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The diagnostic value of transfistular contrast-enhanced ultrasound in branchial cleft fistulas

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Abstract

Aims: This study aimed to assess the diagnostic value of transfistular contrast-enhanced ultrasound (CEUS) for branchial cleft fistulas (BCF), using surgical findings as the reference standard. **Materials and methods:** Fifty-six patients were initially suspected of having BCF. Among them, 35 patients who underwent two-dimensional ultrasound (2D-US), transfistular CEUS, and before surgery were retrospectively enrolled. The diagnostic accuracy of transfistular CEUS and 2D-US for BCF detection was compared. The agreement between CEUS and surgical findings in internal orifice localization and BCF classification was assessed. **Results:** Among the enrolled 35 patients, 29 were surgically confirmed as BCF. Postoperative findings served as the reference standard for all analyses. Transfistular CEUS demonstrated superior diagnostic accuracy compared to 2D-US for BCF detection (91.4% vs. 82.9%). Excellent agreement was observed between CEUS and surgical findings in internal orifice localization (κ=0.814, $p<0.001$). For BCF classification, the accuracy, sensitivity, and specificity of transfistular CEUS were as follows: type I BCF (93.1%, 93.8%, 92.3%), type II BCF (89.7%, 71.4%, 91.3%), and pyriform fossa fistulas (96.6%, 100.0%, 95.7%), respectively. **Conclusions:** As a radiation-free, cost-effective, and non-invasive technique, transfistular CEUS can accurately diagnose BCF, precisely localize its internal orifice, and effectively differentiate its subtypes. For patients clinically suspected of BCF, transfistular CEUS is a reliable preoperative tool for individualized clinical guidance.

Keywords: Branchial cleft fistula; Contrast-enhanced ultrasound; Two-dimensional ultrasound

Introduction

Branchial cleft fistulas (BCF) are congenital anomalies arising from incomplete obliteration of the embryonic branchial apparatus, characterized by persistent communication between the ectodermal branchial cleft and endodermal pharyngeal pouch [1]. BCF typically present in two anatomical variants: complete fistulas with both internal and external openings, and sinuses with a single opening with a blind end [1-3]. Based on embryonic origin and anatomical location, BCF are classified into four types: type I arises from the cartilaginous external auditory canal, penetrates deep into the parotid gland and facial nerve, and terminates at the mandibular angle or the periauricular region; type II arises from the tonsillar fossa, passes through the carotid sheath and terminates at the anterior border of the sternocleidomastoid muscle types III and IV are collectively referred to as pyriform fossa fistulas [4-6]. These fistulas arise from the pyriform fossa or upper esophageal region, extend posteriorly through the thyroid cartilage to the posterior aspect of the carotid sheath, and terminate at the anterior border of the sternocleidomastoid muscle [7].

Clinically, BCF pose significant diagnostic and therapeutic challenges. Studies have shown that BCF patients experience a mean diagnostic delay of 4 years, with a reported aggregate misdiagnosis rate of 15.2% [8-10]. Current evidence shows a postoperative recurrence rate of 3% in primary cases, escalating to 14% in patients with

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prior infection and 21% in those with previous surgical intervention [11-13]. The primary causes of surgical failure and recurrence are incomplete lesion resection or failed internal orifice ligation [14]. Adequate preoperative delineation of the fistula course and internal orifice location via imaging is therefore critical for formulating precise surgical plans and preventing recurrence [15,16].

Current diagnostic modalities for BCF include two-dimensional ultrasound (2D-US), computed tomography (CT), magnetic resonance imaging (MRI), and laryngoscopy [17]. BCF predominantly occur in pediatric populations [18,19]. While CT has been considered a reliable tool for diagnosing occult BCF, its clinical utility is limited by the risks associated with radiation exposure and iodinated contrast media [20]. Although MRI has high soft tissue resolution, it is limited by the need for prolonged sedation in pediatric patients and its relatively high cost [17]. Studies have shown that direct laryngoscopy achieves 90% accuracy in identifying the internal orifice. However, this invasive approach carries inherent risks of general anesthesia and fails to visualize the complete fistula tract [21]. 2D-US is recommended as the preferred method for evaluating pediatric branchial cleft cysts (BCC) patients [15]. However, 2D-US performs poorly in detecting BCF internal orifices and offers limited guidance for clinical decision-making. Currently, transfistular contrast-enhanced ultrasound (CEUS) has shown high accuracy in the diagnosis of anal fistulas, intestinal fistulas in Crohn's disease, and congenital biliary fistulas [22-24]. However, its application to BCF remains to be explored. This study aimed to assess the diagnostic value of transfistular CEUS for BCF through a systematic retrospective analysis.

Materials and methods

Patients

This retrospective study was approved by our institutional review board with a waiver of informed consent. From April 2021 to February 2025, we initially identified 56 consecutive patients who were clinically suspected of having BCF at our institution. Inclusion criteria were: (1) clinical suspicion of BCF; (2) preoperative examination with both 2D-US and transfistular CEUS; and (3) subsequent surgical intervention with available surgical confirmation. Exclusion criteria included: (1) severe coagulation disorders; (2) active acute infection; (3) contraindications to contrast agent; (4) incomplete clinical data. After the condition of the patient was evaluated by clinicians and ultrasonologists, the patient or their family members made the decision to undergo US, transfistular CEUS, and subsequent surgery. Finally, 35 patients were enrolled in the final analysis. Among them, 29 patients were surgically and pathologically confirmed as BCF. The patient enrollment process is summarized in figure 1.

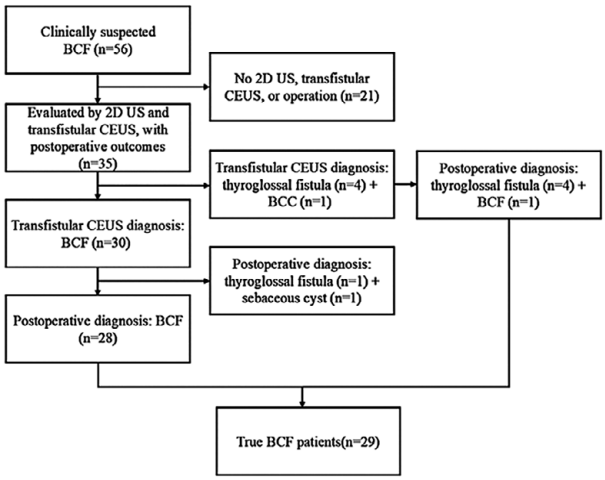

Fig 1. Flowchart for the enrollment of patients. CEUS: Contrast-enhanced Ultrasound; BCF: branchial cleft fistulas; BCC: branchial cleft cyst

Equipment and contrast agent

US was performed using an Aplio i800 system (Canon Medical, Tokyo, Japan), with a high-frequency linear array transducer (i18LX5, frequency range 5–18 MHz). SonoVue (Bracco Imaging, Italy) was reconstituted with 5.0 mL of sterile saline (0.9% sodium chloride) to form the sulfur hexafluoride microbubble suspension. Subsequently, 2.0 mL of the suspension was further diluted in 10.0 mL NS for subsequent use. The manufacturer is supplied with a specialized needle-catheter assembly and matching contrast agent syringe.

2D-US examination

Examination proceeded from the identifiable external orifice inward along the fistula tract. A standardized 2D-US assessment was then conducted, including measuring the internal luminal diameter, evaluating wall morphology, identifying intraluminal debris or obstructions, and assessing spatial relationships to adjacent vessels and tissues. The visible fistula tract was then traced along its course to its suspected termination point.

Transfistular CEUS examination

During the examination, the patient lay supine with the head turned to the healthy side to ensure adequate exposure of the affected neck.

First, the sonographer evaluated the lesion and surrounding skin for signs of local inflammation, including erythema, swelling, local skin warmth, and purulent discharge. If necessary, 2D-US was performed to assess for perifistular abscess formation. For patients in the acute infection phase, transfistular CEUS was postponed, and antibiotic therapy was prioritized. The feasibility of

transfistular catheterization was determined by the diameter of the BCF lumen and its spatial relationship with adjacent organs and vasculature.

When an external opening was present, it was prioritized as the puncture site. For cases without an external orifice, puncture was performed at the most superficial part of the fistula tract under 2D-US guidance, taking care to avoid major blood vessels and nerves.

If the external orifice was clearly visible, direct catheterization was attempted first. If direct catheterization failed, catheterization guided by a puncture needle was attempted. In the absence of a cutaneous external orifice, percutaneous puncture and cannulation were performed under real-time 2D-US guidance. Real-time 2D-US was used to ensure the puncture needle was inserted to the appropriate depth. The needle core was then fully withdrawn, followed by insertion of the catheter. The catheter end was secured to the skin with adhesive tape. After connecting the syringe to the catheter, the sonographer aspirated to check for pus or blood in the lumen. Finally, the prepared contrast agent was injected through the catheter under US guidance.

The dosage of the contrast agent ranges from 1.5 to 10 mL, depending on the following factors: fistula volume, presence of luminal adhesions or obstructions, and patency of the internal orifice. The contrast mode of the instrument was activated, and the contrast agent was rapidly injected into the fistula lumen. It was imperative to meticulously track the entire fistula tract and the internal orifice. For periauricular fistulas, enhancement in the auricular cartilage, parotid gland, and mastoid area was noted. For patients clinically suspected of having a second BCF, contrast extravasation into the oral cavity and enhancement signals within the esophageal lumen were assessed. For patients suspected of having a pyriform fossa fistula, enhancement signals in the thyroid gland, trachea, and esophageal lumen were observed.

Image storage and analysis

Both static images and dynamic video clips (initiated at contrast injection) were acquired and stored for analysis. Frame-by-frame review of the videos was performed to meticulously assess the internal orifice location, fistula tract course, branching patterns, and spatial relationship to adjacent vasculature. Key frames were stored as static images.

All examination findings were independently reviewed by two experienced US physicians. In cases of disagreement, a third senior physician, blinded to the initial assessments, was consulted to reach a consensus. Transfistular CEUS was used to determine the precise location of the internal orifice by tracking the terminal endpoint of enhanced echogenicity along the fistula tract. BCF types were classified based on the fistula tract course and internal orifice location identified during the transfistular CEUS examination or intraoperative exploration.

Statistical analysis

All statistical analyses were conducted using SPSS software (version 27.0 for Windows). Postoperative surgical findings served as the reference standard for all analyses. The accuracy, sensitivity, and specificity of 2D US and transfistular CEUS in detecting BCF were calculated. Inter-rater agreement between transfistular CEUS and postoperative findings for identifying internal orifice location was assessed using the Kappa (κ) statistic. A Kappa Value >0.8 indicated almost perfect consistency, and statistical significance was defined as $p<0.05$. Additionally, the accuracy, sensitivity, and specificity of CEUS for BCF classification were calculated.

Results

Patient characteristics

A total of 35 patients were enrolled in this study. Among them, 29 were diagnosed with BCF postoperatively, including 16 cases of the first BCF, 7 cases of the second BCF and 6 cases of pyriform fossa fistulas. The mean age of patients was 24±15 years. The clinical characteristics of 35 patients are presented in Table I.

US findings

In our cohort, the BCF tracts typically appeared on 2D-US as hypoechoic or anechoic tubular structures. Color Doppler Flow Imaging (CDFI) demonstrated sparse linear vascular signals within the fistula wall or adjacent tissue. Following contrast injection, transfistular CEUS clearly depicted the entire tract as a tube-like enhanced structure (fig 2). While the internal orifice was rarely identifiable on conventional 2D-US or CDFI, it was successfully visualized by transfistular CEUS in cases where contrast agent extravasation into the pharynx was observed.

The value of 2D US and transfistular CEUS for BCF detection

A total of 35 patients underwent 2D US, transfistular CEUS and surgery. Among them, 29 cases were confirmed with BCF postoperatively. A total of 6 cases were postoperatively confirmed as non-BCF, comprising 5 thyroglossal duct fistulas and one sebaceous cyst. Using postoperative findings as the reference standard, the accuracy, sensitivity, and specificity of 2D US for diagnosing BCF were 82.9%, 89.7% and 50.0%, respectively. The accuracy, sensitivity, and specificity of transfistular CEUS for diagnosing BCF were 91.4%, 96.6% and 66.7%, respectively (Table II).

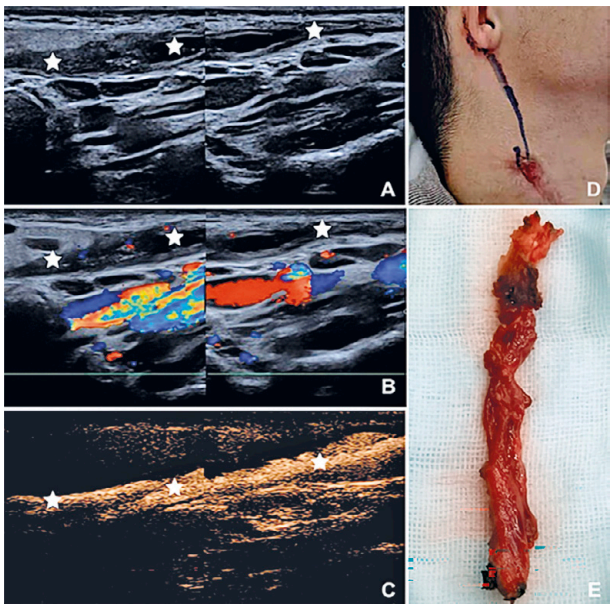

Fig 2. Images of an 18-year-old male patient with type I BCF, who had a recurrent purulent discharge for 18 years: A) 2D US revealed the fistula as a hypoechoic tubular structure (pentagrams); B) on CDFI minimal blood flow signals were observed along the fistula tract (pentagrams); C) transfistular CEUS revealed the fistula as a tubular contrast-enhanced hyperechoic tract (pentagrams); D) body marking of the fistula tract to facilitate preoperative management; E) pathological specimen of the fistula resected during surgery.

Table I. Demographic and clinical characteristics of 35 patients

Demographics	
BCF patients (n=29)	n (%)
Gender, male	17 (58.6)
Age (years)	
≤10	2 (6.9)
11-20	11 (37.9)
21-30	11 (37.9)
≥31	5 (17.2)
Side: left/right	14 (48.3) / 15 (51.7)
Presentations	
Neck mass	5 (17.2)
Neck abscess and recurrent infection	9 (31.0)
Cutaneous discharging fistula	13 (44.8)
Acute suppurative thyroiditis	2 (6.9)
Treatment before admission	
Only antibiotics	6 (20.7)
Incision and drainage	19 (65.5)
Resection	4 (13.8)
Treatment modalities	
Fistulectomy	23 (79.3)
Endoscopic internal orifice ligation	2 (6.9)
Combined surgical procedure*	4 (13.8)
Non-BCF patients (n=6)	
Thyroglossal fistula	5 (83.3)
Sebaceous cyst	1 (16.7)

* Combined surgical procedures: (1) fistulectomy with hemithyroidectomy, (2) fistulectomy with endoscopic electric/ coblation cauterization.

The value of transfistular CEUS in locating the internal orifice

Postoperative findings among the 29 patients with BCF confirmed 21 cases as complete fistulas with identifiable internal orifices and the remaining 8 as sinuses with sealed internal orifices. 2D US failed to identify the internal orifice in any of the cases, whereas transfistular CEUS preoperatively localized the internal orifices in 18 of 21 cases. Using postoperative findings as the reference standard, the findings of transfistular CEUS showed excellent agreement with surgical findings for locating the internal orifice (κ=0.814, p<0.001) (Table III).

Table II. Transfistular CEUS or 2D ultrasound for BCF detection

		Postoperative outcomes		**Accuracy (%)**	**Sensitivity (%)**	**Specificity (%)**
		BCF	**Non-BCF**			
2D US	BCF	26	3	82.9%	89.7%	50.0%
	Non-BCF	3	3			
Transfistular CEUS	BCF	29	2	91.4%	96.6%	66.7%
	Non-BCF	1	4			

Table III. Tranfistular CEUS for BCF internal opening localization

	Internal Orifice Position	**Surgical Outcomes (n)**	**Transfistular CEUS diagnosis (n)**	**Kappa**	**p-value**
Complete fistulas	External auditory canal	10	8	0.814	<0.001
	Tonsillar fossa	5	4		
	Pyriform fossa	6	5		
	Upper esophageal region	0	1		
Sinuses	Absence of an internal orifice	8	11		
Total		29	29		

Table IV. Trans-fistula CEUS for BCF classification

Transfistular CEUS diagnosis	Surgical outcomes			Accuracy (%)	Sensitivity (%)	Specificity (%)
	Type I BCF (n=16)	Type II BCF (n=7)	Pyriform fossa fistulas (n=6)			
Type I BCF (n=16)	15	1	0	93.1	93.8	92.3
Type BCF (n=6)	1	5	0	89.7	71.4	91.3
Pyriform fossa fistulas (n=7)	0	1	6	96.6	100.0	95.7

The value of transfistular CEUS in BCF classification

Using postoperative findings as the reference standard, the accuracy, sensitivity, and specificity of transfistular CEUS for BCF classification were as follows: type I BCF 93.1%, 93.8%, 92.3%, type II BCF 89.7%, 71.4%, 91.3%, and pyriform fossa fistulas 96.6%, 100.0%, and 95.7%, respectively (Table IV).

Discussion

This study demonstrated that transfistular CEUS is a valuable adjunctive imaging technique for the preoperative evaluation of BCF. Compared to conventional 2D-US, which failed to identify the internal orifice in any case, transfistular CEUS showed superior diagnostic performance, has a higher accuracy in detecting BCF (91.4% vs. 82.9%), successfully localizing internal orifices (κ=0.814, $p<0.001$), and precisely classifying fistula types. Transfistular CEUS has significant advantages over 2D-US for diagnosing BCF. However, five cases of misdiagnosis were still recorded. The potential causes of misdiagnosis were analyzed as follows:

Preoperative transfistular CEUS yielded 2 false-positive cases, including one sebaceous cyst and one thyroglossal duct fistula. The misdiagnosis of the sebaceous cyst case may be attributed to sinus formation secondary to spontaneous drainage of the sebaceous cyst after localized infection, which interfered with the judgment of US physicians. Additionally, the thyroglossal duct fistula case was misdiagnosed as sinus-type BCF. This may be attributed to fistula obstruction, which precludes the identification of the internal orifice. Alternatively, it could be due to the physician failing to evaluate the tongue root during transfistular CEUS.

The other case of internal orifice mislocalization, transfistular CEUS showed a tubular enhancement connected with the esophageal wall, with the internal orifice initially localized to the upper esophagus. However, surgical exploration revealed the internal orifice was actually located in the pyriform fossa (fig 3). The sonographer thought that the internal orifice had been adequately identified, and no further tracking was performed. This resulted in the mislocalization. This mislocalization highlights the technical challenge in distinguishing between a fistula terminating in the pyriform fossa and in the adjacent upper esophageal wall, especially when the

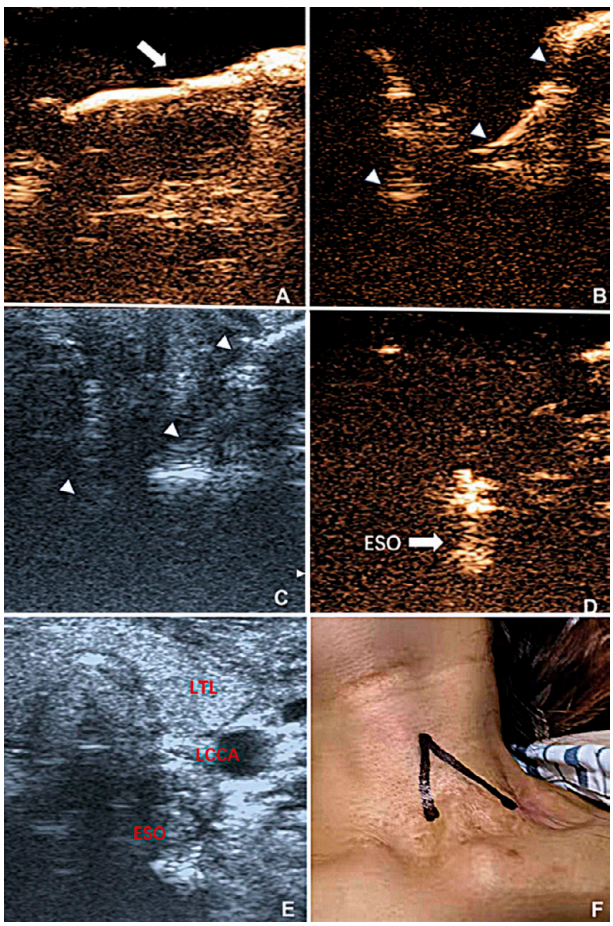

Fig 3. Images of a 27-year-old female patient with a pyriform sinus fistula. She had localized redness, swelling and ulceration in her left supraclavicular fossa for 22 years, with a history of multiple abscess incisions and drains; A) on transfistular CEUS, the puncture needle positioned within the fistula, appearing as a hyperechoic linear structure (arrow); B) and C) contrast agent diffusion along the fistula tract (arrows); D) transfistular CEUS demonstrated that the fistula tract ultimately communicated with the esophageal wall, with contrast enhancement observed within the esophageal lumen (arrow); E) transverse scan, gray scale at the left thyroid lobe (LTL) level; F) body marking of the fistula tract to facilitate preoperative management. LCCA: left common carotid artery; ESO: esophagus.

contrast agent pools at a narrowing or kink in the tract that mimics the true fistula terminus.

Two additional misclassified cases may have resulted from scar tissue formation due to multiple prior surgeries, which obstructed contrast agent diffusion. The incomplete delineation of the fistula tracts led to misdiagnosis. Mural edema and intraluminal inflammatory exudate also can impede contrast agent dispersion, consequently compromising imaging quality. Therefore, transfistular CEUS examination should be performed after inflammatory control in patients during the acute inflammatory phase [19]. In this study, no patients developed contrast agent-related allergic reactions, infection spread, or puncture-related complications.

Treatment management of BCF patients always depends on the type and the internal orifice of BCF [20,25]. Complete surgical resection remains the preferred treatment for BCF patients [21,26]. During surgical dissection along the fistular tract, meticulous care should be taken to preserve adjacent critical neurovascular structures. Type I BCF requires meticulous facial nerve preservation. Management of Type II BCF necessitates meticulous dissection with particular attention to preservation of the carotid artery and hypoglossal nerve. For the pyriform sinus fistulas, attention should be paid to the recurrent laryngeal nerve and its relationship with the thyroid gland. Alternatively, the endoscopic internal orifice ligation may be considered only for patients with uncomplicated fistulas and a clearly well-defined internal orifice. Combined surgery may help reduce the recurrence rate [27,28]. In this study, the efficacy of transfistular CEUS in the internal orifice localization and the BCF classification has been validated. Pre-surgery transfistular CEUS is essential for formulating individualized surgical strategies, achieving complete resection and substantially mitigating risks of nerve injury and recurrence.

Transfistular CEUS is a novel technology for diagnosis of BCF. It is an innovative non-intravascular contrast imaging technique. By directly injecting SonoVue contrast agent into the fistula lumen, it enables real-time, targeted, and dynamic high-resolution visualization of the entire fistula tract and the location of the internal orifice. This technique effectively overcomes the key limitations of conventional intravascular CEUS, including insufficient contrast caused by systemic circulation dilution and the inability to directly visualize fistula drainage pathways [29]. In addition, although transfistular CEUS has been applied in the diagnosis of diseases such as anal fistula, systematic research on its application in BCF diagnosis remains relatively scarce. This study represents the first systematic application of this technique for the preoperative assessment of BCF. Our results confirm its unique diagnostic value in accurately localizing internal orifices and differentiating fistula subtypes, thereby providing effective guidance for clinical surgical planning.

This study has some limits. Firstly, the cohort in this study represented clinically suspected patients rather than an unselected population of BCF. This examination modality based on specific clinical indications may introduce selection bias. Secondly, as a hospital specializing in adult patients, we collected a limited number of cases, given the high prevalence of BCF in the pediatric population. Meanwhile, the restricted sample size precluded a comprehensive comparison of the diagnostic accuracy among transfistular CEUS, CT, and MRI. Prospective multi-center studies involving larger cohorts are required to validate this technique, which should then be increasingly adopted in pediatric populations to optimize early definitive treatment of BCF.

Conclusions

As a radiation-free, cost-effective, and non-invasive technique, transfistular CEUS can accurately diagnose BCF, precisely localize its internal orifice, and effectively differentiate its subtypes. For patients clinically suspected of BCF, transfistular CEUS is a reliable preoperative tool for individualized clinical guidance.

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Conflict of interest: none

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