BMC Cardiovascular Disorders

RESEARCH

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Assisting significance of lncRNA ASB16-AS1 in the early detection and prognosis prediction of patients with deep venous thrombosis

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Abstract

Background Deep venous thrombosis (DVT) is a kind of vascular obstruction, that commonly and widely occurs in lower limbs. Due to the lack of obvious symptoms in the early stage, the rate of misdiagnosis and missed diagnosis is high. This study evaluated the expression and significance of lncRNA ASB16-AS1 (ASB16-AS1) in DVT aiming to identify a novel biomarker for its screening and monitoring.

Methods There were 77 DVT patients and 62 healthy individuals included in this study. Plasma ASB16-AS1 level was evaluated using PCR and compared between DVT and healthy groups. The diagnostic and prognostic values of ASB16-AS1 were assessed with ROC and Cox analyses. The correlation of ASB16-AS1 with patients' conditions, inflammation, and oxidative stress was evaluated by Spearman correlation analysis.

Results ASB16-AS1 was significantly upregulated in DVT ($P < 0.001$), which could discriminate DVT patients from healthy individuals with high sensitivity and specificity (AUC of ROC = 0.858). Increased ASB16-AS1 was associated with the incidence of complications ($P = 0.033$) and especially for pulmonary embolism in patients ($P = 0.029$). ASB16-AS1 was negatively correlated with prothrombin time (PT, r = -0.763), antithrombin level (AT, r = -0.711), and international normalized ratio (INR, r = -0.764), and showed positive correlation with fibrinogen (FIB, $r = 0.793$) and D-dimer (D-D, $r = 0.731$). Additionally, ASB16-AS1 was positively correlated with pro-inflammation cytokines (r_{IL-6} = 0.853, r_{IL-10} = -0.836, r_{hsCRP} = 0.787) and pro-oxidative stress factors (r_{SOD} = -0.751, r_{MDA} = 0.842, $r_{8\text{-}isoPGF2\alpha}$ = 0.840).

Conclusion Upregulated ASB16-AS1 was identified as a diagnostic and prognostic biomarker of DVT and was closely associated with inflammation and oxidative stress during DVT.

Keywords DVT, lncRNA ADB16-AS1, Inflammatory response, Oxidative stress, Occurrence, Development

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Background

Deep venous thrombosis (DVT) is a kind of abnormal clotting of blood in the lower limb vein induced by various factors, where abnormal platelet aggregation and coagulation factors are major risk factors. Only a minority of DVT patients showed obvious symptoms, like lower limb swelling, deep local tenderness, and dorsiflexion of the foot, most DVT cases are without obvious symptoms in the early stage, which makes DVT easy to overlook [1]. DVT without timely intervention would induce pulmonary embolism and threaten patients' lives. It was reported that nearly half of proximal DVT patients developed post-thrombotic syndrome within 2 years after anticoagulant therapies, which behaved as limb pain, swelling, and even the development of refractory venous ulcer [2, 3]. Hence, DVT has also been considered one of the leading causes of cardiovascular disease-related and in-hospital deaths [4, 5]. A venogram has been accepted as a commonly used method for the diagnosis of DVT, which could clear the position, range, and severity of the blocked vein [6]. However, due to invasiveness, high difficulty, and cost, a large number of patients cannot accept it. Ultrasonic Doppler blood flow detector has become a gold standard for DVT and has shown relatively high specificity and accuracy, but its high cost limits its application [7, 8]. Therefore, identifying non-invasive and effective biomarkers for the diagnosis, especially for the early detection of DVT has become an urgent need for its clinical management [9].

Recent studies have noticed the role of long non-coding RNAs (lncRNAs) in the progression of DVT. Previously, the abnormal expression of lncRNA ASB16-AS1 was reported in human cancers and regulates tumor progression, chemotherapy resistance, and cellular processes [10–12]. In a recent transcriptome study on high-altitude-induced thrombosis, ASB16-AS1 was identified as a candidate biomarker [13]. Although the onset of DVT involves a variety of causes and high altitude is just one of the main risk factors for DVT, the dysregulation of ASB16-AS1 in this type of thrombosis also implies its potential function in DVT progression. The routine blood test is a regular examination in the clinic, and serum and plasma samples have also become common biomarker sources. Serum and plasma biomarkers are non-invasive and therefore improve the acceptance of screening examinations. A previous study illustrated the dysregulated plasma ASB16-AS1 in colorectal cancer is of great diagnostic significance that discriminates patients from healthy individuals and benign patients with relatively high sensitivity and specificity [14]. Hence, the plasma ASB16-AS1 in DVT patients might act as a potential biomarker for the early detection and development prediction of patients.

The onset of DVT involves various processes, where inflammation and oxidative stress have been considered critical risk factors [15]. Inflammation induces the secretion of pro-inflammatory and pro-oxidative cytokines, which promote coagulation and inhibit fibrinolysis and also cause vascular wall injury, and deteriorating disease conditions [16–18]. IL-6 and IL-10 are typical inflammation-related cytokines. IL-6 is a pro-inflammation cytokine, while IL-10 showed a significant anti-inflammation feature. Meanwhile, both these two cytokines have been demonstrated to be associated with DVT [19, 20]. While during DVT, vascular stenosis leads to hypoxia of endothelial cells and releases a large number of reactive oxygen species, which causes lipid peroxidation of polyunsaturated fatty acids on cell membranes and produces MDA [21]. In addition, in the antioxidant system, SOD could play an antioxidant defense role on vascular endothelial cells, reducing the susceptibility to venous thrombosis [22]. Hence, both inflammation and oxidative stress are vital indicators associated with the development of DVT, and their correlation with ASB16-AS1 could suggest its significance in the disease progression.

This study first confirmed the abnormal expression of ASB16-AS1 in the plasma of DVT patients, and further established the correlation of ASB16-AS1 with patients' outcomes and disease development to assess its clinical significance.

Materials and methods

Study subjects

This study enrolled 77 DVT patients and 62 healthy individuals from January 2018 to December 2019 at Zibo Central Hospital. All participants have been excluded for the infection of SARS-CoV-2 at their enrollment. Sample size was calculated with the help of GPower 3.1, and the parameters were set as 80% of power and 0.05 level of significance. The calculation result was 64 for each group. To avoid the effect of loss follow-up on the study subjects, we included an additional 20% of the DVT patients. The number of healthy individuals was limited to the scale of the examination center and the inclusion criteria. The study had obtained approval from the Ethics Committee of Zibo Central Hospital (No. 201711010) and informed consent from all subjects. The inclusion criteria of the two groups were as follows:

DVT patients: (1) diagnosed as lower limb DVT based on the color ultrasonic examination; (2) with completed clinical records; (3) aging ≥ 18; (4) had not received anticoagulant therapy within 1 month; (5) had never received dialysis therapy; (6) without the diagnosis of heart diseases, autoimmune diseases, infectious diseases, malignant tumors, and other complications.

Healthy individuals: (1) received routine physical examination during the corresponding period; (2)

without diseases of the lower limb or dermatologically related diseases caused by lower limb diseases.

Sample collection

Fasting peripheral venous blood samples were collected after admission in the anticoagulant tubes with EDTA and stood at 4 °C for 20 min. After centrifuging at 3500 rpm for 10 min, the plasma was obtained and stored at -80 °C for the following analyses.

Follow-up survey

DVT patients were followed up for 3 years after their treatments through telephone and outpatient reviews. The endpoints of follow-up were defined as post-thrombotic syndrome (PTS), chronic venous insufficiency, and recurrence. The incidence of complications in patients was analyzed by Kaplan-Meier and multivariate Cox regression analyses. The follow-up survey in the present study was during the SARS-CoV-2 pandemic. Patients were carefully identified for the infection of SARA-CoV-2, such as testing for SRAS-CoV-2, collection for detailed contact and travel history, and analyses of blood coagulation indexes, and the reported 77 cases in the present study have excluded for SARS-CoV-2 during the follow-up period.

Real-time quantitative PCR

Plasma samples were lysed with Trizol reagent (Invitrogen, USA) for total RNA extraction. The purity and concentration of isolated RNA were evaluated by the value of OD260/280, and then the reversed transcription was conducted with the employment of PrimeScript RT Master Mix kit (TaKaRa, Japan). The relative expression of ASB16-AS1 was analyzed with the SYBR ExScript RT-PCR kit (TaKaRa, Japan) and calculated with the $2^{-\Delta\Delta CT}$ methods normalized to GAPDH. The primer sequences used were as follows: ASB16-AS1 forward 5'-GACAAC AGAATTGGAAGGTCC-3', reverse 5'-CTGTCTGAGG CAGTGAGTAC-3'; GAPDH forward 5'-TGCACCACC AACTGCTTAGC-3', reverse 5'-GGCATGGACTGTGG TCATGAG-3'. The PCR program was: 95 °C for 30 s, 30 cycles of 95 °C for 5 s 60 °C for 30 s, 58 °C for 20s, and 72 °C for 20 s. The variability of GAPDH within (1%) and between (3%) of the groups ensured the expression was stable. Additionally, to ensure the reliability of the PCR assay, the relative expression of ASB16-AS1 was analyzed with both GAPDH and β-actin as internal references, and there were no significant differences observed between different reference genes (Figure S1).

Evaluation of inflammation and oxidative stress indicators

Enzyme-linked immunosorbent assay was used for the analysis of plasma superoxide dismutase (SOD), malondialdehyde (MDA), inflammatory cytokines (IL-6 and IL-10), and 8-iso-prostaglandin$F_{2\alpha}$ (8-iso-PGF2α) with corresponding ELISA kits: the Cu/Zn SOD Human ELISA kit (eBioscience, no. BMS222, USA), Malondialdehyde Assay kit (Abcam, no. ab238537, USA), Human IL-6 ELISA kit (R&D System, no. D6050, USA), Human IL-10 ELISA kit (R&D System, no. DY417E, USA), and 8-iso-PGF2α ELISA kit (Enzo Life Science, USA). OD450 values were measured and a concentration-OD450 curve was established for the calculation of specific concentrations of SOD, MDA, IL-6, and IL-10. The relative sensitivity and specificity of ELISA were both 96–100%.

The serum hs-CRP levels were measure using immunofluorescence assay with the help of i-CHROMATM immune fluorescence analyzer (Boditech Med, South Korea) and corresponding kit (Sangon Biological Engineering Technology, China).

Statistical analyses

Clinical feature comparison between the DVT and healthy group was conducted with a student's t-test using SPSS 26.0 software. Receiver operating characteristic curve (ROC) analysis was carried out to evaluate the diagnostic value of ASB16-AS1 in DVT and the sensitivity and specificity were obtained when the Youden index reached the maximum. The Spearman correlation analysis was used to assess the correlation of ASB16-AS1 with patients' basic clinicopathological features, oxidative stress, and inflammation indicators. Multivariable Cox regression analysis was performed to assess adverse prognostic factors for DVT. Data were expressed as mean ± SD, and statistical significance was indicated by $P < 0.05$.

Results

Basic clinicopathological features between healthy individuals and DVT patients

The study subjects possess matched age and gender compositions with insignificant differences ($P > 0.05$). The healthy individuals included 25 males and 37 females with an average age of 48.74 ± 7.92 years, while the enrolled DVT patients consisted of 32 males and 45 females with an average age of 49.05 ± 7.39 years (Table 1).

There were significant differences observed in the coagulation indicators between the two groups. Specifically, DVT patients possessed lower prothrombin time (PT), antithrombin level (AT), and international normalized ratio (INR) and higher fibrinogen (FIB), and D-dimer (D-D) levels compared with healthy individuals (Table 1). While the blood cell indicators, including eosinophil (EOS), neutrophil (NUE), red blood cell (RBC), and white blood cell (WBC), showed no significant differences ($P > 0.05$). Additionally, DVT patients showed much higher levels of high-sensitivity C-reactive protein (hsCRP) and 8-iso-prostaglandin$F_{2\alpha}$ (8-iso-PGF2α)

Table 1 Baseline information of study subjects

	Healthy	DVT	*P*
Age (years)	48.74 ± 7.92	49.05 ± 7.39	0.812
Sex (male/female)	25/37	32/45	0.173
BMI (kg/m^2)	23.76 ± 3.29	24.29 ± 2.64	0.290
hsCRP (mg/L)	1.40 ± 0.24	11.90 ± 2.43	< 0.001
8-iso-PGF$_{2\alpha}$ (pg/mL)	20.74 ± 5.70	48.43 ± 7.01	< 0.001
Blood cell indicators			
EOS (%)	2.11 ± 0.79	2.34 ± 0.60	0.056
NEU (×10^9/L)	4.73 ± 1.06	4.15 ± 0.81	0.075
RBC (×10^{12}/L)	4.29 ± 1.09	4.64 ± 1.11	0.062
WBC ×10^9/L	5.26 ± 1.42	5.75 ± 1.38	0.141
Coagulation indicators			
PT (s)	12.84 ± 3.12	6.65 ± 1.21	< 0.001
AT (μg/mL)	125.23 ± 12.99	104.40 ± 9.28	< 0.001
TT (S)	20.48 ± 5.88	19.66 ± 3.90	0.268
INR	1.00 ± 0.19	0.33 ± 0.07	< 0.001
FIB (g/L)	3.25 ± 0.58	5.33 ± 0.72	< 0.001
D-D (mg/L)	0.13 ± 0.011	1.81 ± 0.12	< 0.001

hsCRP: high-sensitivity C-reactive protein; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandinF$_{2\alpha}$; EOS: eosinophil; NUE: neutrophil; RBC: red blood cell; WBC: white blood cell; PT: prothrombin time; AT: antithrombin; TT: thrombin time; INR: international normalized ratio; FIB: fibrinogen; D-D: D-dimer

in relative to healthy individuals, indicating the significant inflammation and oxidative stress (both $P < 0.001$, Table 1).

Expression and significance of ASB16-AS1 in DVT patients

Compared with healthy individuals, DVT patients showed a significantly increased plasma ASB16-AS1 expression (Fig. 1A). Upregulation of ASB16-AS1 could discriminate DVT patients from healthy individuals with relatively high sensitivity (81.82%) and specificity (79.03%) and the area under the ROC curve (AUC) was 0.858 (Fig. 1B).

Table 2 Multivariate Cox regression analysis evaluating adverse prognostic factors of DVT

	HR	95% CI	*P*
ASB16-AS1	22.056	3.381–43.895	0.001
PT	0.416	0.139–1.247	0.117
AT	0.459	0.154–1.366	0.162
FIB	2.544	0.758–8.536	0.131
INR	0.253	0.062–1.032	0.055
D-D	2.070	0.594–7.214	0.253
Pulmonary embolism	3.059	0.878–10.657	0.079
TAT	5.508	1.441–21.050	0.013
PIC	2.413	0.672–8.661	0.177

PT: prothrombin time; AT: antithrombin; INR: international normalized ratio; FIB: fibrinogen; D-D: D-dimer; TAT: thrombin-antithrombin complex; PIC: plasmin-α2-antiplasmin complex

DVT patients were grouped into the low-ASB16-AS1 ($n = 38$) and the high-ASB16-AS1 ($n = 39$) groups based on their average expression in DVT patients' plasma. Based on the 3-year follow-up survey, there were 9 cases of PTS, 6 cases of chronic venous insufficiency, and 3 cases of recurrence in the low-ASB16-AS1 group, while the number of the above complications was 10, 6, and 5, respectively in the high-ASB16-AS1 group. The low-ASB16-AS1 group showed a lower incidence rate of complications than that of the high-ASB16-AS1 group (Fig. 1C). Meanwhile, Cox regression analysis demonstrated that ASB16-AS1 served as an independent prognostic factor predicting the incidence of complications of DVT patients (HR = 22.056, 95% CI = 3.381–43.895), as well as TAT (HR = 5.508, 95% CI = 1.441–21.050, Table 2).

Association of ASB16-AS1 with coagulation indicators and disease conditions of DVT patients

Among the abnormal coagulation indicators of DVT patients, ASB16-AS1 showed a significant

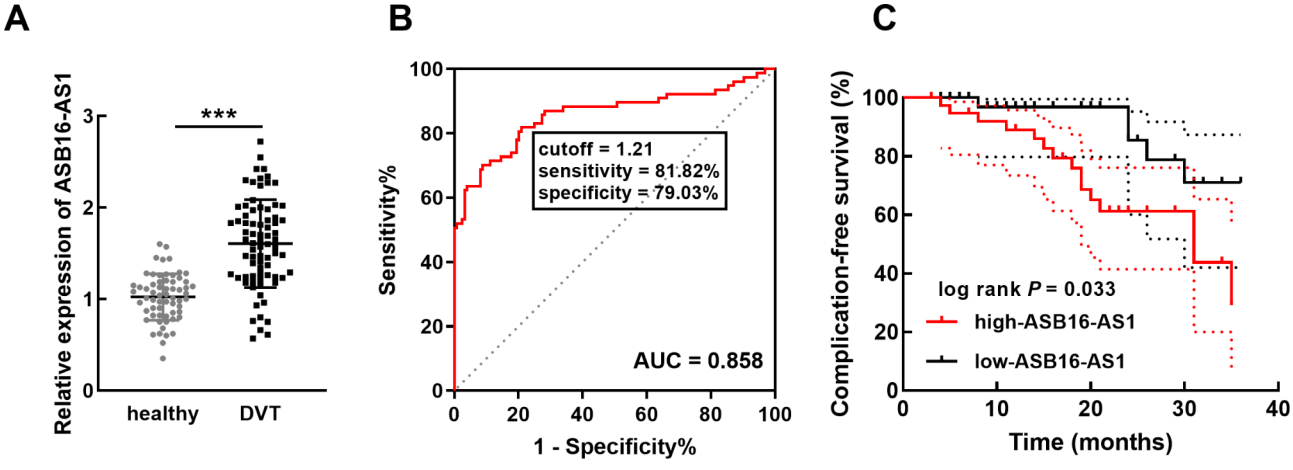

Fig. 1 Expression and significance of ASB16-AS1 in DVT. **A**. ASB16-AS1 was significantly upregulated in the plasma of DVT patients compared with that of healthy individuals. **B**-**C**. Increased ASB16-AS1 could discriminate DVT patients (**B**) and predict the incidence of complications in DVT patients (**C**). ***$P < 0.001$

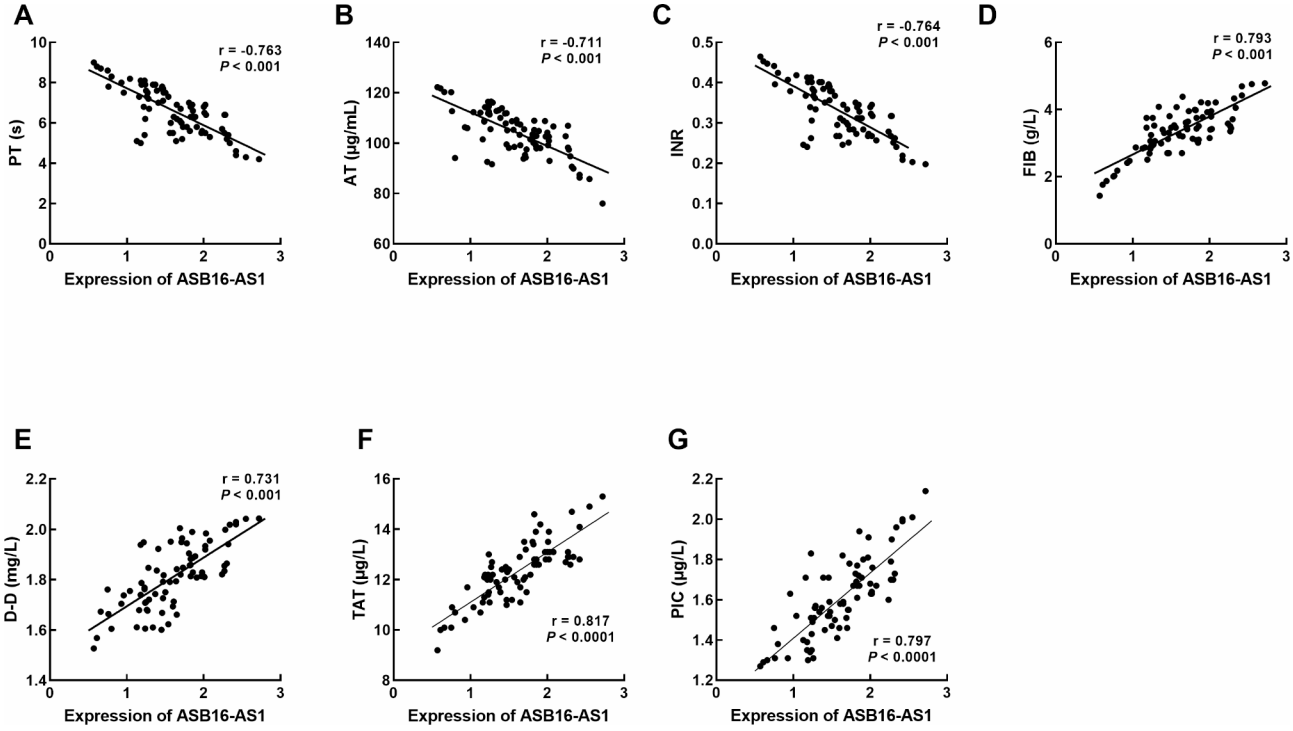

Fig. 2 Correlation of ASB16-AS1 with coagulation indicators of DVT patients. ASB16-AS1 was negatively correlated with prothrombin time (PT, r = -0.763, **A**), antithrombin level (AT, r = -0.711, **B**), and international normalized ratio (INR, r = -0.764, **C**) and positively correlated with fibrinogen (FIB, r = 0.793, **D**), D-dimer (D-D, r = 0.731, **E**), thrombin-antithrombin complex (TAT, r = 0.817, **F**), and plasmin-α2-antiplasmin complex (PIC, r = 0.797, **G**)

Table 3 Association of ASB16-AS1 with disease conditions of DVT patients

		Low-ASB16-AS1	High-ASB16-AS1	*P*
Diseased limb				0.596
Left lower	34	15	19	
Right lower	25	12	13	
Both lower	18	11	7	
Pulmonary embolism				0.029
Present	31	10	21	
Absent	46	28	18	
Thrombus type				0.076
around	38	18	20	
Central	26	10	16	
mixed	13	10	3	

negative correlation with the PT (r = -0.763, Fig. 2A), AT (r = -0.711, Fig. 2B), and INR (r = -0.764, Fig. 2C) of DVT patients. While the FIB (r = 0.793, Fig. 2D), D-D (r = 0.731, Fig. 2E), TAT (r = 0.817, Fig. 2F), and PIC (r = 0.797, Fig. 2G) were positively correlated with ASB16-AS1 expression.

According to the clinical records of DVT patients, the association of ASB16-AS1 with patients' disease conditions was evaluated. It was found that the higher plasma level of ASB16-AS1 was significantly associated with the occurrence of pulmonary embolism in DVT patients (P = 0.029, Table 3). No significant relationship was observed between ASB16-AS1 and patients' diseases limb and thrombus type (P > 0.05, Table 3).

Association of ASB16-AS1 with inflammation and oxidative stress of DVT patients

Upregulation of ASB16-AS1 was significantly correlated with the increasing level of IL-6 (r = 0.853, Fig. 3A), reducing IL-10 (r = -0.836, Fig. 3B), and increasing hsCRP (r = 0.787, Fig. 3C) in DVT patients (P < 0.001). Additionally, a significant negative correlation was also revealed between ASB16-AS1 expression and SOD concentration of DVT patients (r = 0.751, Fig. 3D). MDA (r = -0.842, Fig. 3E) and 8-isoPGF$_{2\alpha}$ (r = 0.840, Fig. 3F) were positively correlated with ASB16-AS1, indicating the potential involvement of ASB16-AS1 in the inflammation and oxidative stress of DVT patients.

Discussion

DVT commonly occurs in patients with fractures, especially after surgery, and is also a common complication in the orthopedics, gynecology, and general surgery departments [23–25]. Vascular endothelium injury, circulation stasis, and blood hypercoagulable state were considered the major pathogenesis of DVT, where hypercoagulation plays a critical role [26–30]. Coagulation factors and the levels of platelets and antithrombin are associated with the clotting of blood. Consistently, DVT patients enrolled

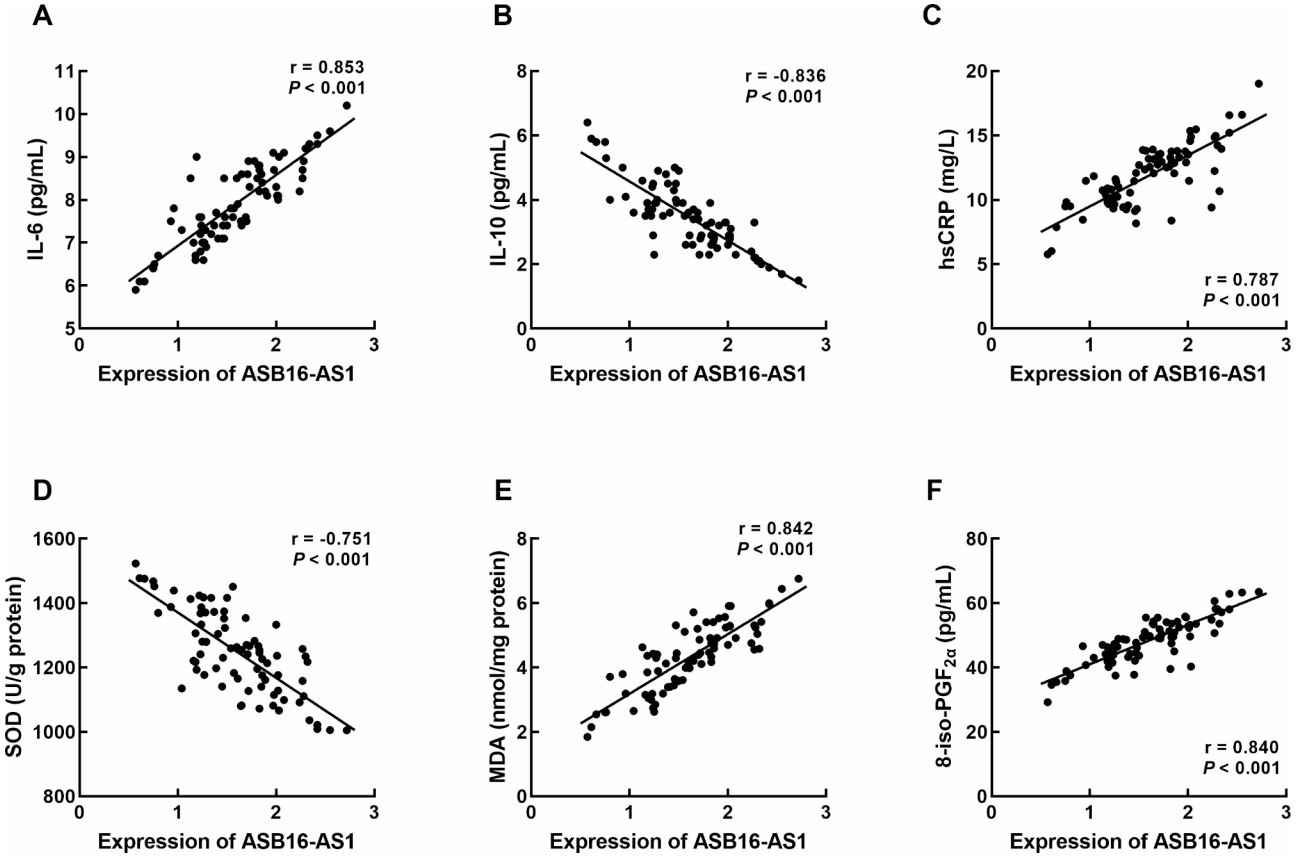

Fig. 3 Correlation of ASB16-AS1 with inflammation and oxidative stress. **A**-**C**. ASB16-AS1 was positively correlated with IL-6 ($r = 0.853$, **A**), negatively correlated with IL-10 ($r = -0.836$, **B**), and positively correlated with hsCRP ($r = 0.787$, **C**). **D**-**F**. ASB16-AS1 was negatively correlated with SOD ($r = -0.751$, **D**), and positively correlated with MDA ($r = 0.842$, **E**) and 8-iso-PGF2α ($r = 0.840$, **F**)

in this study showed lower prothrombin time (PT), antithrombin level (AT), and international normalized ratio (INR), higher fibrinogen (FIB) and D-dimer (D-D) levels. Decreasing PT and AT indicated the weakening anticoagulant function, FIB, and D-D are critical indicators for the formation of thrombus, which represent the hypercoagulable state of blood and the activity of the fibrinolytic system and have been considered as DVT development-related factors [31–34].

With the development of molecular biology, lncRNAs have been accepted as reliable biomarker candidates, and numerous studies have evidenced their significance in the early detection and risk prediction of human disease, including DVT [35]. However, previous studies mainly focused on the roles of lncRNAs in DVT-related cellular processes. For example, upregulated lncRNA XIST in DVT patients regulated the apoptosis of human umbilical vein endothelial cells (HUVECs), which would induce abnormal anticoagulation function [36]. lncRNA MALAT1 is upregulated in DVT, which regulates the viability and apoptosis of HUVECs [37]. In the present study, the upregulation of ASB16-AS1 was observed in the plasma of DVT patients. Previously, ASB16-AS1 was reported to act as a tumor promoter in non-small cell lung cancer accelerating tumor progression and mediating stemness and chemotherapy resistance in gastric cancer [10, 11]. Moreover, the abnormal expression of ASB16-AS1 was previously disclosed in high-altitude-induced thrombosis implying its potential function in thrombosis and correlated diseases [13]. Herein, ASB16-AS1 was evidenced to accurately discriminate DVT patients from healthy individuals. Currently, venogram and ultrasonic examinations are commonly employed methods for DVT, which are of relatively high accuracy, and the accuracy and sensitivity of ASB16-AS1 are not comparable to them. The most significant advantage of plasma ASB16-AS1 is that it can achieve the purpose of non-invasion for allergic patients who cannot accept invasive examinations. Although the present findings are not enough to suggest that it should be used for clinical screening of DVT, it does confirm the potential of plasma ASB16-AS1 in assisting screening of DVT.

A 3-year follow-up survey was conducted in this study to summarize the disease development and recurrence of DVT patients. It was found that increasing plasma ASB16-AS1 indicated the incidence of complications in

DVT patients and was identified as an independent prognostic indicator. The prognostic value of TAT was also confirmed in this study. TAT is a direct indicator of the activation of the coagulation system, and the relatively high TAT level suggests a hypercoagulable state, which is associated with the severe development of DVT. The prognostic value of ASB16-AS1 identified in the present study was more significant than TAT. Therefore, ASB16-AS1 is of great potential to serve as a diagnostic and prognostic biomarker for DVT, which needs further clinical validation.

Moreover, ASB16-AS1 showed a close association with DVT development-related factors, including PT, AT, FIB, INR, and D-D. In the clinical diagnosis, the increasing level of D-D could indicate the formation of thrombosis and the reducing level could represent the therapeutic efficacy [38–40]. Additionally, increasing FIB and INR are also considered indirect indicators for the onset of DVT, which indicates the hypercoagulable state of blood [41, 42]. The significant correlation of ASB16-AS1 with these factors suggested that ASB16-AS1 was associated with decreasing anticoagulation and the increasing coagulation of blood, which were indirectly related to the development of DVT. Inflammatory response and oxidative stress are always accompanied by disease occurrence and are also related to disease development and severity [15]. Although the formation of thrombosis and inflammation are two independent processes, inflammatory reaction has been considered a major etiology of DVT in recent research, which promotes the formation of thrombosis and increases the risk of adverse cardiovascular events [43–45]. Previous studies demonstrated that IL-6 was active during the onset and development of DVT and was induced by oxidative stress during the formation of thrombosis [19, 20]. Additionally, oxidative stress could induce vascular endothelial cell oxygenation damage, leading to the formation of thrombosis, which served as one of the major etiologies of DVT [22]. Numerous studies have also demonstrated the close association between oxidative stress indicators and DVT development [21, 46, 47]. ASB16-AS1 was positively correlated with pro-inflammation cytokines and MDA and negatively correlated with anti-inflammation cytokines and SOD. Moreover, 8-iso-$PGF_{2\alpha}$ was also observed to show significant positive correlation with ASB16-AS1. 8-iso-$PGF_{2\alpha}$ is the product of lipid peroxidation and has been considered an ideal indicator for oxidative stress. Hence, these observations indicated the involvement of ASB16-AS1 in inflammation and oxidative stress during DVT progression. Previous studies have also developed an anti-inflammation therapeutic strategy that could prevent DVT and improve anticoagulation function. Hence, ASB16-AS1 can be considered a potential therapeutic target for DVT. However, there was a lack of comparison of inflammation and oxidative stress between healthy individuals and DVT patients, which could enhance the significance of ASB16-AS1 in these processes during DVT progression. Immunity is associated with the activation of the coagulation system and indirectly influences the formation of thrombosis. Therefore, future studies would deeply investigate the effect of ASB16-AS1 on immunity to further disclose its regulatory mechanism on DVT development.

However, the limitations of the study should also attract attention in future investigations. Due to the various risk factors of DVT, this study did not refine patients' pathogenesis to ensure the sample size. More targeted study subjects are needed in future studies to deeply evaluate the significance of ASB16-AS1 in discriminating different causes-induced DVT. Moreover, multiple-center investigations and a larger sample size should also be taken into the study design to improve the universality of the study results. The discrimination of DVT patients from arterial thrombosis is also a critical process for the clinical diagnosis of DVT. However, due to the limited enrollment of study subjects, the significance of ASB16-AS1 in discrimination between DVT and arterial thrombosis has not been revealed in the present study. Additionally, DVT-related adverse prognosis was judged mainly based on the therapeutic response of patients. If the patient receives treatment that targets the clot (such as anticoagulant therapy) and the symptoms alleviated, these patients are considered to possess a DVT-caused adverse prognosis. Therefore, a long-term follow-up survey might be necessary to further validate the observed prognostic significance of ASB16-AS1 in DVT.

Conclusion

According to the above findings, upregulated ASB16-AS1 in DVT is of great diagnosis and prognosis significance, which can be considered as a potential biomarker for the screening and monitoring of DVT.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-025-04487-0.

Supplementary Material 1

Author contributions

DW Z, XM Y and M W designed the research study. ML L, YY L, C C, MY Y and B L performed the research. ML L, YY L, XY Z and B L analyzed the data. DW Z, XM Y and M W wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Funding

No funding was received for conducting this study.

Data availability

Corresponding authors may provide data and materials.

Declarations

Ethics approval and consent to participate
The study protocol was approved by The Ethics Committee of Zibo Central Hospital (No. 201711010) and followed the principles outlined in the Declaration of Helsinki. In addition, informed consent has been obtained from the participants involved.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not Applicable.

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Received: 7 March 2024 / Accepted: 8 January 2025
Published online: 08 February 2025

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