ORIGINAL RESEARCH

Comparison of Endoscopic Ultrasound-Guided Fine Needle Aspiration with 19-Gauge and 22-Gauge Needles for Solid Pancreatic Lesions

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Purpose: We aimed to compare the histological and/or cytological diagnostic outcomes of EUS-FNA using 19G and 22G needles for solid pancreatic lesions and to evaluate the feasibility and safety of 19G needle.

Patients and Methods: Data from patients with solid pancreatic lesions, who underwent EUS-FNA, were retrospectively retrieved from a single tertiary center from June 2017 to January 2021. The sensitivity, specificity, and accuracy of diagnosis, sample adequacy, number and time of punctures, and adverse events, were compared between the 19G and 22G groups. Univariate and multivariate logistic regression analyses were used to identify optimal factors for a correct histological diagnosis.

Results: A total of 186 patients (19G group, n = 90; 22G group, n = 96) were analyzed in the study. The higher sensitivity and accuracy were observed in 19G group than those in the 22G group both in histological evaluation (89.3% vs 76%, p = 0.031; 91.1% vs 79.2%, p = 0.023; respectively) and in the combined histological and cytological evaluations (93.3% vs 81.3%, p = 0.027; 94.4% vs 84.3%, p = 0.027, respectively). However, there were no significant differences in specificity, positive predictive value (PPV), and negative predictive value (NPV). The number of needle passes and the puncture time were significantly lower in the 19G group than that in the 22G group (1.66 ± 0.07 vs 2.25 ± 0.08, p < 0.001; 125.4 ± 4.93s vs 169.0 ± 5.6s p < 0.001; respectively). Only 2 cases were failed in the 19G group and no serious complications occurred. Univariate and multivariate logistic analyses suggested that CA199 levels and needle types are related to the accuracy of the EUS-FNA histological diagnosis.

Conclusion: EUS-FNA using a 19G needle is effective and safe for solid pancreatic lesions. Compared with the 22G needle, EUS-FNA with a 19G needle can obtain a better histological diagnostic accuracy of solid pancreatic lesions, and with fewer needle passes and in a shorter time.

Keywords: sensitivity, specificity, diagnostic accuracy, histology, cytology

Introduction

Endoscopic ultrasound (EUS) guided fine-needle aspiration (FNA) is an effective and safe technique for the obtention of tissues for cytopathological evaluation of lesions adjacent to the digestive tract. For the sampling tissues from pancreatic solid masses, EUS-FNA has been used as the standard technique.[1] Its diagnostic yield is highly variable with a reported sensitivity, specificity and accuracy of 73–96%, 93–100%, and 74–94%, respectively, for solid pancreatic lesions.[2–4] This diagnostic variation is strictly dependent on the availability of onsite cytopathology, the adequacy of the tissue core for histology, location of the mass, technical variability

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(eg, needles used, number of passes), and the experience of the endoscopist.[5,6] Additionally, diseases such as auto-immune pancreatitis, solid pseudopapillary tumors, and neuroendocrine tumors, may be difficult to diagnose without a large amount of tissues for immunohistochemical analysis.[7,8]

Among the most adopted devices, 19G/22 G/25 G needles have been proved to be effective and safe in clinical practice, and consequently, most published studies have used these devices. Theoretically, smaller needles result in less blood aspirates, and the to-and-fro transversal movements are easier through pancreatic tissues. A meta-analysis of randomized controlled trials showed a similar specificity and sensitivity between 22G and 25G EUS-FNA.[4] For patients with unresectable pancreatic cancer, tissue samples obtained by EUS-FNA can be used for molecular testing, xenotransplantation, and organ culture to further guide a personalized therapy.[9,10] However, limited tissue yields, and cytological evaluations cannot meet these requirements.

Studies have shown that the use of EUS-FNA with a standard 19G needle for liver biopsies,[11] gastrointestinal stromal tumors,[12] and lymphomas[13] allows the collection of sufficient samples for histological analyses to reach accurate diagnoses. The European Society of Gastrointestinal Endoscopy (ESGE) recommends[14] the use of FNA and fine-needle biopsy (FNB) needles for the sampling of solid masses, and suggests using 19G FNA or FNB needles to obtain a core tissue specimen. However, few and controversial reports exist regarding the use of EUS-FNA with a 19G needle for the diagnosis of pancreatic diseases. Hence, we retrospectively compared the sensitivity, specificity and accuracy of the diagnosis, sample adequacy, number and time of passes, and adverse events, using 19G and 22G needles for solid pancreatic lesions, based on cytological or histological assessment and the combination of cytological and histological assessments.

Patients and Methods
Study Design and Patients
This was a retrospective study conducted at the Second Hospital of Hebei Medical University. All consecutive patients with solid pancreatic lesions, who underwent EUS-FNA from June 2017 to January 2021, were identified from our clinical and endoscopy database. The inclusion criteria involved patients with solid pancreatic lesions who underwent EUS-FNA, were aged ≥18

years, and who used 19G or 22G needles (Echo-Tip Ultra, Wilson-Cook Medical Inc., Winston-Salem, USA). Patients, with incomplete data or using other types of needles, were excluded. The clinical, endoscopy, and pathology data were collected, including patients' baseline characteristics (sex, age, CT/MRI results, and serum CA199), features of the pancreatic lesions (location, size, shape, and echogenicity), and procedural information related to EUS-FNA (needle type, puncture route, puncture time, and immediate and delayed complications). All patients signed informed consent for EUS-FNA and were followed up for at least 6 months. The study was conducted according to the Declaration of Helsinki and approved by the Research Ethics Committee of the second hospital of Hebei Medical University.

EUS-FNA Procedure
All EUS-FNAs were performed by experienced endoscopists using a linear array echoendoscope (EG-530UT or EG-580UT, FUJIFILM, Tokyo, Japan) on patients under conscious sedation using propofol and dezocine. Both echoendoscopes used a SU-8000 processor (FUJIFILM, Tokyo, Japan). The pancreatic lesion was fully visualized with EUS, and the absence of blood vessels in the optimal puncture site was ensured using the color Doppler function. EUS-FNA for pancreatic head and neck lesions were performed from the duodenum or stomach, while the pancreatic body and tail were performed from the stomach. The endoscopist punctured the pancreatic lesion using a 19G or 22G needle under EUS guide, then the needle was moved more than 10 times to-and-fro within the pancreatic mass, while the stylet was slowly withdrawn by an assistant. Finally, the puncture needle was removed, and the aspirated specimen was expelled onto a glass slide by reinserting the needle stylet. The puncture time was calculated through a operation video. Without rapid on-site evaluation (ROSE), some whitish parts of the specimen were placed in 10% formalin solution for histopathological evaluation. Smears for cytological evaluation were made from the residual specimen by an endoscopist who had been trained to prepare slides. If the endoscopist macroscopically assessed the tissue samples to be insufficient, the procedure was repeated. Technical success was defined as performing the procedure without difficulty.

Cytohistological Evaluation
The tissue specimens were stained with hematoxylin and eosin for evaluation. If necessary, immunohistochemical procedures were performed. The cytologic smears were

stained using Papanicolaou staining. Cytological and histological reports were retrospectively reviewed by two experts in digestive pathology who were blinded to the needle sizes. The results of the cytohistological evaluation were classified into four categories: positive, suspicious, negative for malignancy, and non-diagnostic. Adequate samples were defined as those sufficient for histological evaluation. Insufficient specimens were considered as non-diagnostic cases in analyzing the histological diagnostic accuracy.

Study Endpoints and Measures

The primary outcomes were sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of EUS-FNA using 19G and 22G needles for solid pancreatic lesions. The initial diagnosis was determined by the cytohistological results of EUS-FNA, and the final diagnosis was based on the 6-month follow-up. For a final diagnostic reference, malignant lesions were determined via a surgical histopathological diagnosis or EUS-FNA findings for malignancies with compatible clinical symptoms and imaging examinations. Benign lesions were determined by negative for malignancy according to EUS-FNA and no malignant progression that was clinically followed up for at least 6 months. Sensitivity was defined as the number of EUS-FNA accurately diagnosed malignancies divided by the number of total malignant cases at the final diagnosis. Specificity was defined as the number of EUS-FNA accurately diagnosed benign lesions divided by the number of total benign cases at the final diagnosis. The diagnostic accuracy rate was defined as the ratio of the sum of the true–positive and true–negative cases divided by the total number of evaluated cases. Secondary outcomes were defined as the rate of adequately obtained tissues, the number of needle passes, puncture time, technical success, and complications due to EUS-FNA between the groups.

Statistical Analysis

All data were evaluated using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY). Continuous variables were presented as the means ± standard deviation and compared using the Student's t-test or Wilcoxon rank-sum test. Categorical parameters were expressed as the number and percentages and compared using the chi-square or Fisher's exact tests. Univariate and multivariate logistic regression analyses were performed to identify EUS-FNA optimal factors for a correct histological diagnosis. Variables with p <0.2 according to univariate analysis entering the multivariate analysis, the results were presented as odds ratios (OR) with 95% CIs. Two-tailed p-values were calculated using the Fisher's exact test for measurement data; p-values of <0.05 were considered statistically significant.

Results

Patient Characteristics and Final Diagnosis

A total of 199 patients with solid pancreatic lesions who underwent EUS-FNA were enrolled, of whom 5 patients were excluded due to EUS-FNA incomplete data records, 6 patients were excluded due to the usage of other needles, and 2 cases were excluded due to loss of follow-up. The flow chart is presented in Figure S1. Finally, a total number of 186 patients were analyzed. Among them, 90/186 (48.4%) patients were in the 19G group, and 96/186 (51.6%) were in the 22G group. The mean patient age was 58.8 ± 0.97 years (22–86 years), 108/186 (58.1%) were male patients, and 78/186 (41.9%) female patients. A total of 108/186 (58.1%) lesions were in the head and neck, 78/186 (41.9%) in the pancreatic body and tail. The mean of solid pancreatic lesions' size was 3.63 ± 0.10 cm (0.9–7.6 cm). Sex, age, lesion location, and lesion size did not significantly differ between the 19G and 22G groups (Table 1). Only 2/90 (2.2%) cases in the 19G group needed re-aspiration at a later date due to the needle unfeasibility associated with the overbending of the endoscope in the duodenum. Apart from 4 patients who had elevated pancreatic enzymes, no serious complications, such as bleeding, perforation, pancreatitis, or needle tract seeding, were observed during the follow-up.

A total of 150 malignant lesions and 36 benign lesions were finally diagnosed based on pathologic evaluation and follow-up data, and only 56/186 (30.1%) patients underwent surgery. The final diagnosis showed that pancreatic adenocarcinoma (123/186, 66.1%) was dominant in all cases, followed by autoimmune pancreatitis (20/186, 10.8%), chronic pancreatitis (16/186, 8.6%), neuroendocrine tumors (9/186, 4.8%), solid pseudopapillary tumors of the pancreas (8/186, 4.3%), pancreatic adeno-squamous carcinoma (5/186, 2.7%), and pancreatic metastases (5/186, 2.7%) (Table 2).

Diagnostic Yield

According to the histological diagnosis of EUS-FNA for malignancies, the sensitivity and diagnostic accuracy of the histological evaluation in the 19G group were significantly

Table I Patient Characteristics and Data of EUS-FNA Procedure

	19G Group (n = 90)	22G Group (n = 96)	P-value
Sex (male/female)	51/39	57/39	0.708
Age (mean±SD)	58.7±1.29	58.9±1.45	0.933
Location			0.825
Head and neck, n (%)	53/90 (58.9%)	55/96 (57.3%)	
Body and tail, n (%)	37/90 (41.1%)	41/96 (42.7%)	
Size median (cm, mean±SD)	3.67±0.13	3.59±0.15	0.687
CA199			0.837
<37 U/mL, n (%)	42/90 (46.7%)	47/96 (49%)	
37–1000 U/mL, n (%)	32/90 (35.6%)	35/96 (36.5%)	
>1000 U/mL, n (%)	16 /90(17.7%)	14/96 (14.5%)	
Puncture route			0.46
Duodenum, n (%)	44/90 (46.7%)	50/96 (52.1%)	
Stomach, n (%)	46/90 (53.3%)	46/96 (47.9%)	
Elevated pancreatic enzymes, n (%)	2/90 (2.2%)	2/96 (2.1%)	0.948
Final diagnosis (malignant/benign)	75/15	75/21	0.369

Table 2 Final Diagnosis

	19G Group (n = 90)	22G Group (n = 96)
Pancreatic adenocarcinomas, n (%)	61 (67.8%)	62 (64.6%)
Adenosquamous carcinoma, n (%)	3(3.3%)	2 (2.1%)
Solid pseudopapillary neoplasm, n (%)	4 (4.4%)	4 (4.2%)
Neuroendocrine tumor, n (%)	4 (4.4%)	5 (5.2%)
Metastasis, n (%)	3 (3.3%)	2 (2.1%)
Autoimmune pancreatitis, n (%)	9(10.0%)	11 (11.5%)
Chronic pancreatitis, n (%)	6 (6.7%)	10 (10.4%)

higher than those in the 22G group (89.3% vs 76%, p = 0.031; 91.1% vs.79.2%, p = 0.023; respectively). The specificity, PPV, and NPV of the two groups were 100% vs 90.5%, p = 0.623; 100% vs 96.6%, p = 0.421; and 65.2% vs 51.4%, p = 0.292; respectively (Table 3). According to the cytologic diagnosis, there were no significant differences between the two groups (p > 0.05) (Table S1). Furthermore, a higher

sensitivity (93.3% vs 81.3%, p = 0.027) and a higher accuracy (94.4% vs 84.3%, p = 0.027) were observed in the 19G group compared to those in the 22G group and when the histological evaluation was combined with the cytological evaluation. However, there were no significant differences in specificity, PPV and NPV (Table 3).

The number of needle passes was significantly lower in the 19G group compared to that in the 22G group (1.66 ± 0.07 vs 2.25 ± 0.08, p < 0.001). Time spent for puncturing was 125.4 ± 4.93s per lesion in the 19G group and 169 ± 5.6s in the 22G group (p < 0.001) and the time saved was 34.6 ± 5.4 s (Table 4). Adequate tissues for histological evaluation were obtained in 88/90 (97.8%) of patients in the 19G group and in 87/96 (90.6%) of patients in the 22G group (p = 0.079). However, there was no statistical difference in technical successes' rate in the 19G and the 22G group (97.8% vs 100%, P = 0.5).

Furthermore, subgroup analysis of puncture route showed that the advantage of 19G in needle passes and puncturing

Table 3 Diagnosis Yield of EUS-FNA

	Histological			Histological and Cytological		
	19G (n = 90)	22G (n = 96)	P-value	19G (n = 90)	22G (n = 96)	P-value
Sensitivity	67/75 (89.3%)	57/75 (76.0%)	0.031	70/75 (93.3%)	61/75 (81.3%)	0.027
Specificity	15/15 (100%)	19/21(90.5%)	0.623	15/15 (100%)	20/21 (95.2%)	1
PPV	67/67 (100%)	57/59 (96.6%)	0.421	70/70 (100%)	61/62 (98.4%)	0.951
NPV	15/23 (65.2%)	19/37 (51.4%)	0.292	15/20 (75%)	20/34 (58.8%)	0.229
Accuracy	82/90 (91.1%)	76/96 (79.2%)	0.023	85/90 (94.4%)	81/96 (84.3%)	0.027

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

Table 4 Puncturing Parameters of EUS-FNA

	19G Needle	22G Needle	P-value
Needle passes (number)	1.66±0.07	2.25±0.08	<0.001
Puncturing time (second)	125.4±4.93	169± 5.6	<0.001
Technical success rate, n (%)	88 (97.8%)	96 (100%)	0.5
Adequate sampling rate, n (%)	88 (97.8%)	87 (90.6%)	0.079

time in both puncture routes when compared to than 22G (p < 0.001). A higher accuracy of histology was also observed in trans-gastric route when using the 19G needle (95.7% vs 80%, p = 0.045). There were no significant differences in diagnosis accuracies associated with cytology, technical success, and adequate sampling for both groups (Table 5).

EUS-FNA Related Factors for a Histological Diagnostic Accuracy

EUS-FNA related factors for the histological diagnostic accuracy were assessed using univariate and multivariate logistic regression (Table 6). In the univariate and multivariate analyses, CA199 (>1000 vs <37, OR 16.066 (2.335–110.5), p = 0.000; OR 8.133 (1.578–41.91), p = 0.012 respectively), and needle sizes of EUS-FNA (19G vs 22G: OR 5.436 (1.14–26.513), p = 0.036; OR 2.697 (1.122–6.486), p = 0.027, respectively) were related to EUS-FNA diagnosis accuracy. A puncture site (stomach vs duodenum: OR 3.950, p = 0.019) was only significant in the univariate analysis.

Discussion

In recent years, endoscopists have made great efforts to improve the adequacy of samples by using EUS-FNA. They also improved endoscopic diagnostic accuracy through the use of different sizes and types of puncture needles, better sampling techniques, rapid on-site examination (ROSE), and by applying contrast-enhanced ultrasound and elastography.[15–17] Some reports have shown that ROSE can improve diagnostic accuracy rates and reduce the number of needle passes during EUS-FNA.[18–20] However, ROSE is time-consuming, costly, and unapplicable in many hospitals due to a lack of pathologists. Currently, puncture needle selection remains the main focus of many researchers.

Histologic analysis is increasingly becoming the diagnostic standard due to the emerging field of neoadjuvant treatment strategies for patients suspected of pancreatic ductal adenocarcinoma.[21] In this study, we respectively compared the outcomes of EUS-FNA using 19G and 22G needles for solid pancreatic lesions. The 19G group obtained sufficient tissue samples and achieved an accurate histological diagnosis in 82/90 (91.1%) of patients with an average of 1.66 ± 0.07 needle passes. By contrast, the 22G group got a correct histological diagnosis in 76/96 cases (79.2%) with an average of 2.25 ± 0.08 needle passes. The combination of histological and cytology evaluations improved the final diagnosis accuracy of pancreatic lesions when compared with histological or cytology evaluation alone.

Based on subgroup analysis of puncture routes, significantly less needle passes and puncturing time in both puncture routes were observed in the 19G group compared to those in the 22G group (p < 0.001); however, a significantly higher histologic accuracy was only observed with trans-gastric route. Furthermore, univariate analysis and multivariate logistic analysis showed that 19G needle improves the histological diagnosis accuracy of solid pancreatic lesions when compared with that of the 22G needle, especially for lesions in the pancreatic body and tail.

By using 22G needles for solid pancreatic lesions, EUS-FNA has an 82–94% cytological diagnostic accuracy[22–24] and a 71–80% histological diagnostic accuracy.[25,26] The larger caliber of 19G needle improves the rate of obtaining adequate specimens when compared with that of the 22G puncture needle, which is important for achieving an accurate diagnosis and performing

Table 5 Diagnosis Yield of the Needle by Puncture Route

	Trans-Duodenal			Trans-Gastric		
	19G n = 44	22G n = 46	P-value	19G n = 46	22G n = 50	P-value
Needle passes (number)	1.75±0.11	2.41±0.11	<0.001	1.56±0.12	2.09±0.11	<0.001
Puncturing time (second)	131±8.23	180±8.25	<0.001	117±9.10	157±8.27	<0.001
Accuracy of histology (%)	38 (86.4%)	36 (78.3%)	0.315	44 (95.7%)	40 (80%)	0.045
Accuracy of cytology (%)	36 (81.8%)	37 (80.4%)	0.867	39 (84.8%)	40 (80%)	0.540
Technical success rate, n (%)	42 (95.5%)	46 (100%)	0.455	46 (100%)	50 (100%)	1
Adequate sampling rate, n (%)	42 (95.5%)	40 (87%)	0.296	46 (100%)	47 (94%)	0.271

Table 6 Uni- and Multi-Variable Logistic Analyses of Factors Associated with Histological Diagnosis Accuracy of EUS-FNA

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P	OR (95% CI)	P
Male sex	2.25 (0.89–5.686)	0.086	–	–
Age	1.014 (0.979–1.051)	0.441	–	–
Size	0.945 (0.653–1.369)	0.766	–	–
Puncture site (stomach vs duodenum)	3.950 (1.255–12.43)	0.019	–	–
Needle passes	0.751 (0.385–1.463)	0.400	–	–
Needle size (19G vs 22G)	5.436 (1.14–26.513)	0.036	2.697 (1.122–6.486)	0.027
CA199		0.014		0.034
CA199 (37–1000 vs <37)	4.94 (0.972–25.11)	0.054	1.061 (0.452–2.491)	0.892
CA199 (>1000 vs <37)	16.066 (2.335–110.5)	0.000	8.133 (1.578–41.91)	0.012

ancillary analysis for personalized therapy. As demonstrated in a report of a randomized controlled trial[27] that included 117 solid pancreatic and peripancreatic masses, Per-Protocol (PP) analysis (excluding patients with technical failure) revealed that the cytological diagnostic accuracy was significantly higher in the 19G group than it is in the 22G group. Intention-To-Treat analysis revealed unsignificant differences in the cytological diagnostic rates between the groups (86.7% vs 78.9%, p = 0.268). However, the histological diagnostic values of the two needles were not evaluated. Larghi et al[28] assessed the histological diagnostic value of EUS-FNA using a standard 19G needle in 120 cases of digestive system diseases, including esophageal diseases, mediastinal disease, enlarged lymph nodes, abdominal masses, and pancreatic lesions. Among the 120 cases, the technical success rate reached 98.9% with only one technical failure. Adequate samples were obtained in 97.5% of the EUS-FNA cases, with a diagnostic accuracy of 93.2%. Nevertheless, the study included 13 patients with pancreatic body or tail lesions and excluded pancreatic head or uncinate lesions. In our study, the sensitivity and diagnostic accuracy of histological assessment were significantly higher in the 19G needle group, which were consistent with the previous study. EUS-FNA with 19G needles enabled the obtention of sufficient tissue samples than did the EUS-FNA with 22G needles. Thus, 19G needles can meet the needs of pathological examinations and immunohistochemical analyses. More importantly, the correct diagnosis of benign lesions can avoid unnecessary surgery and significantly improve patients' prognosis.

With the increasing need for higher quantity and quality tissue specimens to perform molecular analysis and guide a precision cancer therapy, the new generation of fine-needle biopsy (FNB) needles, is expected to facilitate the collection of more tissue, and therefore, achieve optimal diagnostic accuracy. ESGE recommends the equal use of FNA and FNB needles for the tissue sampling of solid pancreatic masses.[29] Research reported that the acquisition rates and diagnostic yields did not significantly differ between the 19G FNA and 19G/20G/22G FNB needles, as both types of needles have excellent feasibility.[30,31] In this study, EUS-FNA with a 19G needle provides good diagnosis outcomes for solid pancreatic lesions and requires significantly fewer passes when compared with that of the 22G needle. Notably, most of patients required 1–2 passes in the 19G group, which was consistent with that of the previously reported results on FNB needles. Fewer needle passes per patient results in shorter procedure times which may reduce the risk of complications.

In our study, EUS-FNA was successfully performed in all cases of the 22G group, whereas 2/90 (2.2%) patients with pancreatic head lesions failed in the 19G group, due to the needle unfeasibility associated with the overbending of the endoscope in the duodenum. However, the technical success rates did not significantly differ between the two groups. No serious complications occurred, and only two patients per group had a slightly increased amylase but with no clinical symptoms after the procedure. In summary, EUS-FNA with a 19G needle is safe for solid pancreatic lesions.

Although our study showed that 19G needles were superior to 22G needles in many ways, the study had some limitations. First, this was a single-center retrospective study with limited cases. We compared the diagnostic values of both needles in solid pancreatic lesions only. Whether the results are applicable to other solid lesions is unknown.

Second, the adequacy of the tissue specimens was assessed based on whether they were sufficient for histological evaluation, and the quantity and quality of the histological core were not accurately measured. Future studies should use larger samples and assess whether the tissue obtained via EUS-FNA using a 19G standard needle is sufficient for molecular analysis and precision therapy for cancer.

Conclusion

In summary, EUS-FNA with a 19G needle is feasible and safe for tissue sampling associated with the diagnosis of solid pancreatic lesions. Compared with 22G needles, 19G needles enable the obtention of adequate tissue specimens for higher histological diagnostic accuracy and with fewer needle passes and in a shorter time.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

These authors report no conflicts of interest in this work.

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