

ORIGINAL ARTICLE

Serum PDK4 Level Changes and Clinical Implications in Lower-Limb Arterial Occlusion with Secondary Thrombosis Post-Intervention

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ABSTRACT

Background: The aim is to investigate the dynamic changes in serum pyruvate dehydrogenase kinase 4 (PDK4) levels and their predictive value for treatment outcomes in patients with lower extremity arteriosclerosis obliterans (LEASO) and secondary thrombosis undergoing catheter-directed thrombolysis (CDT).

Methods: A prospective cohort of 252 LEASO patients with secondary thrombosis treated with CDT between January 2022 and January 2025 was enrolled. Serum PDK4 levels were measured at baseline and at 8-hour intervals up to 72 hours post-CDT. Patients were classified as Effective (n = 175) or Ineffective (n = 77) based on third-day DSA imaging and clinical response. Generalized estimating equations (GEEs), ROC curve, and multivariable logistic regression were applied.

Results: The effective group showed significantly lower PDK4 levels at every time point ($p < 0.001$). GEEs revealed significant time, group, and group $\times$ time effects (all $p < 0.001$). Baseline PDK4 ≥ 7.059 ng/mL predicted ineffective thrombolysis with AUC 0.728 (70.13 % sensitivity, 68.00 % specificity). Independent risk factors for poor outcome were smoking, hypertension, diabetes, coronary artery disease, and higher baseline PDK4; pro-urokinase use was protective.

Conclusions: Serum PDK4 levels decline rapidly after successful CDT and baseline PDK4 ≥ 7.059 ng/mL is an independent predictor of ineffective thrombolysis in LEASO patients with secondary thrombosis. PDK4 monitoring may guide early prognostic assessment and individualized therapy.

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KEYWORDS

lower extremity arteriosclerosis obliterans, secondary thrombosis, interventional therapy, pyruvate dehydrogenase kinase 4

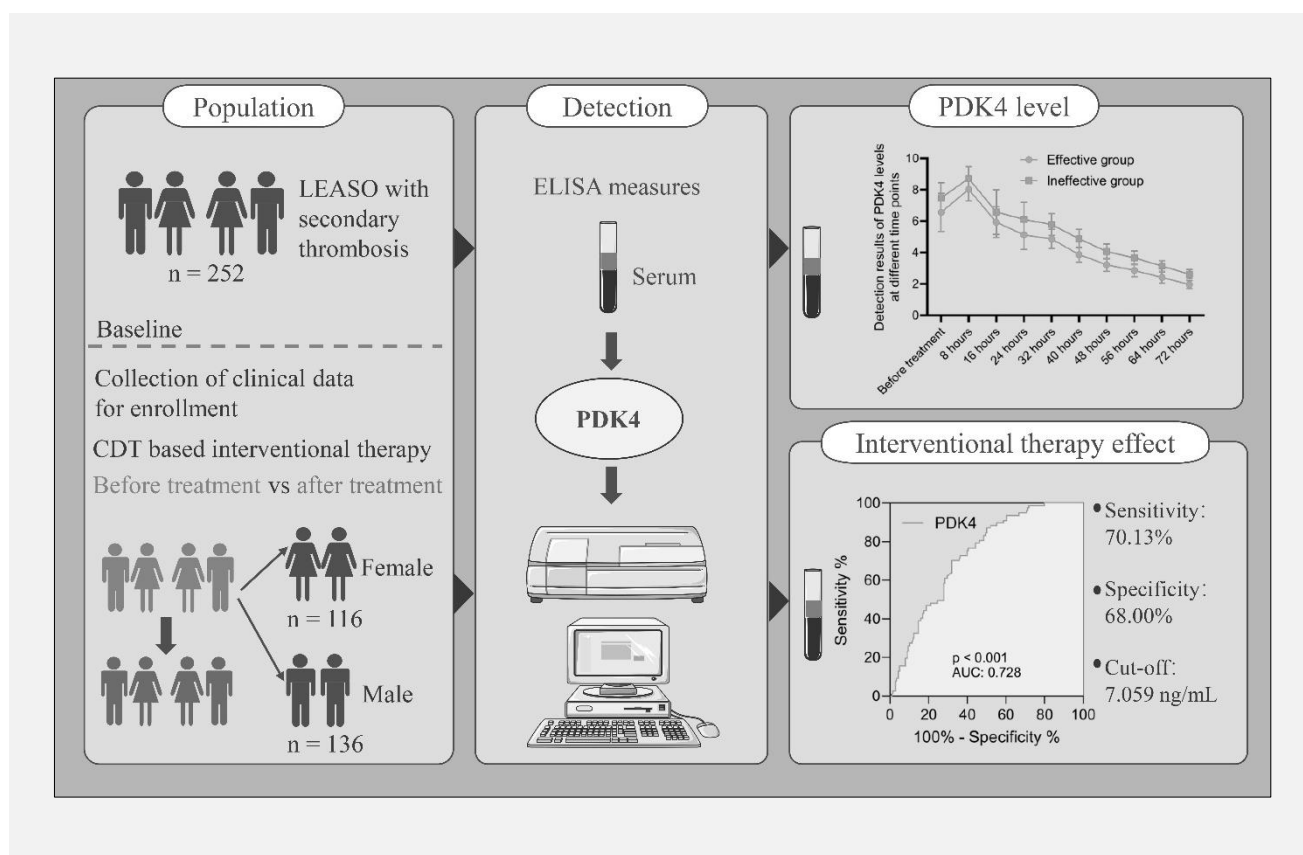


Figure 1. The graphical abstract of present research.

INTRODUCTION

Lower extremity arteriosclerosis obliterans (LEASO) is a chronic condition characterized by the stenosis or occlusion of lower limb arteries due to atherosclerotic plaques, leading to chronic limb ischemia [1]. Its pathogenesis involves endothelial injury, lipid infiltration, hemodynamic disturbance and genetic predisposition [2]. Clinically, LEASO presents with coldness, numbness, weakness, intermittent claudication, and - when advanced - rest pain, ischemic ulcers or gangrene, markedly impairing quality of life and increasing cardiovascular risk [3]. Superimposed thrombosis can precipitate acute limb ischemia [4-6]. Catheter-directed thrombolysis (CDT), which delivers thrombolytic agents such as pro-urokinase directly into the thrombus, is the primary intervention for LEASO complicated by secondary thrombosis [7,8]. CDT effectively recanalizes acute/sub-acute and some chronic occlusions, reduces amputation rates and improves quality of life [9]. However, predicting individual outcome and tailoring therapy remain challenging.

Pyruvate dehydrogenase kinase 4 (PDK4) is a key mitochondrial enzyme that phosphorylates the pyruvate-dehydrogenase complex and thereby governs cellular energy metabolism [10]. PDK4 promotes inflammation,

plaque instability and vascular calcification, linking it to atherosclerosis progression [11,12]. Thrombolytic therapy restores blood flow and improves tissue perfusion, thereby provoking metabolic reprogramming in ischemic tissue [13,14]. Indeed, higher serum PDK4 correlates with tissue-injury severity and predicts adverse outcomes in cardiovascular diseases [11]. However, whether PDK4 serves as a prognostic indicator after CDT in LEASO with secondary thrombosis remains unexplored.

Here, we tracked serial PDK4 levels in LEASO patients with secondary thrombosis undergoing CDT and tested whether baseline or post-treatment PDK4 change predicts clinical prognosis.

MATERIALS AND METHODS

Bioinformatics analysis

The Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov>) was queried to select the peripheral artery disease (PAD)-related microarray dataset GSE113873 for analysis. This dataset documents differentially expressed genes in the blood of patients with PAD compared to healthy controls. Differentially expressed molecules in PAD patients were identified

using a cutoff of adjusted p-value less than 0.05 and $|\log_2(\text{fold change})|$ greater than 1.

Study population

LEASO with secondary acute thrombosis is the most common pathological type of adult acute lower limb ischemia. Previous studies have reported an efficacy rate of 93.33% for catheter-directed thrombolysis in treating acute limb ischemia [15]. To ensure a margin of error no greater than 5% (i.e., a 10% difference between the upper and lower limits of the confidence interval), a sample size calculation was performed using PASS 15.0 software with a power of $1 - \alpha = 0.9$ (two-sided test). The calculation determined that a minimum of 87 subjects were required to ensure the scientific rigor of the study design.

A total of 252 patients with LEASO and secondary thrombosis who received treatment at our hospital between January 2022 and January 2025 were prospectively enrolled in the study.

Inclusion criteria were as follows: 1) Patients met the diagnostic criteria for LEASO outlined in the *Peripheral Artery Disease Management Guidelines* [16], which include typical symptoms such as intermittent claudication, rest pain, limb ulceration, or gangrene, along with high-risk factors such as smoking, diabetes, hypertension, or hyperlipidemia; physical examination revealed diminished or absent distal arterial pulses; ankle-brachial index (ABI) ≤ 0.9 ; and imaging studies (Doppler ultrasound, computed tomography angiography [CTA], magnetic resonance angiography [MRA], or digital subtraction angiography [DSA]) demonstrated arterial stenosis or occlusion. 2) Preoperative CTA showed the presence of secondary thrombosis in the lower limb arteries, and the patients were suitable for endovascular interventional therapy. 3) The onset of disease was within 14 days. 4) The affected limb was limited to one side. 5) Complete clinical and follow-up data were available. 6) Patients or their legal guardians provided informed consent and agreed to participate in the full course of observation and follow-up for the study.

Patients were excluded if they met any of the following criteria: 1) Presence of dysfunction or failure of vital organs such as the heart, liver, or kidneys; 2) Presence of immune system or hematologic disorders; 3) Presence of malignant tumors; 4) Underwent major surgical treatment within 3 months prior to admission; 5) Allergy to any of the medications used in this study; 6) Presence of uncontrollable hypertension (e.g., systolic blood pressure (SBP) ≥ 180 mmHg or diastolic blood pressure (DBP) ≥ 110 mmHg that cannot be reduced below these levels with pharmacologic therapy); 7) Presence of schizophrenia or other psychiatric disorders; 8) Pregnant or breastfeeding women; 9) Presence of severe cognitive impairment that would prevent the patient from cooperating with relevant examinations or follow-up during the study process; 10) Presence of any other condition deemed by the investigators as unsuitable for participation in this study.

A total of 252 patients were ultimately included in the analysis, comprising 136 males and 116 females, with ages ranging from 62 to 80 years and symptom duration from 7 to 13 days. The flowchart of participant enrollment is shown in Figure 2.

Study methods

Collection of clinical data: Clinical data of the enrolled patients were prospectively collected, including gender, age, body mass index (BMI), smoking and alcohol consumption status, ischemia status [17], duration of symptoms, site of arterial injection, length of thrombolytic catheter, use of pro-urokinase, and comorbidities such as hypertension, diabetes, hyperlipidemia, and coronary artery disease.

Treatment methods

All patients with LEASO underwent interventional therapy centered on CDT. The surgical approach involved either contralateral or ipsilateral common femoral artery puncture (either retrograde or antegrade), with lesion localization confirmed via catheter angiography. Under the guidance of DSA, an ultra-slippy loach guidewire was used in conjunction with a catheter to navigate through the lesion. Ensuring that the guidewire and catheter remained within the true lumen of the artery and successfully traversed the thrombus was crucial for the initial success of thrombolysis. If the guidewire and catheter could not pass through the occluded segment, a 4F single-bend catheter could be used to deliver a 15-minute thrombolytic treatment directly adjacent to the thrombus before attempting passage again; if this was still unsuccessful, a micro-puncture needle technique for retrograde puncture was employed.

During the procedure, pro-urokinase was initially administered via a bolus injection of 250,000 U, followed by a continuous infusion at a rate of 10,000 - 20,000 U/minute, with a total dose not exceeding 3 million U within 48 hours. DSA was repeated 24 - 48 hours after thrombolysis; if residual stenosis was $\geq 50\%$, percutaneous transluminal angioplasty was performed at 8 - 14 atmospheres of pressure, with balloon inflation lasting 30 - 60 seconds. For patients who experienced elastic recoil or dissection after angioplasty, drug-eluting stents were immediately implanted for treatment.

Close monitoring for bleeding complications was essential throughout the treatment process. Systemic anticoagulation with unfractionated heparin was administered, with coagulation tests performed every 6 hours and heparin dosage adjusted based on activated partial thromboplastin time (aPTT) to maintain levels between 50 and 70 seconds. After removal of the thrombolytic catheter, antiplatelet therapy was initiated.

Detection methods for PDK4

Blood samples (5 mL each) were collected from all enrolled patients before treatment and at 8, 16, 24, 32, 40, 48, 56, 64, and 72 hours after interventional therapy. The samples were allowed to clot at room temperature

for 30 minutes, followed by centrifugation at $1,000 \times g$ for 15 minutes. The serum was immediately harvested for measurement or aliquoted and stored at $\leq -80^{\circ}\text{C}$.

The levels of PDK4 in serum samples were quantitatively determined using a PDK4 Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog Number: ml104-865) produced by Shanghai Enzyme-linked Biotechnology Co., Ltd. Before the experiment, all reagents were equilibrated to room temperature for 30 minutes and prepared according to the kit instructions, including wash buffer, diluent, standards, biotinylated antibody working solution, and enzyme conjugate working solution. A pre-coated microplate was used, and 100 μL of standards and 2-fold diluted serum samples were added to the wells, followed by incubation at 37°C in the dark for 1.5 hours. Subsequent steps, including washing, incubation, and color development, were performed according to the kit instructions, and the reaction was terminated by adding stop solution. The absorbance was measured within 5 minutes using a microplate reader at 450 nm. The concentration of PDK4 in the samples was calculated based on a standard curve generated from the standards' concentrations and absorbance values.

Grouping

Patients were categorized into different groups based on the immediate DSA imaging results on day 3 post-interventional therapy, the status of distal arterial pulsation, and the improvement in clinical symptoms. The criteria for evaluation were as follows [15]: If DSA showed complete recanalization of the occluded segment, with palpable dorsalis pedis and/or posterior tibial artery pulsation, and complete resolution of lower limb pain, the outcome was classified as highly effective. If DSA showed complete or partial recanalization of the occluded segment, with non-palpable dorsalis pedis and/or posterior tibial artery pulsation but with warm skin temperature and alleviated pain in the lower limb, the outcome was classified as effective. If DSA showed persistent obstruction of the occluded segment, with non-palpable dorsalis pedis and/or posterior tibial artery pulsation and no improvement in lower limb pain, the outcome was classified as ineffective. The overall effective rate was calculated using the formula: Overall effective rate = Highly effective rate + Effective rate.

Statistical analysis

Data analysis was performed using SPSS software version 26.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess the normality of the distribution. Normally distributed continuous variables were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$), and intergroup comparisons were conducted using the *t*-test. Non-normally distributed continuous variables were described using the median and interquartile range [*M* (*P*₂₅, *P*₇₅)], with intergroup comparisons performed using the Mann-Whitney U test. Categorical variables were represented by frequency (*n*) and percentage (%),

with intergroup comparisons conducted using the chi-squared test.

The PDK4 repeated-measures data obtained from the two groups during the study were longitudinal, with each data point not being independent and the within-group correlation being significant. To derive more accurate conclusions, the Generalized Estimating Equations (GEEs) statistical method, based on the gamma distribution, was used to analyze the changes in serum PDK4 levels at different time points between the two groups.

The diagnostic value of pre-treatment PDK4 levels for the interventional treatment outcomes in patients with LEASO and secondary thrombosis was assessed using the Receiver Operating Characteristic (ROC) curve, and the PDK4 level with the maximum Youden index (sensitivity + specificity - 1) was used as the optimal cutoff value. Logistic regression analysis was employed to identify risk factors influencing the interventional treatment outcomes in patients with LEASO and secondary thrombosis.

RESULTS

Bioinformatics analysis results

Analysis of the GSE113873 microarray dataset revealed that, compared with the healthy control group, PDK4 was most significantly upregulated in the serum of patients with PAD, as shown in Figure 3. This study will further investigate whether there is an association between serum PDK4 levels and treatment outcomes in patients with PAD.

Comparison of baseline characteristics between the effective and ineffective groups

On day 3 after interventional therapy, based on the assessment of DSA imaging results, a total of 95 patients were identified as highly effective, 80 as effective, and 77 as ineffective, with an overall effective rate of 69.44%. Patients were divided into the effective group (*n* = 175) and the ineffective group (*n* = 77) according to the treatment outcomes. There were no significant differences between the two groups in terms of gender, age, BMI, alcohol consumption history, length of thrombolytic catheter, hypertension, diabetes, and hyperlipidemia (*p* > 0.05). Significant differences were observed between the two groups in smoking history, ischemia status, duration of symptoms, site of arterial injection, use of pro-urokinase, and coronary artery disease (*p* < 0.05). See Table 1.

Serum PDK4 levels at different time points in the effective and ineffective groups

Significant differences in PDK4 levels were observed between the effective group (*n* = 175) and the ineffective group (*n* = 77) at each time point (*p* < 0.001). To better understand these differences, we calculated the relative change in PDK4 from baseline at each time

Table 1. Comparison of baseline characteristics between the effective and ineffective groups.

Variable	Effective group (n = 175)	Ineffective group (n = 77)	<i>t</i> / <i>Z</i> / χ^2	p
Gender (n/%)				
Male	98 (56.00)	38 (49.35)	0.952 *	0.329
Female	77 (44.00)	39 (50.65)		
Age [<i>M</i> (<i>P</i> 25, <i>P</i> 75), years]	68.00 (63.00, 71.00)	68.00 (63.00,72.00)	-1.432 ^	0.152
BMI ($\bar{x} \pm s$, kg/m ⁻²)	24.02 ± 0.86	23.80 ± 1.03	-1.758	0.080
Smoking history (n/%)	92 (52.57)	53 (68.83)	5.786 *	0.016
Alcohol consumption history (n/%)	38 (21.71)	25 (32.47)	3.298 *	0.069
Ischemia status (n/%)				
Class I	19 (10.86)	7 (9.09)	6.405 *	0.093
Class IIa	43 (24.57)	16 (20.78)		
Class IIb	108 (61.71)	46 (59.74)		
Class III	5 (2.86)	8 (10.39)		
Duration of symptoms [<i>M</i> (<i>P</i> 25, <i>P</i> 75), d]	10.00 (9.00, 11.00)	12.00 (11.00, 13.00)	-8.566 ^	< 0.001
Site of arterial injection (n/%)				
Common femoral artery	97 (55.43)	41 (53.25)	0.105 *	0.949
Popliteal artery	48 (27.43)	22 (28.57)		
Brachial artery	30 (17.14)	14 (18.18)		
Length of thrombolytic catheter (n/%)				
10 cm	48 (27.43)	22 (28.57)	0.664 *	0.882
20 cm	28 (16.00)	13 (16.88)		
30 cm	52 (29.71)	25 (32.47)		
40 cm	47 (26.86)	17 (22.08)		
Use of urokinase ($\bar{x} \pm s$, × 10 ⁴ U)	257.60 ± 19.17	235.16 ± 18.64	-8.634 ^	< 0.001
Comorbidities (n/%)				
Hypertension	101 (57.71)	55 (71.43)	4.265 *	0.039
Diabetes mellitus	110 (62.86)	60 (77.92)	5.528 *	0.019
Hyperlipidemia	78 (44.57)	41 (53.25)	1.615 *	0.204
Coronary artery disease	51 (29.14)	42 (54.55)	14.819 *	< 0.001

* represents the chi-squared (χ^2) value, ^ represents the t-value, and # represents the Z-value.

point using the formula:

$\Delta\text{PDK4(T)} = (\text{PDK4(T)} - \text{PDK4(T}_0))/\text{PDK4(T}_0) \times 100\%$. Results from the GEEs analysis indicated that within 72 hours after thrombolytic therapy, serum PDK4 levels changed significantly over time (time effect, $p < 0.001$). There were significant differences in serum PDK4 levels between the two groups after treatment (group effect, $p < 0.001$). Moreover, the differences in measured values between the effective and ineffective groups increased progressively over time after treatment (group time interaction effect, $p < 0.001$). The relative PDK4 changes from baseline differed between the two groups. At 8 hours post-treatment, the effective

group had a 26.17% increase, and the ineffective group had an 18.33% increase. Over time, the effective group showed a steeper decline in PDK4 levels.

By 72 hours, both groups had decreased markedly, with the effective group showing a significantly greater reduction (-69.09%) than the ineffective group (-64.29%). These results underscore the potential of PDK4 as a biomarker of treatment efficacy. See Table 2.

Table 2. Detection results of PDK4 levels at different time points in the effective and ineffective groups.

Time	Effective group (n = 175)	Ineffective group (n = 77)	<i>t</i>	<i>p</i>	Relative change from baseline (%)
Pre-treatment	6.56 ± 1.21	7.48 ± 0.97	6.410	< 0.001	
8 h	8.02 ± 0.73	8.71 ± 0.75	6.818	< 0.001	26.17/18.33
16 h	5.93 ± 0.98	6.58 ± 1.43	3.604	< 0.001	-6.80/-10.41
24 h	5.13 ± 0.94	6.11 ± 1.08	7.250	< 0.001	-19.55/-16.97
32 h	4.86 ± 0.61	5.78 ± 0.69	10.572	< 0.001	-23.46/-15.76
40 h	3.87 ± 0.48	4.88 ± 0.59	14.383	< 0.001	-39.08/-33.91
48 h	3.22 ± 0.42	4.06 ± 0.48	13.951	< 0.001	-49.42/-44.93
56 h	2.86 ± 0.40	3.66 ± 0.44	14.228	< 0.001	-54.89/-50.50
64 h	2.41 ± 0.36	3.14 ± 0.34	15.096	< 0.001	-62.08/-57.25
72 h	1.96 ± 0.25	2.62 ± 0.29	18.506	< 0.001	-69.09/-64.29
Time effect	Wald $\chi^2 = 20,822.110$ $p < 0.001$				
Group effect	Wald $\chi^2 = 126.936$ $p < 0.001$				
Group × time effect	Wald $\chi^2 = 42.250$ $p < 0.001$				

Table 3 Risk factors influencing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis.

Variable	β	SE	Walds	<i>p</i>	OR	95% CI
Smoking status	0.888	0.380	5.453	0.020	2.431	1.153 - 5.123
Use of pro-urokinase	-0.066	0.011	36.651	< 0.001	0.936	0.916 - 0.956
Hypertension	0.807	0.386	4.366	0.037	2.241	1.051 - 4.778
Diabetes mellitus	0.840	0.415	4.105	0.043	2.316	1.028 - 5.219
Coronary artery disease	1.062	0.362	8.614	0.003	2.893	1.423 - 5.880
Pre-treatment PDK4 levels	0.677	0.155	18.952	< 0.001	1.968	1.451 - 2.669

Diagnostic value of pre-treatment PDK4 levels for the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis

Results from the ROC curve analysis showed that the Area Under the Curve (AUC) for diagnosing the effectiveness of interventional therapy based on pre-treatment PDK4 levels was 0.728, with a sensitivity of 70.13 % and a specificity of 68.00 %. The optimal cut-off value was determined to be 7.059 ng/mL. See Figure 4.

Risk factors influencing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis

Logistic regression analysis was conducted to identify risk factors affecting the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis, using the outcome of interventional therapy (effective = 0, ineffective = 1) as the dependent variable and smoking status (yes = 1, no = 0), use of pro-uroki-

nase (continuous variable), hypertension (yes = 1, no = 0), diabetes (yes = 1, no = 0), coronary artery disease (yes = 1, no = 0), and pre-treatment PDK4 levels (continuous variable) as independent variables.

The results indicated that smoking, hypertension, diabetes, coronary artery disease, and pre-treatment PDK4 levels were independent risk factors influencing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis. The use of pro-urokinase was identified as a protective factor. See Table 3 and Figure 5.

DISCUSSION

Acute limb ischemia is a pathological condition characterized by a sudden interruption or drastic reduction in blood flow to the lower limbs, leading to acute ischemia and hypoxia of the tissues, which in turn triggers a series of clinical symptoms and signs [5]. LEASO is

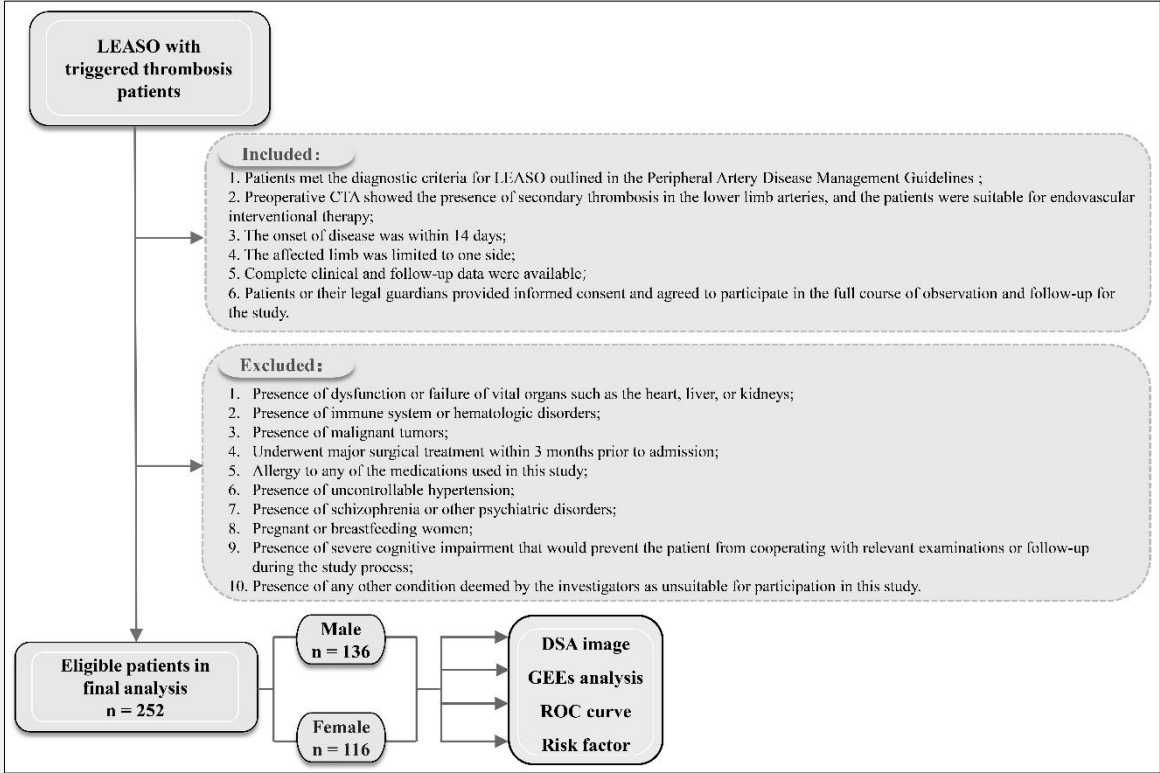


Figure 2. Flowchart of participant enrolment.

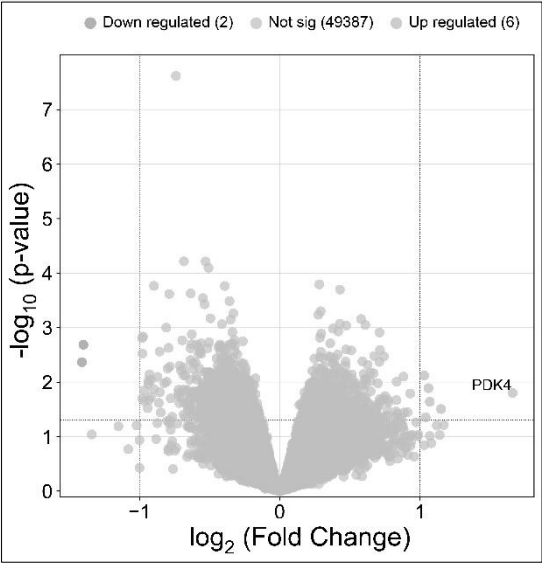


Figure 3. Analysis results of the GSE113873 microarray dataset.

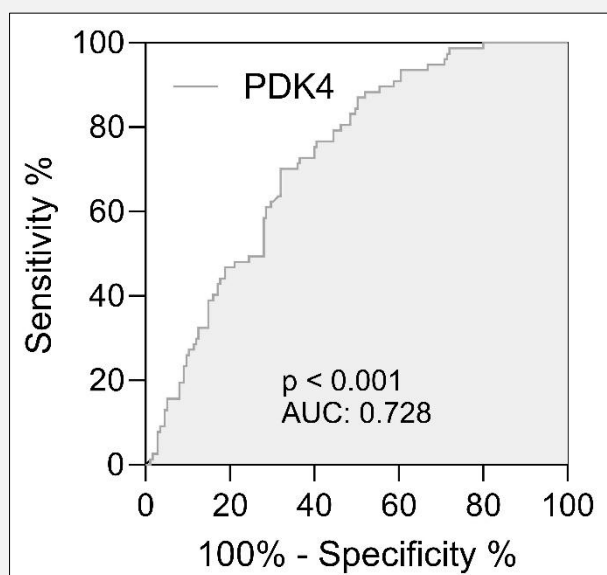


Figure 4. ROC curve for assessing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis based on pre-treatment PDK4 levels.

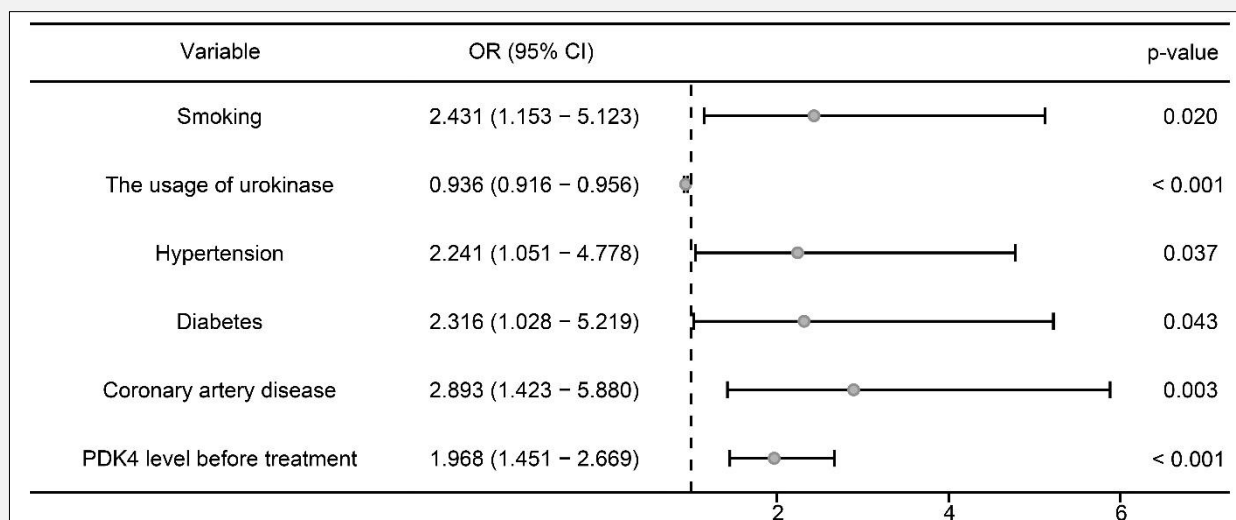


Figure 5. Forest plot of risk factors influencing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis.

one of the common causes of lower limb ischemia, and when secondary thrombosis occurs on this basis, the already compromised blood flow in the lower limb arteries due to stenosis can be further impeded by the thrombus, resulting in a drastic reduction or even cessation of blood flow and causing acute limb ischemia [18].

Inflammatory responses and thrombus formation jointly drive the acute exacerbation of LEASO, with PDK4 playing a key role in both processes. On one hand, PDK4 promotes the proliferation of smooth muscle cells and the progression of atherosclerosis by regulating mitochondrial metabolism. On the other hand, PDK4 expression is upregulated 3.2-fold in activated platelets, where it enhances glycolysis and ROS production, accelerating the release of thromboxane A₂ and fibrin deposition [19]. Additionally, PDK4-mediated formation of neutrophil extracellular traps further increases thrombus stability [20], suggesting that PDK4 is a pivotal molecule linking atherosclerosis and acute thrombotic events. In CD14⁺ monocytes treated with low-density lipoprotein (LDL), PDK4 mRNA expression is significantly upregulated, indicating that PDK4 may be involved in the induction of abnormal glycolysis in LDL-treated CD14⁺ monocytes and is associated with the abnormal activation of CD14⁺ monocytes in patients with coronary artery disease [21]. In the pathogenesis of atherosclerosis, after endothelial cell injury, smooth muscle cells migrate to and proliferate within the intima [22,23]. The upregulation of PDK4 may influence the proliferation and migration of smooth muscle cells by affecting cellular energy metabolism [24, 25]. Although current research has primarily focused on the role of PDK4 in coronary artery disease, given the similarities in the pathogenesis of lower extremity arterial occlusive disease and coronary atherosclerosis, PDK4 may also play a role in the proliferation of smooth muscle cells in lower extremity arterial occlusive disease.

In this study, by monitoring the changes in PDK4 levels after interventional therapy, it was found that PDK4 levels rapidly increased 8 hours after thrombolytic treatment and then continuously decreased within 72 hours, with the differences in PDK4 levels between the two groups gradually increasing. This suggests that the clearance rate of PDK4 is directly related to the degree of thrombus dissolution, indicating that changes in PDK4 levels can serve as a potential indicator for assessing the effectiveness of interventional therapy.

Results from the ROC curve analysis showed that the AUC for diagnosing the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis based on pre-treatment PDK4 levels was 0.728, with a sensitivity of 70.13% and a specificity of 68.00%. The cutoff value was determined to be 7.059 ng/mL. This indicates that a pre-treatment PDK4 level $\geq$ 7.059 ng/mL is associated with an increased risk of failed recanalization and can serve as an important reference indicator for predicting treatment outcomes, as-

sisting clinicians in making preliminary assessments of patient prognosis before treatment.

Previous studies have identified smoking, diabetes, and hypertension as risk factors influencing the occurrence of LEASO [26-28]. In the present study, results from logistic regression analysis indicated that smoking, hypertension, diabetes, coronary artery disease, and pre-treatment PDK4 levels were independent risk factors affecting the effectiveness of interventional therapy in patients with LEASO and secondary thrombosis, while the use of pro-urokinase was a protective factor influencing the effectiveness of interventional therapy in these patients. These findings suggest that in clinical practice, these risk factors should be closely monitored, and targeted interventions should be implemented to enhance the efficacy of thrombolytic therapy. For patients who smoke, smoking cessation interventions should be intensified; for those with hypertension and diabetes, strict control of blood pressure and glucose levels should be maintained; and for patients with coronary artery disease, comprehensive cardiovascular disease management should be provided. In addition, the rational use of thrombolytic agents such as pro-urokinase, ensuring the scientific dosing and administration of these drugs, is also an important measure to improve therapeutic outcomes.

As a prospective study, the present research, through its rigorous design and inclusion and exclusion criteria, has minimized the impact of selection bias and confounding factors, thereby more accurately assessing the significance of PDK4 in the effectiveness of interventional therapy for patients with LEASO and secondary thrombosis. However, despite the enhanced scientific rigor conferred by the prospective study design, certain limitations remain. First, this study measured only serum PDK4 levels and did not investigate its expression in tissues. Additionally, the study had a small sample size and a short duration; future research should expand the sample size and extend the study period to enhance the reliability and generalizability of the results. Finally, future studies could further explore the combined application of PDK4 with other biomarkers or clinical indicators to more comprehensively evaluate thrombolytic efficacy and predict prognosis.

In summary, this study has thoroughly investigated the changes in serum PDK4 levels and their clinical significance in patients with LEASO and secondary thrombosis undergoing interventional therapy, revealing the important role and potential clinical applications of PDK4 in thrombolytic treatment. Monitoring changes in PDK4 levels can provide clinicians with an early indicator for assessing the prognosis of patients with LEASO and secondary thrombosis, thereby optimizing treatment strategies and improving patient outcomes and quality of life.

Ethical Statement:

This study involving human participants was conducted in accordance with the Declaration of Helsinki and received ethical approval from The Affiliated Hospital of Bei-hua University (Approval No. 2022025). Written informed consent was obtained from all participants prior to their inclusion in the study, ensuring their confidentiality and anonymity throughout the research process.

Data Availability Statement:

Data will be available upon request.

Declaration of Generative AI in Scientific Writing:

All authors affirm that no artificial intelligence was utilized in the writing of this manuscript.

Declaration of Interest:

The authors declare no competing interests.

References:

- Huang Q, Shu H, Zeng C, Qiu P, Xiong X, Lu X. Endovascular revascularisation versus surgical revascularisation in patients with lower limb atherosclerosis obliterans: a protocol for systematic review and meta-analysis with trial sequential analysis and meta-regression. *BMJ Open* 2022;12(12):e066903. (PMID: 36600343)
- Daugherty A, Fisher EA, Taubman MB, Heistad DD, Fogelman AM. Forty-Year Anniversary of Arteriosclerosis, Thrombosis, and Vascular Biology. *Arterioscler Thromb Vasc Biol* 2021; 41(9):2353-6. (PMID: 34432483)
- Firnhaber JM, Powell CS. Lower Extremity Peripheral Artery Disease: Diagnosis and Treatment. *Am Fam Physician* 2019; 99(6):362-9. (PMID: 30874413)
- McNally MM, Univers J. Acute Limb Ischemia. *Surg Clin North Am* 2018;98(5):1081-96. (PMID: 30243449)
- Natarajan B, Patel P, Mukherjee A. Acute Lower Limb Ischemia-Etiology, Pathology, and Management. *Int J Angiol* 2020;29(3): 168-74. (PMID: 33100802)
- Olinic DM, Stanek A, Tătaru DA, Homorodean C, Olinic M. Acute Limb Ischemia: An Update on Diagnosis and Management. *J Clin Med* 2019;8(8):1215. (PMID: 31416204)
- Fleck D, Albadawi H, Shamoun F, Knuttinen G, Naidu S, Oklu R. Catheter-directed thrombolysis of deep vein thrombosis: literature review and practice considerations. *Cardiovasc Diagn Ther* 2017;7(Suppl 3):S228-s237. (PMID: 29399526)
- Goldhaber SZ, Magnuson EA, Chinnakondepalli KM, Cohen DJ, Vedantham S. Catheter-directed thrombolysis for deep vein thrombosis: 2021 update. *Vasc Med* 2021;26(6):662-9. (PMID: 34606385)
- Güneş Y, Sincer I, Erdal E. Catheter-directed intra-arterial thrombolysis for lower extremity arterial occlusions. *Anatol J Cardiol* 2019;22(2):54-9. (PMID: 31375648)
- Wang X, Shen X, Yan Y, Li H. Pyruvate dehydrogenase kinases (PDKs): an overview toward clinical applications. *Biosci Rep* 2021;41(4):BSR20204402. (PMID: 33739396)
- Forteza MJ, Berg M, Edseldt A, et al. Pyruvate dehydrogenase kinase regulates vascular inflammation in atherosclerosis and increases cardiovascular risk. *Cardiovasc Res* 2023;119(7):1524-36. (PMID: 36866436)
- Ma WQ, Sun XJ, Zhu Y, Liu NF. PDK4 promotes vascular calcification by interfering with autophagic activity and metabolic reprogramming. *Cell Death Dis* 2020;11(11):991. (PMID: 33203874)
- Ascher E, Kibrik P, Rizvi SA, Alsheekh A, Marks N, Hingorani A. Fast-track thrombolysis protocol for acute limb ischemia. *J Vasc Surg* 2021;73(3):950-9. (PMID: 32437952)
- Funaki B. Thrombolysis for acute limb-threatening ischemia: a practical approach. *Semin Intervent Radiol* 2012;29(3):201-3. (PMID: 23997413)
- Lian WS, Das SK, Hu XX, Zhang XJ, Xie XY, Li MQ. Efficacy of intra-arterial catheter-directed thrombolysis for popliteal and infrapopliteal acute limb ischemia. *J Vasc Surg* 2020;71(1):141-8. (PMID: 31327613)
- Gornik HL, Aronow HD, Goodney PP, et al. 2024 ACC/AHA/AACVPR/APMA/ABC/SCAI/SVM/SVN/SVS/SIR/VESSE Guideline for the Management of Lower Extremity Peripheral Artery Disease: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol* 2024;83(24):2497-604. (PMID: 38752899)
- Hardman RL, Jazaeri O, Yi J, Smith M, Gupta R. Overview of classification systems in peripheral artery disease. *Semin Intervent Radiol* 2014;31(4):378-88. (PMID: 25435665)
- Ferrer C, Cannizzaro GA, Borlizzi A, Caruso C, Giudice R. Acute ischemia of the upper and lower limbs: Tailoring the treatment to the underlying etiology. *Semin Vasc Surg* 2023;36(2): 211-23. (PMID: 37330235)
- Flora GD, Nayak MK, Ghatge M, Kumskova M, Patel RB, Chauhan AK. Mitochondrial pyruvate dehydrogenase kinases contribute to platelet function and thrombosis in mice by regulating aerobic glycolysis. *Blood Adv* 2023;7(11):2347-59. (PMID: 36971790)
- Flora GD, Ghatge M, Nayak MK, Barbhuyan T, Kumskova M, Chauhan AK. Deletion of pyruvate dehydrogenase kinases reduces susceptibility to deep vein thrombosis in mice. *Blood Adv* 2024;8(15):3906-13. (PMID: 38838230)
- Du P, Guo R, Gao K, et al. Identification of differentially expressed genes and the role of PDK4 in CD14⁺ monocytes of coronary artery disease. *Biosci Rep* 2021;41(4):BSR20204124. (PMID: 33739370)
- Grootaert MOJ, Bennett MR. Vascular smooth muscle cells in atherosclerosis: time for a re-assessment. *Cardiovasc Res* 2021; 117(11):2326-39. (PMID: 33576407)
- Miano JM, Fisher EA, Majesky MW. Fate and State of Vascular Smooth Muscle Cells in Atherosclerosis. *Circulation* 2021; 143(21):2110-6. (PMID: 34029141)
- Thoudam T, Chanda D, Sinam IS, et al. Noncanonical PDK4 action alters mitochondrial dynamics to affect the cellular respiratory status. *Proc Natl Acad Sci U S A* 2022;119(34): e2120157119. (PMID: 35969774)

25. Sarkar A, Pawar SV, Chopra K, Jain M. Gamut of glycolytic enzymes in vascular smooth muscle cell proliferation: Implications for vascular proliferative diseases. *Biochim Biophys Acta Mol Basis Dis* 2024;1870(3):167021. (PMID: 38216067)
26. Xu Y, Pouncey AL, Zhou Z, Woodward M, Harris K. Smoking as a risk factor for lower extremity peripheral artery disease in women compared to men: A systematic review and meta-analysis. *PLoS One* 2024;19(4):e0300963. (PMID: 38656947)
27. Chase-Vilchez AZ, Chan IHY, Peters SAE, Woodward M. Diabetes as a risk factor for incident peripheral arterial disease in women compared to men: a systematic review and meta-analysis. *Cardiovasc Diabetol* 2020;19(1):151. (PMID: 32979922)
28. Eid MA, Mehta K, Barnes JA, et al. The global burden of peripheral artery disease. *J Vasc Surg* 2023;77(4):1119-26.e1. (PMID: 36565779)

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