Application of long non-coding RNA RBAT1 in improving diagnosis and prognosis of ovarian carcinoma

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Tumorigenesis of bladder cancer and retinoblastoma is correlated with long non-coding RNA (lncRNA) RBAT1. However, the role of RBAT1 in ovarian carcinoma (OC) is unclear. Thus, the study explored the role of RBAT1 in OC. This research enrolled patients with OC (*n*=68), irritable bowel disease (IBD, *n*=68, females), digestive tract inflammation (DTI, *n*=68, females), urinary tract infection (UTI, *n*=68, females), endometriosis (EM, *n*=68, females), and healthy controls (HCs, *n*=68) to collect plasma sampled. OC and paired non-tumor tissues were collected from patients with OC. RBAT1 accumulation in all samples was analyzed using RT-qPCR. The role of plasma RBAT1 in OC diagnosis was examined using the ROC curves with OC patients as the true positive cases and other patients and HCs as the true negative cases. The role of RBAT1 in predicting the survival of OC patients was analyzed using the survival curve study. RBAT1 was overexpressed in both OC plasma and tissues. Plasma RBAT1 levels were correlated with RBAT1 levels in OC tissues but not in non-tumor tissues. Plasma RBAT1 could distinguish OC patients from other patients and HCs. Patients with high plasma RBAT1 levels had a shorter survival. RBAT1 is overexpressed in OC and might be applied to improve the diagnosis and prognosis of OC. *Anti-Cancer Drugs* 34: 9–14 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Ovarian carcinoma (OC) is a solid tumor formed by malignant cells originating from the cells covering the ovary and is the second most frequently detected gynecologic tumor [1–3]. Due to the aggressive and fast-growth nature of OC, delayed diagnosis usually leads to distant tumor metastasis, which is related to a high mortality rate [4,5]. For instance, about 1 in 78 women will be affected by OC, and more than 2 of 3 of these patients will die of OC during their lifetime [5]. Without distant metastasis, 90% of OC patients survive 5 years, while once distant metastasis has occurred, the 5-year survival rate drops to below 30% [6]. Unfortunately, the early detection of OC in most cases is difficult.

Although OC patients at early stages may present symptoms, such as abdominal and pelvic pain, indigestion, and bloating [7,8], these symptoms can be easily recognized as clinical signs of other clinical disorders, such as irritable bowel disease (IBD), digestive tract inflammation (DTI), urinary tract infection (UTI), and endometriosis (EM) [9,10]. Therefore, misdiagnosis of OC is common in clinical practice. To this end, efforts have been made to develop biomarkers for OC [11–13]. However, the accuracy of currently available cancer biomarkers is not satisfactory. Although no protein-coding information was observed in the sequence of long non-coding RNAs (lncRNAs), they indirectly affect protein accumulation to regulate cancers [14]. To date, some lncRNAs have been identified as potential biomarkers for OC [15]. Previous studies have shown that tumorigenesis of bladder cancer and retinoblastoma requires the involvement of lncRNA RBAT1 [16]. However, the role of RBAT1 in OC is unclear. Our preliminary sequencing analysis has shown altered RBAT1expression in OC but not in other diseases that can mimic the symptoms of OC. Therefore, RBAT1 may serve as a biomarker to avoid the misdiagnosis of OC. Therefore, this study explored the role of RBAT1 in OC, with a focus on its clinical values.

Materials and methods

Enrollment of participants

This research enrolled patients with OC ($n=68$, 55.9 ± 7.4 years), IBD ($n=68$, females, 55.8 ± 7.1 years), DTI ($n=68$, females, 55.4 ± 7.9 years), UTI ($n=68$, females, 55.8 ± 7.0 years), and EM ($n=68$, females, 55.7 ± 7.1 years) and healthy controls (HCs, $n=68$, 55.8 ± 7.6 years) at the

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First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University after the hospital ethics committee approved this study and informed consent was obtained. Our study was performed in compliance with the Declaration of Helsinki. OC patients were all diagnosed with transvaginal ultrasound and confirmed by histopathological analysis of the tumor tissues. Other patients were diagnosed through standard methods, and those with malignancies were excluded. HCs showed normal physiological functions during the systemic physiological examination.

Preparation of samples

The present research included both plasma and tissue samples. Briefly, OC and paired non-tumor tissues were obtained from the resected tumors from OC patients (48 cases of stages 1–III and 6 cases of stage IV) and biopsies (14 cases of stage IV) and confirmed by histopathological analysis. Tissue samples were stored in liquid nitrogen before the subsequent experiments. Plasma samples were collected from fastened blood samples extracted from both patients and controls in EDTA tubes after centrifugation at 12 000*g* for 12 min and stored in liquid nitrogen before the subsequent experiments.

Survival analysis

After blood extraction, patients received standard treatments and were released except OC patients. OC patients' death and survival were monitored for 5 years to summarize their survival conditions.

RNA samples and RT-qPCR

High-quality RNA samples were extracted from tissues by mixing with TRIzol (Invitrogen, Carlsbad, CA, USA) at a ratio of no more than 1:10 after being cleaned and washed three times with PBS. To completely break the cell membrane, the mixtures were incubated at room temperature for 30 min. After that, cell debris was removed by centrifugation at 12 000*g* for 20 min. After removal of the contaminated proteins by 10% chloroform, RNAs were precipitated using 50% methanol, washed with 70% ethanol three times, and dissolved in RNase-free water.

After examining RNA integrity and concentration using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), cDNA samples with high quality were prepared using Takara Prime Script 1st Strand cDNA Synthesis Kit (Takara, Kusatsu, Japan). The quality of cDNA samples was analyzed by amplifying GAPDH. cDNA samples yielded no GAPDH DNA were trashed and prepared. RBAT1 accumulation was then determined through qPCRs with 18S rRNA as the internal control, and Ct values were normalized using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were expressed as the average values of three technical replicates and analyzed using GraphPad Prism (v7; GraphPad Software Inc., San Diego, CA, USA). Differences between two groups and among multiple groups were examined using Student's *t*-test and ANOVA Tukey's test, respectively. To study the role of RBAT1 in OC diagnosis, ROC curves were analyzed with OC patients as the true positive cases and IBD patients, DTI patients, UTI patients, EM patients, or HCs as the true negative cases. To explore the value of plasma RBAT1 level in predicting OC patients' survival, the 68 patients were grouped into high and low RBAT1 level groups ($n = 34$) with the median plasma RBAT1 level as the cut-off value, and their survival curves were plotted and compared. Differences with a P value <0.05 were considered statically significant.

Results

RBAT1 accumulation in patients and controls

This study included OC patients, IBD patients, DTI patients, UTI patients, EM patients, and HCs, with 68 cases in each group. Plasma samples from all participants and OC and paired non-tumor tissues were used to analyze RBAT1 accumulation using RT-qPCR. Ct values from three technical replicates were normalized using the $2^{-\Delta\Delta Ct}$ method to the sample with the highest ΔCt value, which was set to value '1'. Our experimental data analysis showed that RBAT1 was overexpressed in both OC plasma (Fig. 1a, $P < 0.01$) and tissues (Fig. 1b, $P < 0.01$). Therefore, altered expression of OC is a contributor or consequence of OC. Interestingly, no altered plasma RBAT1 expression was observed in other groups of patients.

Correlations between RBAT1 in plasma and tissue samples

To analyze the source of plasma RBAT1, the correlation of RBAT1 in plasma to RBAT1 in OC or non-tumor tissue samples was analyzed by performing Pearson's correlation coefficient. Plasma RBAT1 was only correlated with RBAT1 in OC tissues (Fig. 2a) but not in non-tumor tissues (Fig. 2a). Therefore, plasma RBAT1 is mainly from OC tissues rather than non-tumor tissues.

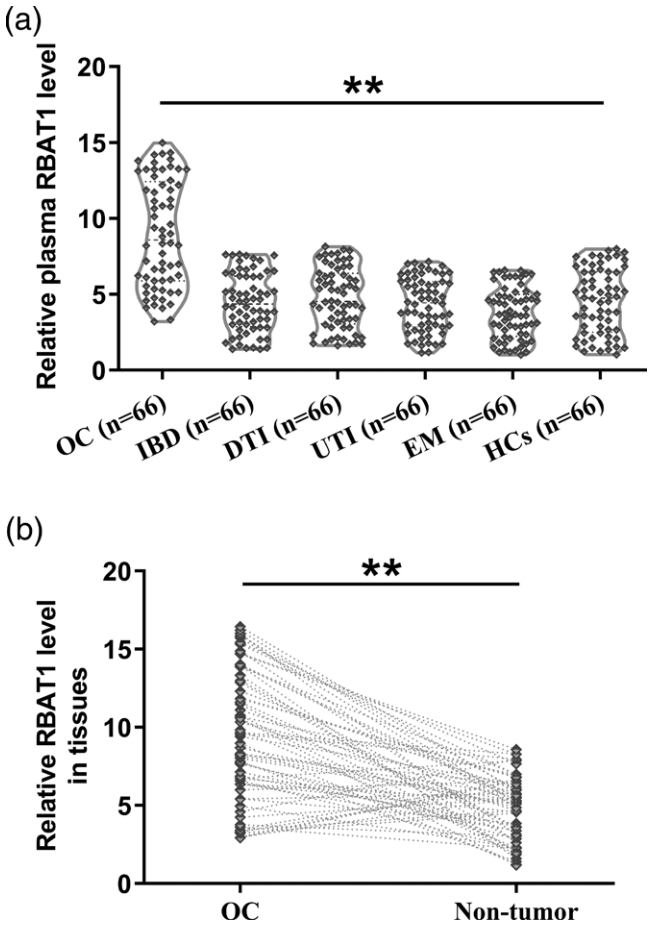
The role of RBAT1 in diagnosing ovarian carcinoma

To study the value of plasma RBAT1 in diagnosing OC, ROC curves were compared using OC patients as the true positive cases and IBD patients (Fig. 3a), DTI patients (Fig. 3b), UTI patients (Fig. 3c), EM patients (Fig. 3d), or HCs (Fig. 3e) as the true negative cases. The area under the curve greater than 0.65 indicates a potential diagnostic value. As shown in Fig. 3, plasma RBAT1 distinguished OC patients from other patients and HCs (all areas >0.8 and $P < 0.0001$).

The role of plasma RBAT1 in predicting ovarian carcinoma patients' survival

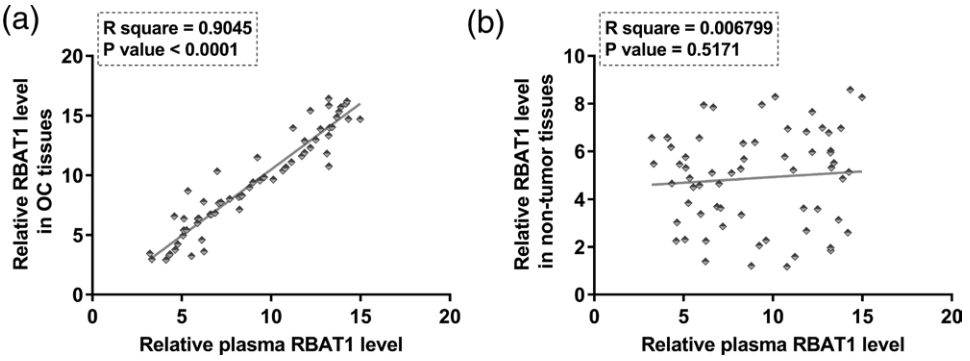
To explore the value of plasma RBAT1 in predicting OC patients' survival, the 68 patients were grouped into high and low RBAT1 level groups ($n = 34$). Their survival curves were plotted and compared using by log-rank test. It was observed that high levels of RBAT1 in plasma were closely

Fig. 1



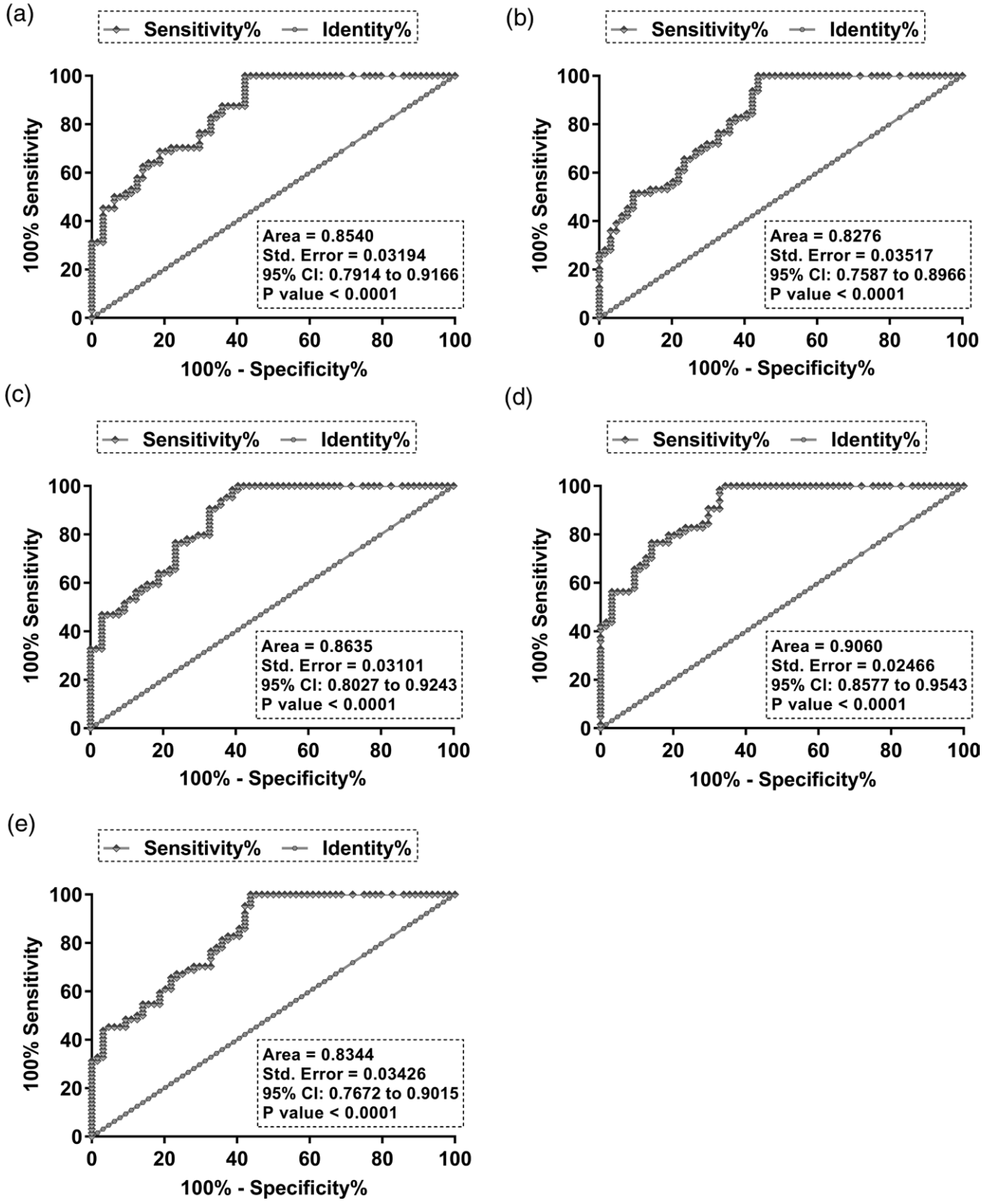
RBAT1 accumulation in patients and controls. This study included OC patients, IBD patients, DTI patients, UTI patients, EM patients, and HCs, with 68 cases in each group. Plasma from all participants (a) and OC and paired non-tumor tissues (b) were used to analyze RBAT1 accumulation. $^{**}P<0.01$. DTI, digestive tract inflammation; EM, endometriosis; IBD, irritable bowel disease; OC, ovarian carcinoma; UTI, urinary tract infection.

Fig. 2



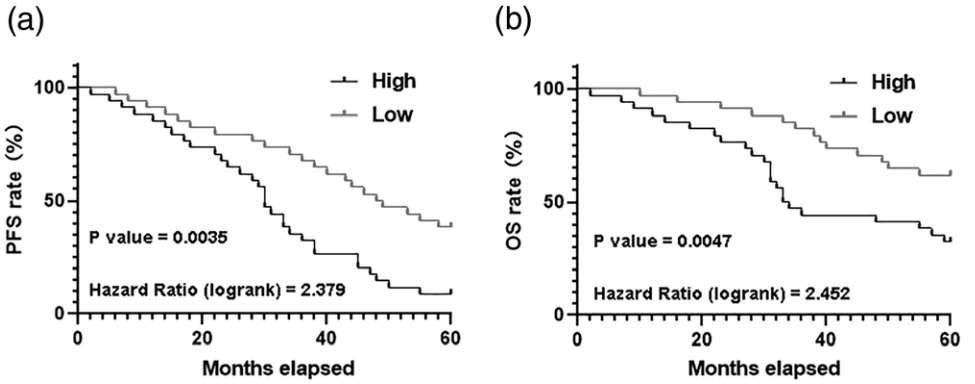
Correlations between RBAT1 in plasma and tissue samples. To analyze the source of plasma RBAT1, the correlation of RBAT1 in plasma to RBAT1 in OC (a) and non-tumor (b) tissue samples were analyzed. OC, ovarian carcinoma.

Fig. 3



The role of RBAT1 in diagnosing OC. To study the value of plasma RBAT1 in diagnosing OC, ROC curves were plotted and compared with OC patients as the true positive cases and IBD patients (a), DTI patients (b), UTI patients (c), EM patients (d), or HCs (e) as the true negative cases. DTI, digestive tract inflammation; EM, endometriosis; HCs, healthy controls; IBD, irritable bowel disease; OC, ovarian carcinoma; UTI, urinary tract infection.

Fig. 4



The role of RBAT1 in predicting OC patients' survival. To explore the value of RBAT1 in predicting OC patients' survival, including PFS (a) and OS (b), the 68 patients were grouped into two high and low RBAT1 level groups (n=34). Survival curves were plotted and compared. OC, ovarian carcinoma

Table 1 Associations between plasma RBAT1 and patients' clinical features

Features	Cases	High	Low	*P*
Age (years)				0.81
<55	31	16	15	
≥55	37	18	19	
Tumor size (cm)				0.46
≤8	41	22	19	
>8	27	12	15	
Ascites				0.33
≤100	30	17	13	
>100	38	17	21	
Federation International of Gynecology and Obstetrics stage				0.004
I + II	36	12	24	
III + IV	32	22	10	
Distant metastasis				0.02
Yes	14	11	3	
No	54	23	31	

correlated with poor PFS (Fig. 4a) and OS (Fig. 4b). Therefore, increased accumulation of plasma RBAT1 may serve as an indicator of poor survival of patients with OC.

Associations between plasma RBAT1 and patients' clinical features

Associations between plasma RBAT1 and patients' clinical features were studied using Chi-squared test and Student's *t*-test. Our data revealed a close association between plasma RBAT1 and patients' distance tumor metastasis and clinical stages, but not age, tumor size, and ascites (Table 1). Therefore, RBAT1 may mainly participate in the metastasis of OC.

Discussion

The present study explored the participation of RBAT1 in OC and analyzed the value of its expression in predicting OC and patients' survival. The results revealed an altered RBAT1 accumulation in OC and its potential role in improving the diagnosis and prognosis of OC.

RBAT1 is highly expressed to promote both bladder cancer and retinoblastoma progression by activating E2F3 and interacting with HNRNPL [16]. At present, the involvement of RBAT1 in OC has not been reported. The present research revealed increased RBAT1 accumulation in both tumor tissues and plasma samples from OC patients, but not from patients with IBD, DTI, UTI, and EM. Therefore, increased RBAT1 accumulation is likely specific to OC. Moreover, RBAT1 is only closely correlated with RBAT1 in OC tissues but not in non-tumor tissues. Therefore, plasma circulating RBAT1 is mainly from OC tissues. We also observed the close association between plasma RBAT1 and tumor metastasis, but not tumor size. Our data indicated the potential role of RBAT1 in OC tumor metastasis, but this speculation should be verified by function assays.

Symptom-based diagnosis of OC is not accurate. Although certain biomarkers, such as mesothelin and lactate dehydrogenase, have been developed to predict OC, these biomarkers usually show low sensitivity or specificity [15,17]. For instance, lactate dehydrogenase level is also altered in EM, which is frequently misdiagnosed as OC [18]. This study showed that plasma RBAT1 could be applied to effectively separate OC patients from IBD, DTI, UTI, and EM patients. Therefore, plasma RBAT1 may be applied to improve the diagnostic accuracy of OC. Moreover, patients with high plasma RBAT1 levels usually had shorter survival periods, suggesting plasma RBAT1 as a prognostic biomarker for OC. Although previous studies have characterized many lncRNAs as potential biomarkers for the diagnosis of OC, most of these studies only included HCs as the true negative cases in ROC curve analysis. In contrast, our study included patients with IBD, DTI, UTI, or EM as the true negative cases and showed that plasma RBAT1 itself is sufficient to avoid the misdiagnosis of OC.

Conclusion

RBAT1 accumulation was increased in OC and could be applied in clinical practice to improve the diagnosis and prognosis of OC.

Acknowledgements

All patients signed informed consent. This study was approved by the ethics committee of The First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University and carried out in accordance with the World Medical Association Declaration of Helsinki.

The datasets generated during or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Conflicts of interest

There are no conflicts of interest.

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