General Hospital, Salahuddin General Hospital, private clinics, and ear healthcare centers. The control group comprised 80 healthy individuals matched with the study participants. Throat swabs were collected and inoculated on blood agar and MacConkey media for microbial examination. Blood samples were also collected from patients and controls to determine ASO and ADNB levels using ELISA-based assays.
- ***Result:*** The study's results indicate that 30.28% of the children were infected with *streptococcus pyogenes*, with varying percentages of other bacterial infections observed. A significant association was found between *S. pyogenes* infection and urban residence. Additionally, elevated levels of ADNB (171.4±33.5 pg/ml) and ASO (390.5±108.1 IU/ml) were detected in *S. pyogenes* infected children compared to the control group (p-value = 0.0001). The lower limit of ASO in infected children was 209.4 IU/ml, while the upper limit in the control group was 172.1 IU/ml.
- ***Conclusions:*** The study highlights significant alterations in immune markers ASO and ADNB levels among children infected with *streptococcus pyogenes*, implicating their potential role in the pathogenesis of the infection.
- **Keywords:** Anti-streptolysin O, anti-DNase B, *streptococcus pyogenes*, sore Throat.

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INTRODUCTION

Sore throat is a common occurrence in pediatric healthcare settings, including clinics and emergency departments, and it is predominantly caused by viral infections. However, approximately 25% of sore throat cases in children can be attributed to a bacterial infection known as *streptococcus pyogenes* [(1)]. *Streptococcus pyogenes* is a significant bacterial pathogen that affects humans and can manifest in various ways, ranging from mild localized infections to severe life-threatening invasive infections [(2)].

Among children aged 5-14 years, group A streptococci (GAS) pharyngitis, caused by *streptococcus pyogenes*, is the most prevalent form of pharyngitis, accounting for approximately 37% of all cases in this age group. Globally, there are 18.1 million reported cases of severe *S. pyogenes* infections, with an estimated 1.78 million new cases occurring annually [(3)]. *Streptococcus pyogenes* typically colonizes the pharynx, anus, and genital mucosa. Infections caused by this bacterium are highly contagious and can be transmitted through airborne droplets, contact with nasal discharge, contaminated objects or surfaces, skin contact with infected lesions, or contaminated food sources [(4)]. Crowded environments such as military camps, nursing homes, and schools facilitate the transmission of the organism, leading to epidemics of group A streptococci infections [(5)].

Multiple virulence factors contribute to the clinical manifestations of *streptococcus* pyogenes. The bacterial capsule, composed of hyaluronic acid, provides protection against phagocytosis. M protein, lipoteichoic acid, and protein F are responsible for the attachment of the bacteria to host cells. Streptococcus pyogenes also produces exotoxins, including pyrogenic (erythrogenic) toxin, which causes the characteristic rash seen in scarlet fever and toxic shock syndrome. Other virulence factors include streptokinase, streptodornase, hyaluronidase, and streptolysins, which facilitate tissue invasion [(6, 7)].

The diagnosis of *streptococcus pyogenes* (group A streptococci) infection can be made through bacterial culture or serological testing. The widely accepted serodiagnostic tests for this infection are antistreptolysin O (ASO) and antideoxyribonuclease B (ADNB) [(7)]. ASO is the most popular and standardized serological test, offering not only diagnostic value but also assistance in follow-up processes and the evaluation of treatment effectiveness. ASO is particularly useful when throat culture techniques are inadequate or when the patient has already taken antibiotics [(8)]. Anti-deoxyribonuclease B (ADNB) titers measure the concentration of serologic antibodies specific to the DNase B enzyme produced by streptococcus pyogenes. These titers provide quantitative information regarding the presence of these antibodies in the blood [(5)]. The aim of this study was to evaluate the immunological biomarkers, specifically the ASO titer and ADNB, in patients presenting with sore throat.

MATERIALS and METHODS:

Prior to the commencement of the cross-sectional study in Salah Al-din Governorate from December 15, 2022, to March 15, 2023, written consent approval was obtained from the parents or legal guardians of all patients and control group participants, ensuring ethical compliance and protecting the rights of the participants involved in the research. The study focused on 317 sore throat patients, aged between 5 and 17 years old, who were selected from Samarra General Hospital, Salah Al-din General Hospital, private clinics, and health care centers. Additionally, 80 healthy individuals within the same age range and without any acute or chronic diseases were carefully matched as the control group. These control group participants were drawn from the aforementioned hospitals and health care centers, ensuring a comprehensive and representative study population.

Methods

- Ask the person to tilt their head back slightly, open their mouth as wide as possible.
- Depress the person's tongue and ask them to say, "Ahh."
- Collect the throat culture by rubbing the sterile swab tip on the surface of one or both tonsils, the tonsillar pillars, or the posterior pharyngeal wall. Other areas of the oral pharynx and mouth are not acceptable sites and could lead to false negative results.
- Gently move the swab without touching the teeth, gums or tongue.
- Place the swab immediately into the sterile tube or collection device using aseptic technique.
- Label the collection tube with patient name, date of birth, source and date of collection.
- Throat swabs from children were collected and aseptically inoculated on Petri plates containing blood agar and MacConkey media. After incubation, the colonies were morphologically and microscopically examined. The colonies were subcultured on solid media several times and preserved at 4 °C for further investigations. Microorganisms were identified on the basis of their morphological characteristics on selective and differential media. API Strept kits were used for biochemical testing to confirm the identity of microorganisms.

Five ml of blood was collected by vein puncture using a five ml syringe from each patient and control persons. Blood samples were placed into in plane tubes, left for 30 minutes at 37 °C for clotting and centrifuged at 3000 rpm for 15 minutes, sera from were then aspirated and transferred into Eppendorf tubes for determination of ASOT, ADNB, Vitamin D and IL-37 by Enzyme linked immunosorbent assay (ELISA).

This study was conducted in accordance with the principles of the Declaration of Helsinki and the local regulations governing research involving human subjects. The data were entered and analyzed using IBM SPSS Statistics 26 software. Descriptive statistics, including frequency and percentage for categorical data, and mean and standard deviations for continuous data, were calculated. The normality of the data was assessed using the Kolmogorov-Smirnov test. To compare the means of ASO and ADNB between the study and control groups, two-sample t-tests were utilized. Statistical significance was determined at a p-value of less than 0.05.

RESULTS

The findings of the study revealed the presence of various bacteria in the throat swabs of the examined children. Among the 317 isolated bacteria, 30.28% (96 out of 317) were identified as *Streptococcus pyogenes*, while 14.20% were classified as Group *G Streptococci*. Moreover, 6.94% of the samples tested positive for *H. influenzae*, 8.52% were found to be infected with S. aureus, and 10.73% showed the presence of *E. coli*. Additionally, 3.15% of the samples were identified as *K. pneumoniae*. Notably, 17.03% of the children exhibited negative growth in their throat swabs, as indicated in Figure 1. These results shed light on the distribution and prevalence of bacterial strains among the studied population.

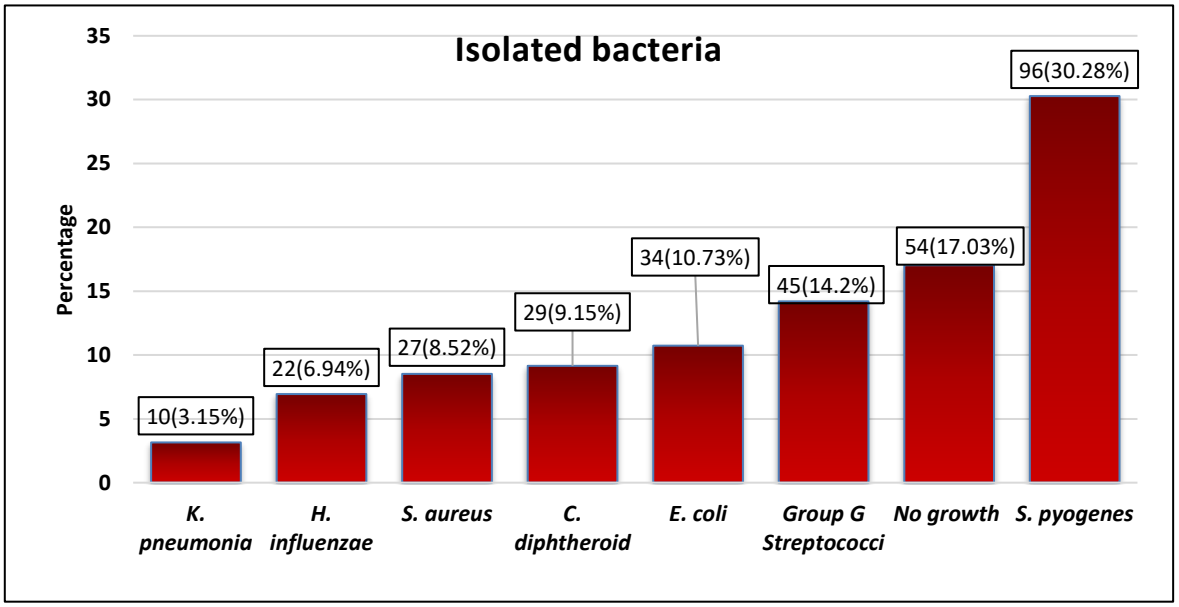

Figure 1: Isolated bacteria from throat swabs of studied children

According to this study, it was found that 75 (78.13%) of the children diagnosed with *S. pyogenes* infection resided in urban areas, while 21 (21.88%) came from rural areas, as depicted in Figure 2.

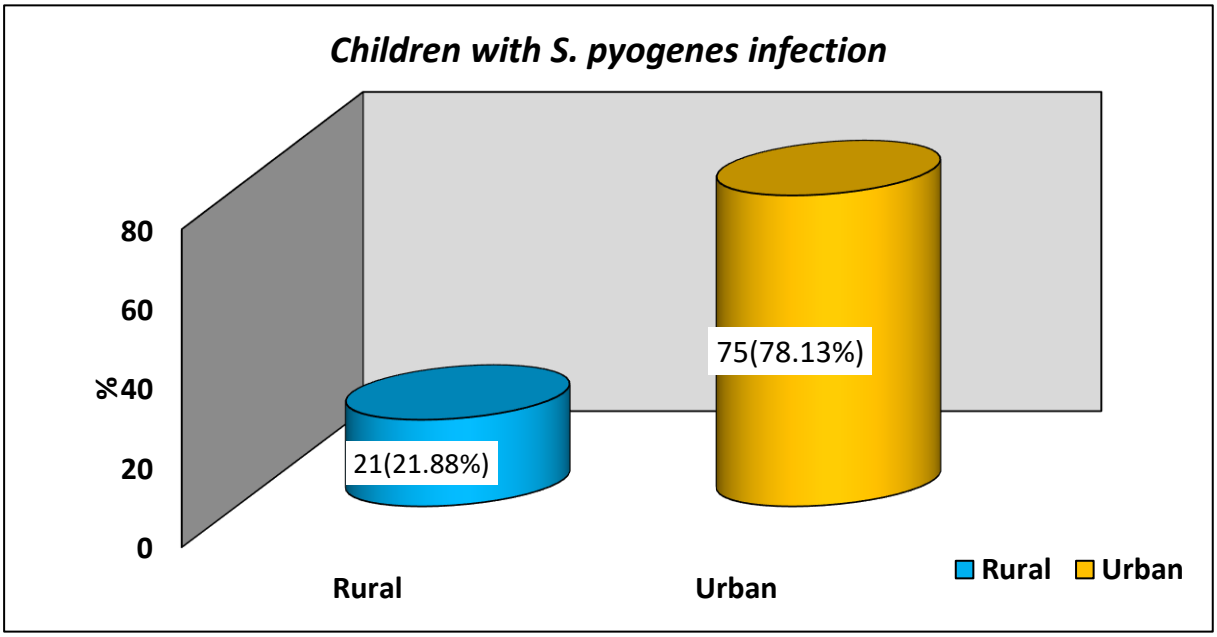

Figure 2: Residence distribution of children with *S. pyogenes infection*

Figure 3 illustrates that a significant proportion of S. pyogenes isolates obtained from children with throat infections exhibited susceptibility to various antibiotics. Cefepime, vancomycin, ceftriaxone, carbenicillin, clindamycin, and co-trimoxazole displayed favorable efficacy rates of 87.5%, 83.33%, 81.25%, 71.87%, 68.75%, and 65.63%, respectively. Conversely, a considerable resistance rate was observed against amikacin (60.42%) and ampicillin (79.17%).

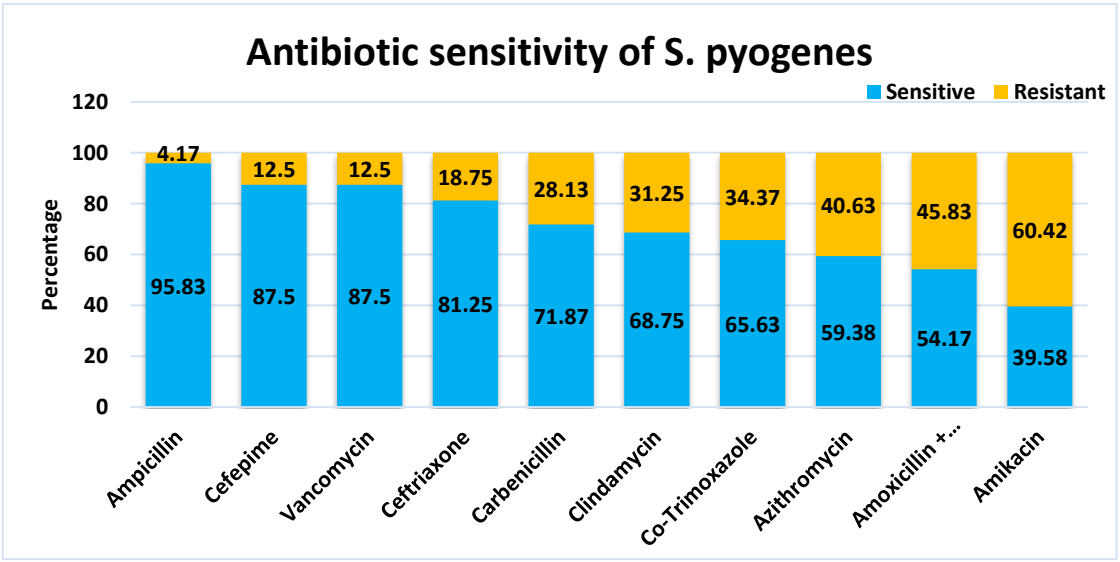

Figure 3: Antibiotics sensitivity test of S. pyogenes isolates.

The study revealed that all children who tested positive for S. pyogenes infection displayed a range of symptoms, including fever and hyperemia. Furthermore, they experienced edema, exudate, painful glands, petechiae, and dysphagia, as indicated in Table 1.

Table 1: Clinical features distribution of children with *S. pyogenes* infection

Associated clinical features	Children with *S. pyogenes* infection.	
	No.	%
Fever	96	100
Hyperemia	96	100
Edema	88	91.67
Exudate	88	91.67
Painful glands	90	93.75
Petechiae	91	94.79
Dysphagia	93	96.88

The results presented in Table 2 showed a significant increase (P= 0.0001) in the mean level of ASO (Antistreptolysin O) among children with S. pyogenes infection (390.5±108.1 IU/ml) compared to the control group (98.6±18.4 IU/ml). Furthermore, there is a significant increase (P= 0.0001) in the mean serum ADNB value among children with S. pyogenes infection (171.4±33.5 pg/ml) compared to the control group (56.8±10.4 pg/ml).

Table 2: Level of ASO and ADNB in children with *S. pyogenes* infection and control group

Studied groups	No.	ADNB (pg/ml)		P Value†	ASO (IU/ml)		P Value†
		Mean	SD		Mean	SD	
***Streptococcus pyogenes* patients**	96	171.4	33.5	0.0001*	390.5	108.1	0.0001*
Control group	80	56.8	10.4		98.6	18.4	

† *two-sample t-test, *Significant P<0.05.*

DISCUSSION

The study revealed that 30.28% of the children examined had Streptococcus pyogenes bacteria, while 14.20% had Group G Streptococci, 6.94% were infected with *H. influenzae*, 8.52% tested positive for *S. aureus*, 10.73% had *E. coli*, and 3.15% had *K. pneumonia.* Additionally, 17.03% of the throat swabs showed negative growth (Figure 1). These findings align with previous studies conducted in Iraq [1,2] that have identified group A beta-hemolytic streptococci (GABHS) as a major cause of tonsillitis based on positive culture results for these microorganisms.

Among the bacteria isolated from throat swabs, Streptococcus species, especially S. pyogenes, had the highest occurrence rate. This is consistent with the study conducted by Ho et al. [3], which found that S. pyogenes was the most frequently isolated bacteria in patients with throat infections. This correlation suggests that Streptococcus species, particularly *S. pyogenes*, have a preference for thriving in the throat's mucus membranes. The throat's mucus membranes provide an environment conducive to the growth and colonization of certain bacteria. Streptococcus species have developed adaptations that allow them to survive and multiply in this specific niche. These bacteria possess adhesion factors that enable them to attach to the surface of throat epithelial cells, facilitating their colonization [4]. Furthermore, Streptococcus species employ mechanisms to evade or counteract the host's immune responses (GAS uses S protein to bind to RBC fragments which it "expresses" on it surface, shielding it from phagocytosis by macrophages), which further aids their persistence in the throat [5]. The ability of Streptococcus species, including *S. pyogenes*, to flourish in the throat's mucus membranes is also influenced by their virulence factors. For instance, *S. pyogenes* produces various toxins and enzymes that contribute to tissue damage and inflammation, allowing the bacterium to establish infection and evade the immune system [6,7,8]. It is important to note that while Streptococcus species, particularly S. pyogenes, are commonly associated with throat infections, other bacteria and even viral pathogens can also cause similar symptoms. Accurate identification of the causative agent is crucial for appropriate treatment and management of throat infections [9].

Several reasons may explain why *Streptococcus pyogenes* had the highest occurrence rate among bacteria isolated from throat swabs in this study. First, *S. pyogenes* can easily spread through respiratory droplets when an infected person coughs or sneezes, making streptococcal infections highly contagious [9]. Second, *S. pyogenes* can colonize the throat and persist without causing symptoms. This asymptomatic carrier state can contribute to the high occurrence rate when throat swabs are taken [10]. Third, throat swabs are specifically designed to detect bacteria in the throat, including Streptococcus species. Therefore, when throat swabs are performed, *S. pyogenes* is more likely to be detected compared to other types of bacteria [6]. Finally, throat infections due to *S. pyogenes* often exhibit seasonal patterns, with higher occurrence rates during colder months. This could be due to factors such as increased indoor crowding, close contact, and decreased immune response during the winter season [10].

The study findings revealed that a significant majority of children (78.13%) with *S. pyogenes* infection were from urban areas, while a smaller proportion (21.88%) were from rural areas. This result is consistent with the findings of Abd Al-Kareem et al. [11], who reported that the majority of children with *S. pyogenes* infection lived in crowded urban areas. Factors such as population density, increased human interaction, and limited access to healthcare resources in urban settings could contribute to higher transmission rates. Urban areas often have higher population densities and may exhibit socioeconomic disparities, which can affect healthcare access, hygiene practices, and living conditions, thereby influencing the prevalence of *S. pyogenes* infection [12,13]. The close contact between individuals in urban areas, such as in schools, daycares, and crowded living conditions, may contribute to a higher transmission rate of the bacteria [14,15].

In agreement with our findings, Mahdi [16] observed the highest sensitivity of antibiotics, such as cefepime, vancomycin, ceftriaxone, and carbenicillin, in treating *S. pyogenes* infections. These results are similar to those reported by Kebede et al. [17], who found that Cefotaxime and Cephalexin had high sensitivity (80.6% and 72.5%, respectively), while *S. pyogenes* showed complete resistance to Amikacin. The high resistance to Amikacin may be due to the vulnerability of the bacteria to enzyme modification, rendering the antibiotic ineffective, or the loss of outer membrane proteins, reducing the antibiotic's permeability within the cell [18].

Consistent with previous studies, the clinical manifestations of streptococcal infections were observed in the current study. Symptoms such as fever, malaise, sore throat, difficulty swallowing, headaches, and abdominal pain were commonly reported. Tonsillitis was characterized by edematous and hyperemic tonsils with confined purulent exudates, while peritonsillar abscess, although rare, caused a toxic appearance, a fluctuant mass, and asymmetric uvula deviation [16,17]. Fever is a common symptom in bacterial infections, including those caused by S. pyogenes, indicating an immune response to the infection and aiding in distinguishing it from other illnesses.

Streptolysin O, produced by *Streptococcus pyogenes*, induces beta-hemolysis, leading to the complete lysis of red blood cells. This characteristic is often used to identify and differentiate group A streptococci on blood agar plates. The formation of a clear zone around colonies on the blood agar indicates beta-hemolysis and the presence of streptolysin O [21]. ASO antibodies in the blood are commonly measured to diagnose recent or previous streptococcal infections, such as strep throat or scarlet fever. Elevated levels of ASO antibodies indicate recent exposure to streptolysin O and suggest an immune response against a streptococcal infection [22]. Bennett et al. [23] observed increased ASO levels in the sera of children with tonsillitis due to S. pyogenes infection compared to healthy controls. The increase in ASO antibodies can serve as an indicator of recent or ongoing streptococcal infection, particularly in cases of tonsillitis. Similar studies have also reported elevated ASO levels among children with tonsillitis due to S. pyogenes infection [24].

In this study, children with S. pyogenes infection exhibited ASO levels with a lower limit of 209.4 IU/ml, while the upper limit in the control group (presumably healthy individuals

without S. pyogenes infection) was 172.1 IU/ml. A similar study conducted by Abdou et al. [23] reported standard values below 200 IU/ml, and the lower limit of normal was even higher at 398.5 IU/ml. This increase in ASO levels could be attributed to widespread, large untreated streptococcal infections. Additionally, geographic location and specific climatic and socioeconomic circumstances may contribute to variations in ASO levels among populations [24].

The upper limit of normal ASO titers frequently varies according to geographic area, season, and site of infection. Determining the upper limit of normal ASO titer is crucial to prevent overdiagnosis of streptococcal infection based on elevated titers, which is a common challenge in daily practice [25]. Previous studies have reported different upper normal limits of ASO titer, such as <200 IU/ml in Iraq [26], 326 IU/ml in Korea [27], 305 IU/ml in India (Mumbai) [28], 239 IU/ml in another study from India [29], and 200 IU/ml in Tanzania [30]. These variations may be attributed to specific geographic locations and their unique climatic and socioeconomic circumstances [26].

Streptococcus pyogenes produces several nucleases that are crucial for the bacteria's escape from neutrophil extracellular traps. Among the four streptococcal deoxyribonucleases (DNase), DNase B elicits the most consistent immunologic host response [29]. The study findings suggest that children with S. pyogenes infection exhibited higher levels of ADNB compared to the control group. The statistical significance indicates that the observed difference in mean ADNB levels between the two groups is unlikely to have occurred by chance alone, but rather suggests a genuine association between S. pyogenes infection and increased ADNB levels. Consistent with these findings, Karmarkar et al. [30] reported elevated levels of anti-DNase antibodies among patients with tonsillitis. Previous studies also support the elevation of ADNB antibody levels among children with tonsillitis due to S. pyogenes infection [32-34].

CONCLUSION:

This study presents compelling evidence of noteworthy changes in immune markers, specifically ASO and ADNB levels, among children infected with Streptococcus pyogenes compared to the control group. The findings indicate the potential involvement of these immune markers in the pathogenesis of S. pyogenes infection. Further investigations are necessary to delve into the underlying mechanisms and explore potential therapeutic implications associated with these immune marker alterations in children infected with S. pyogenes. Understanding the role of ASO and ADNB in the immune response to S. pyogenes infection could contribute to the development of targeted therapies and improved management of this common bacterial infection in children.

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Conflicts of interest:
There are no conflicts of interest**.**

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