Abnormalities of the Serum Proteomic in Thrombosis After Central Venous Catheter (CVC) Insertion in Patients with End-stage Kidney Disease

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Introduction. This study utilized serum proteomics with tandem mass tags (TMT) to investigate potential biomarkers associated with femoral central venous catheter (CVC) thrombosis in end-stage kidney disease (ESKD) patients. TMT proteomics analysis on serum samples was conducted to identify proteins with distinct expression levels that may be linked to thrombosis. The findings have important implications for enhancing anticoagulant procedures, catheter closure techniques, and determining optimal intervention timing for post-catheterization dialysis.

Methods. Thirty ESKD patients with CVC receiving hemodialysis between May 2021 and October 2022 at the First Affiliated Hospital of Chengdu Medical College were included in the study, and grouped according to vascular color Doppler ultrasound results, including 23 patients in the thrombo-positive group and 7 patients in the thrombo-negative group. Selection criteria were: 1) Patients with ESKD candidate for hemodialysis initiation; 2) no dialysis access has been placed previously, and CVC needs to be inserted as a temporary access; 3) patients volunteered to participate in this clinical study. Clinical data, blood tests, coagulation function, and biochemical parameters were collected and analyzed on the 14th day after catheterization. Color ultrasonography was conducted on the same day to categorize patients into two groups: those with thrombus-positive results and those with thrombus-negative results.

Results. TMT proteomics analysis identified twenty-eight differently expressed proteins, including 16 upregulated and 12 downregulated proteins. Enrichment analysis demonstrated nine proteins that were significantly enriched in four pathways within the thrombus-positive group after CVC insertion. Enzyme-linked immunosorbent assay (ELISA) test confirmed the TMT proteomics findings, specifically highlighting significant differences in human plasma kallikrein B1 (KLKB1) and angiopoietin-like protein 3 (ANGPTL3) levels on the 14th day after CVC insertion. Additionally, KLKB1, fibrinogen (FIB), D-dimer, and fibrinogen degradation products (FDP) levels were significantly elevated, while ANGPTL3 levels were decreased on the 14th day after CVC insertion in the thrombus-positive ESKD patient group.

Conclusion. Monitoring coagulation status post-CVC catheterization and evaluating potential biomarkers like KLKB1 and ANGPTL3 can contribute to the development of personalized treatment plans, improving the quality of hemodialysis and the overall quality of life for ESKD patients.

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Original Paper

INTRODUCTION

Chronic kidney disease (CKD) has emerged as a significant global health issue, contributing to the rising number of deaths worldwide.[1] End-stage kidney disease (EKSD) manifests with severe uremic symptoms and causes major problems in patients. Studies indicate that the incidence of CKD in China is 10.8%, and approximately 1 to 2 million individuals suffer from ESKD.[2] Patients diagnosed with ESKD require kidney replacement therapy (KRT), including kidney transplantation, hemodialysis (HD), or peritoneal dialysis (PD), is necessary for the rest of their life. However, due to the high cost of kidney transplantation, most ESKD patients in China primarily rely on HD and PD, with HD being the most commonly chosen method.[3] These annual dialysis treatment costs for ESKD patients impose a substantial economic burden on the country and society. Arteriovenous fistula (AVF) is the preferred form of vascular access for HD, but when the embedded AVF is not yet functional or is immature, central venous catheter (CVC) insertion becomes necessary.[4] Despite the increased risks associated with the use of CVCs, they continue to be a commonly chosen option for initiating dialysis.[5-7] However, the precise mechanisms and factors that contribute to thrombosis formation following CVC insertion have not yet been fully deciphered.[8-10] Proteomics, a comprehensive scientific field dedicated to the analysis of proteins, has been extensively employed in different aspects of kidney health, while its application in exploring thrombosis after CVC insertion in ESKD patients remains unexplored.[11-13] To date, there is a lack of proteomics analyses that have examined the occurrence of thrombosis following CVC insertion in patients with ESKD. Tandem Mass Tag (TMT) proteomics is an in vitro polypeptide labeling technique that employs 2-plex, 6-plex, or 10-plex isotope labels to specifically mark amino groups of polypeptides. TMT proteomics has found extensive applications in drug target identification, disease screening, and understanding the molecular mechanisms of biological growth and development. By employing Tandem Mass Tag (TMT) proteomics, we aim to identify potential biomarkers associated with thrombosis and provide a broader method for predicting thrombosis, thereby improving patient care and outcomes.

MATERIALS AND METHODS

Research Participants

The study consisted of 30 end-stage kidney disease (ESKD) patients who underwent their first temporary CVC insertion for hemodialysis between May 2021 and October 2022 at the First Affiliated Hospital of Chengdu Medical College (FAHCMC). This study was supported by the Key Discipline Construction project of Sichuan Province, Key project of Education Department of Sichuan Province (17ZA0137). Vascular ultrasound examinations using a color Doppler flow imaging ultrasound instrument (Model: EPIQ7, PHILIPS, USA) were performed in the ultrasound department on day fourteen post-CVC implantation to assess the presence of thrombosis. Diagnostic criteria for positive catheter-related thrombus included direct signs of recent thrombus at the catheter tip, filling defect observed on ultrasound blood flow signal, and absence of normal periodic changes on blood flow frequency spectrum.

Inclusion criteria were: 1) Patients with ESKD candidate for hemodialysis initiation; 2) Patients with no dialysis access has been placed previously, and CVC needs to be inserted as a temporary access; 3) Patients volunteered to participate in this clinical study. Exclusion Criteria were: 1) Patients with arteriovenous fistula without the need for catheterization; 2) patients who have had a central venous catheter placed and subsequently removed due to infection or other reasons, and received an re-catheterization; 3) patients with gastrointestinal bleeding, intracranial hemorrhage and other active bleeding within two weeks of catheterization; 4) patients whose long-term oral medications have a major influence on coagulation function; and 5) patients with a history of deep vein thrombosis.

Blood Sample Collection and Biochemical Indicators Detection Methods

The blood samples obtained from patients were collected on the 14th day following catheterization. All biochemical measurements including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB) platelet count (PLT), blood biochemical indices including: Total cholesterol (TC), Triglycerides (TG), Serum albumin (ALB), High density lipoprotein (HDL), Low density lipoprotein (LDL), and indicators related to coagulation function, including D-dimer, Fibrinogen

degradation product (FDP), Prothrombin time (PT), International normalized ratio (INR), Activated partial thromboplastin time (APTT), Thrombin time (TT), Fibrinogen (FIB), were performed in our clinical laboratory (Beckman Coulter, Inc, Bria, California, USA).

Differential Protein Screening

According to the TMT kit (TMT 10-plex Isobaric Label Reagent Set, Thermo Fly, Inc., USA) instructions, the labeled samples of each group were mixed in equal quantities. Peptide samples were subjected by reverse-phase HPLC and separated at pH = 10, as shown in Table 1. Nano-uplc liquid system Easy-NLC1200 (Thermo Fisher Scientific, Waltham, MA, USA) was used to separate 2μg polypeptides from each group, and then q-EXactive (Thermo Fisher Scientific, Waltham, MA, USA) was used for detection, as shown in Table 2.

Serum Proteomic Analysis and Bioinformatics Analysis

The protein database used in this study was uniprothuman_20181016_iso. The marker sites were polypeptide N-terminal and Lys(K), and the PIF was set at 0.75. The quantitative method used was secondary reporter sub-quantification with four TMT markers, and nonstandard intensity-based absolute-protein-quantification (IBAQ) quantification was performed at the same time. The six samples were then standardized so that the total protein, or median, of each group was consistent.

All differentially expressed proteins were identified by statistical analysis of the results. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed on proteins with significant differences (multiple > 1.5, P value ≤ .05, and unique peptide > 2). The experiment involved biological repetition.

ELISA Validation Test

The concentration of the screened differential proteins was measured with an enzyme-linked immunosorbent assay (ELISA; Shanghai Enzyme Linked Biotechnology Co., LTD, Shanghai, China). The optical density (OD) of each microplare was measured at 450 nm by using a microplate reader (syergyH1, American Berten Instrument Co., LTD, VT, USA).

Statistical Analysis

Statistical analysis of the data was carried out using SPSS 22.0 (Authorization code: X5YW7GM2SW). All measurement data in this study are statistically described by the mean ± standard deviation or median. A t-test of two independent samples was used for the measurement data conforming to the normal distribution and homogeneity of variance. For the measurement data that did not conform to a normal distribution or homogeneity of variance, the nonparametric Mann-Whitney U test was adopted. Enumerative data in the study are described as percentages, and Fisher's exact probability test or the χ^2 test was used ($P < .05$).

RESULTS

Comparision of General Data of Participants

Based on the color ultrasound results, patients were classified into thrombus positive group (n = 23) and thrombus negative group (n = 7). Detailed medical history and clinical data were collected and summarized in Table 3 to 5.

Table 1. Composition of Chromatographic Column, Mobile Phase A and B

The Name of the Reagent	Composition
The chromatographic column	150 mm*2.1 mm (water, XBridge BEH C18 XP Column)
Mobile phase A	10 mM ammonium formate aqueous solution, pH = 10
Mobile phase B	Ammonium formate 10 mM, 10% H2O, 90% ACN, pH = 10

Table 2. Composition of Reversed-phase Chromatographic Column, Mobile Phase A and B

The Name of the Reagent	Composition
Reverse-phase chromatographic column	Reprosil-Pur 120 C18-AQ, 1.9 μm, Dr. Math
Mobile phase A	0.1% formic acid acetonitrile aqueous solution (acetonitrile is 2%)
Mobile phase B	0.1% formic acid acetonitrile aqueous solution (acetonitrile is 80%)

Table 3. The Basic Disease Composition of the Study Participants

Cause of ESKD	Number of Cases	Percentage (%)
Primary glomerulonephritis	19	63.33
Diabetic Nephropathy	4	13.33
Lupus nephritis	1	3.33
Hypertensive nephropathy	1	3.33
Renal damage in multiple myeloma	1	3.33
Amyloidogenic nephropathy	1	3.33
Unknown cause	3	10.00
Total	30	100

Differential Proteins in Thrombus Positive Group and Thrombus Negative Group

The analysis of blood revealed the following parameters. The quality inspection results of the protein samples showed that the protein samples were qualified and there was no obvious degradation of the protein samples, as shown in Figure 1. In our study, expression levels of Δ ratio > 1.5 or < 1/1.5, *P* value ≤ 0.05, and the protein

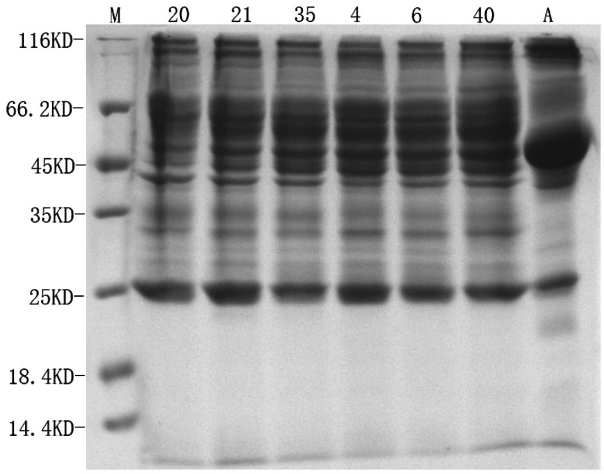

Figure 1. Gel Electrophoresis Pattern (There are a lot of high abundance proteins in blood proteins) [It is the gel image of protein before the high abundance treatment, and the remaining six bands are the gel image of protein after the high abundance treatment. Coomath bright blue results showed no obvious protein degradation of polyacrylamide gel electrophoresis sample loading was 20 μg and the protein samples qualified without obvious protein degradation.]

Table 4. Description of Baseline Characteristics of the General Data of the Study Participants

	Positive Group (n = 23)	Negative Group (n=7)	*P*
Male [n (%)]	15 (65.2)	4 (57.1)	> .999
Female [n (%)]	8 (34.8)	3 (43.9)	> .999
Age ($\bar{x} \pm S$)	57.43 ± 18.07	64.29 ± 20.44	.401
Diabetes history (with / without)	9/14	2/5	.618
Smoking history (with / no)	11/12	4/3	.671

note: There were no significant differences in the basic information of the two groups (*P* > .05).

Table 5. Description of Laboratory Data in the Study Participants

	Positive Group (n = 23)	Negative Group (n = 7)	*P*
WBC, $\times 10^9$/L	7.11 ± 2.82	7.12 ± 2.11	.993
RBC, $\times 10^{12}$/L	2.61 ± 0.74	2.55 ± 0.61	.860
HB, g/L	72 (59 to 86)	71.43 ± 14.00	.924
PLT, $\times 10^9$/L	124 (97 to 170)	136.71 ± 68.26	.701
PT, sec	11 (10.10 to 11.70)	11.37 ± 1.32	.631
TNR	1.02 (0.94 to 1.08)	1.05 ± 0.65	.631
APTT, sec	29.90 (28.30 to 31.00)	29.29 ± 4.00	.631
TT, sec	15.40 (14.40 to 16.40)	15.60 ± 1.33	.886
FIB, g/L	5.14 ± 0.91	4.32 ± 0.83	.041*
D-dimer, ng/mL	929 (508 to 2765)	323.43 ± 198.44	< .001*
FDP, ug/mL	8.4 (5.48 to 13.24)	4.31 ± 1.42	.002*
ALB, g/L	28.56 ± 4.13	28.04 ± 2.93	.763
TG, mmol/L	1.92(1.18 to 3.27)	1.48 ± 0.64	.311
TC, mmol/L	4.15 ± 0.90	3.23 (2.84 to 5.18)	.598
HDL, mmol/L	1.14 (0.70 to 1.29)	1.09 ± 0.50	> .999
LDL, mmol/L	2.41 ± 0.72	2.60 ± 1.62	.778

note: *P* values were compared between the positive group and negative group (**P* < .05 vs. negative group)

of unique peptide > 2 were defined significant. Among the results, 28 differential proteins were identified, and the identification and quantitative results are summarized in Table 6 and Table 7. For comparison between groups, the volcano map is displayed. Dots distributed over the two transverse boundaries and outside the longitudinal boundaries represent significantly different proteins and are labeled accordingly. As shown in Figure 2, there were sixteen up-regulated proteins and 12 down-regulated proteins. The top 10 Gene Ontology (GO) items are shown in pictures as follows, as shown in Figure 3.

KEGG Pathway Analysis of Differential Proteins

KEGG enrichment analysis showed that nine proteins were significantly enriched in four pathways in the thrombus positive group compared with the thrombus negative group after CVC catheterization, and which have six different protein F12/C1S/C7/KLKB1/C6/CFHR3 associated with complement level and blood coagulation unicom road, three differences between protein APOE/LPL/ANGPTL3 associated with cholesterol metabolism, two differences between protein C7/C6 and prion disease, three differential proteins C1S/C7/C6 were associated with systemic lupus erythematosus, as shown in Figure 4.

Comparison Results of Screened Differential Proteins at Different Time Points Between Two Groups

Without considering the interaction between group and time, the comparison between each indicator at different time points indicated that there was no significant difference in the measured values of KLKB1, C1S, CFHR3 or ANGPTL3 between the two groups on the first day or the seventh day and the 14th day (Table 8). The statistical results showed that there was an interaction between two groups and time for the KLKB1 detection results, as shown in Table 8.

Repeated Measure ANOVA for KLKB1 and C1S

The findings suggest that KLKB1 levels vary among different patient groups, displaying distinct trends over time, as depicted in Figure 5. Conversely, the detection results of C1S, as shown in Figure 6, did not exhibit any interaction between group and time. Notably, the positive group consistently demonstrated higher C1S values compared to the negative group across all time points. However, statistical analysis revealed no significant differences in C1S values between the two groups at different time intervals, as presented in Tables 9 and 10.

Repeated Measure ANOVA for CFHR3

In the detection results of CFHR3, as shown in

Table 7. Differential Protein List

Protein Number	Protein Name	Ratio of differential protein in thrombus positive group/thrombus negative group
A0A024QZN4	VCL; HEL114	Lower0.607928226
G3V3H8	WARS	Lower0.577856398
A0A080YV01	LYZG; LALBA	Higher1.67989788
B3GN61	CDH1	Higher2.618055425
Q8IZZ5	F12	Higher1.685660873
A0A0S2Z3D5	APOE	Higher1.524573392
B2R6M6	EFEMP1	Lower0.560009837
A0A182DWH7;	SEPP1	Higher1.641577131
B3KRV7	LPL	Lower0.386107272
A2NB45	—	Higher1.753972035
A8K061	ANGPTL3	Lower0.507455073
A8K2N0	C1S	Higher2.556047147
A8K2T4	C7	Lower0.475691608
G3V0E5	TFRC	Higher1.59015403
B0YJC6	PROZ	Higher1.559307578
Q96PQ9	LOX	Lower0.577348894
D6RF35	GC; HEL-S-51	Lower0.604720306
H0YAC1	KLKB1	Higher1.524425942
O43866	CD5L	Higher1.548938901
P00738	HP	Higher2.717490266
P00739	HPR	Higher1.67602254
P10645	CHGA	Lower0.580949301
P13671	C6	Lower0.476386565
P27169	PON1	Higher2.15835729
Q53XB4	RAB1;RNASE4	Lower0.652410891
P80108	GPLD1	Higher1.547530985
Q02985-2	CFHR3	Lower0.4172791
V9HW34	HEL-213	Higher1.749903749

note: The identification and quantitative results are summarized in Table 7.

Table 6. Protein Identification and Quantitative Statistical Results

The Total Number of Peptides	Proteins	Quantifiable Proteins	Differential Proteins
4565	970	928	28

note: Among the results, twenty-eight differential proteins were identified.

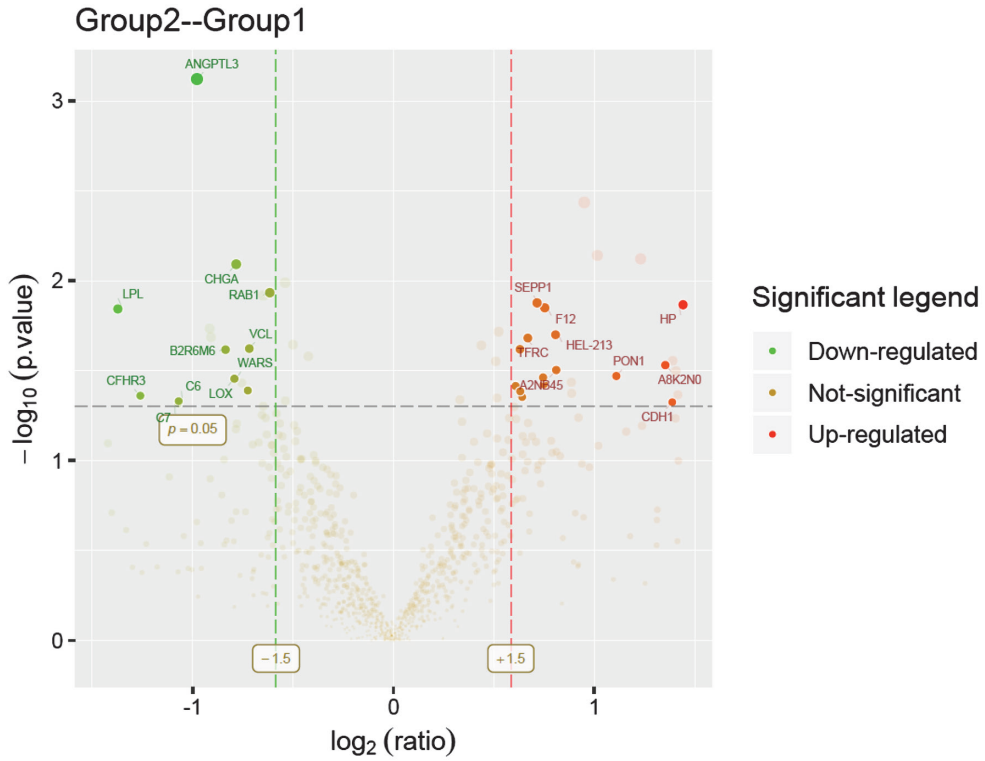

Figure 2. Differential Protein Levels Between the Two Groups [The proteins with significant difference between the thrombus positive group and the thrombus negative group were dark green (down-regulated) and orange yellow (up-regulated) respectively, while the proteins with no statistical difference were light yellow.]

Table 11, the statistical results showed that there was no interaction between group and time. The CFHR3 value in the positive group was lower than in the negative group at all time points

Table 8. Comparison of Different Detection Indexes Between the Two Groups

Project ($\bar{x} \pm S$)	The Positive (n = 23)	The Negative (n = 7)	*P*
KLKB1 (the first day)	4.43 ± 1.38	4.64 ± 1.29	.724
KLKB1 (the seventh day)	4.53 ± 0.99	4.97 ± 1.02	.313
KLKB1 (the fourteenth day)	4.94 ± 0.67	3.86 ± 0.65	.001*
C1S (the first day)	128.20 ± 30.94	127.37 ± 28.80	.950
C1S (the seventh day)	128.20 ± 26.55	128.07 ± 35.18	.992
C1S (the fourteenth day)	131.96 ± 19.29	130.42 ± 25.94	.866
CFHR3 (the first day)	1154.68 ± 213.42	1227.13 ± 244.68	.453
CFHR3 (the seventh day)	1064.54 ± 239.75	1207.15 ± 387.43	.245
CFHR3 (the fourteenth day)	1005.96 ± 151.80	1125.94 ± 151.80	.078
ANGPTL3 (the first day)	42.63 ± 11.74	41.11 ± 8.20	.754
ANGPTL3 (the seventh day)	40.47 ± 10.63	48.12 ± 16.69	.157
ANGPTL3 (the fourteenth day)	31.92 ± 6.68	44.45 ± 10.38	.001*

note: *P* values were compared negative group vs. positive group (*$P < .05$)

Table 9. Paired Test of Different Groups of C1S

(I) group	(J) group	Mean difference(I-J)	Standard error	*P*	95% CI	
					The lower limit	The higher limit
The negative	The positive	-0.832	9.888	.934	-21.086	19.423

note: There were no significant differences in the pair test of different groups of C1S ($P > .05$).

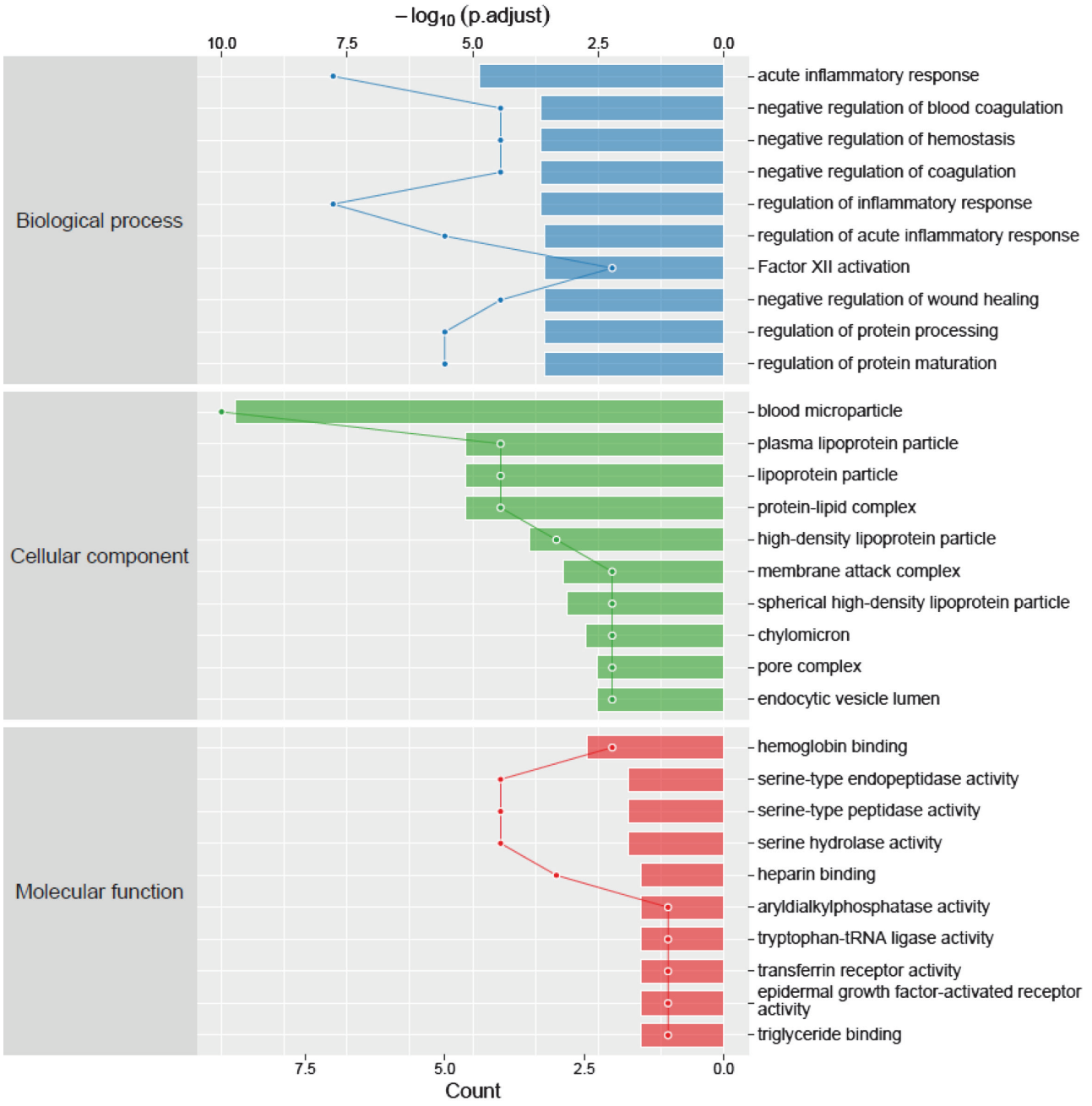

Figure 3. Differential Protein GO Enrichment Analysis [The Gene Ontology (GO) includes Cellular Component (CC), Molecular Function (MF) and Biological Process. BP and other three aspects describe the attributes of genes and their products, and have a dynamically updated standardized vocabulary, which is considered as a standardized gene functional classification system for GO analysis of differential proteins.]

(Figure 7). The CFHR3 value showed no significant statistical difference between two groups (Table 12). Paired comparisons between time points showed that the CFHR3 on the 14th day was significantly lower than that on the first day, and Day 7 vs. Day 14 with no significant difference (Table 13).

Evaluation of the Diagnostic Value of KLKB1 and ANGPTL3

Compared to the thrombo-negative group after CVC catheterization, levels of KLKB1 in the thrombo-positive group were significantly increased on the 14th day, as shown in figure 8. Meanwhile the levels of ANGPTL3 in the thrombo-positive

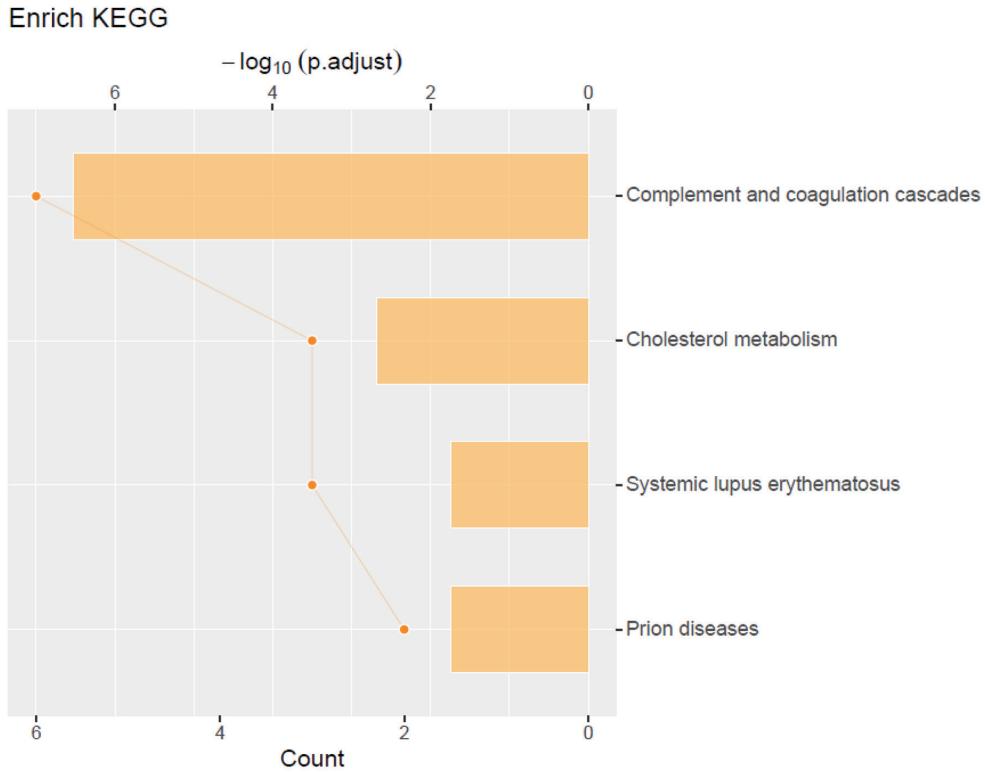

Figure 4. Differential Protein KEGG Enrichment Analysis

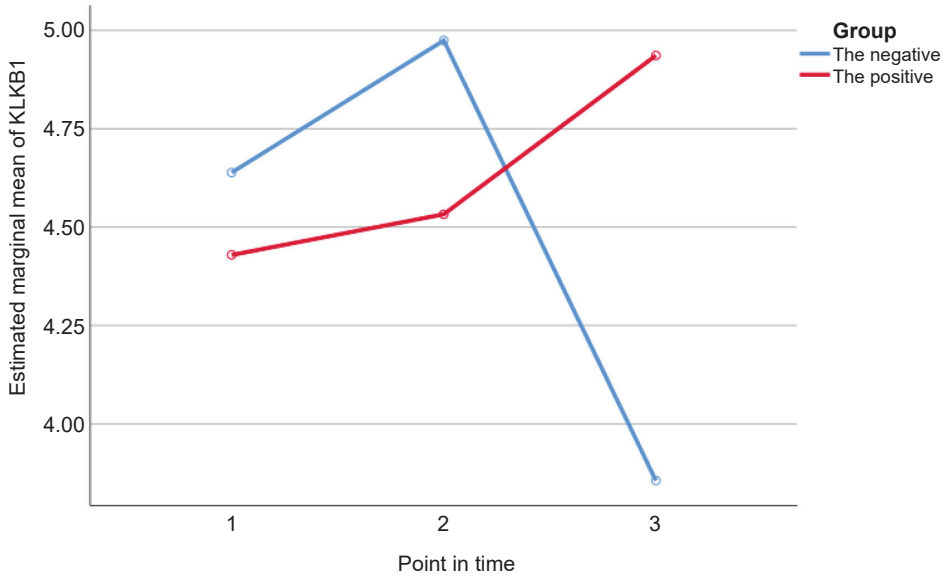

Figure 5. Graph of the Group and Time Interaction for KLKB1 [As shown in Figure 5, KLKB1 in the positive group increased over time, while that in the negative group decreased. There was no significant difference in KLKB1 between the two groups]

group decreased significantly on the 14th day, as shown in Figure 9.

DISCUSSION

TMT technology is a technique for in vitro labeling of polypeptide, in which 10 isotopes are used to mark the amino group of the peptide, and the relative protein content of 10 groups of samples can be compared by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.[14]

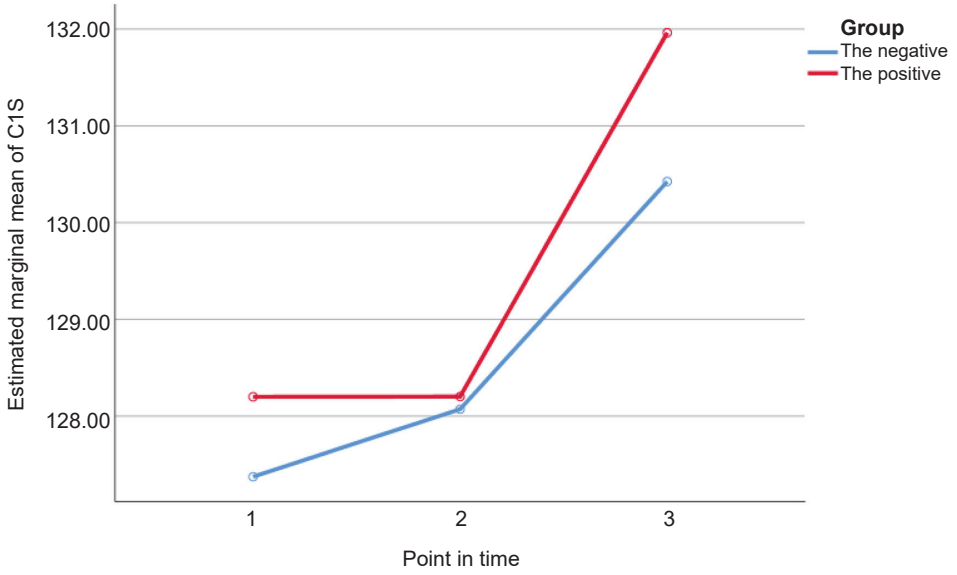

Figure 6. Graph of Group and Time Interaction for C1S [As shown in Figure 6, the C1S value of the positive group increased at all time points compared with the negative group.]

Currently TMT technology is widely used and is primarily used as drug action targets, screening of disease markers, molecular mechanisms of biological growth and development. This approach has been used for malignant tumors, including lung adenocarcinoma, thyroid cancer, and esophageal cancer, as well as other disease states, such as atrial fibrillation, aortic dissection, neurodegenerative diseases, and epilepsy.[15-21] In our study, different expressions of proteins in the serum were screened, using TMT proteomics to identify potential biomarkers that may be linked

Table 10. Pair Test of Different Times of C1S

(I) time	(J) time	Mean difference (I-J)	Standard error	*P*	95% CI	
					The lower limit	The higher limit
the 1st day	The 7th day	-0.350	6.393	.957	-13.446	12.745
the 1st day	The 14th day	-3.407	5.311	.526	-14.287	7.473
the 7th day	The 14th day	-3.057	4.047	.456	-11.347	5.233

note: There were no significant differences in the pair test of different times of C1S (*P* > .05).

Table 11. Repeated Measures ANOVA for CFHR3

Sources of variation	Df	SS	MS	F	*P*
Between groups	29	2474101.802			
Group	1	200807.117	200807.117	2.473	.127
The error between groups	28	2273294.685	81189.096		
Within group (Repeated)	45.057	2080491.348			
Time	1.502	168374.793	112108.732	2.483	.109
Group interactions over time	1.502	13764.425	9164.746	0.203	.753
Error in the groups	42.053	1898352.130	45142.032		

note: There were no significant differences in repeated measures ANOVA of CFHR3 (*P* > .05).

Table 12. Pair Test of Different Groups for CFHR3

(I) group	(J) group	Mean difference (I-J)	Standard error	*P*	95% CI	
					The lower limit	The higher limit
The negative	The positive	111.680	71.013	.127	-33.783	257.143

note: There was no significant difference in the value of CFHR3 between on the 14th day and the first day (*P* > .05).

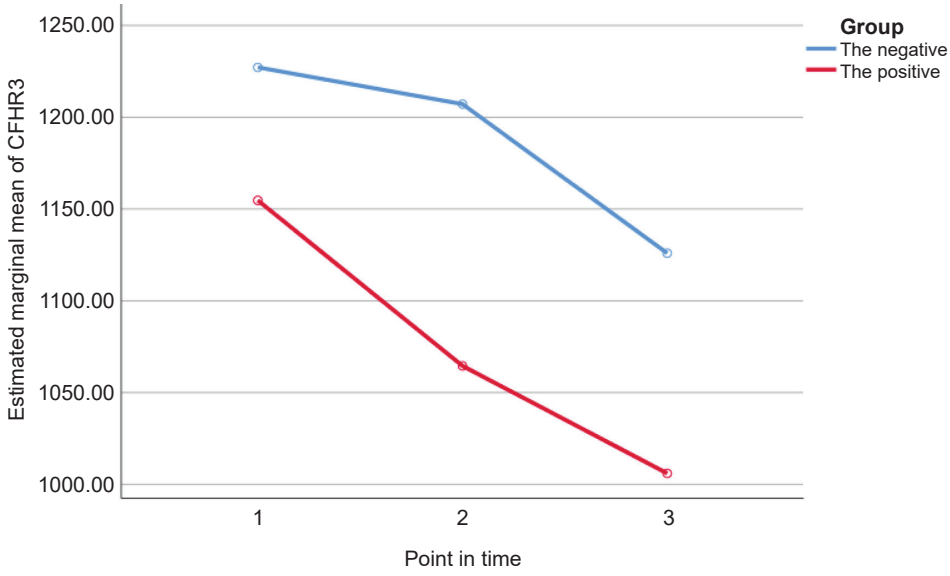

Figure 7. Graph of the Group and Time Interaction for CFHR3
note: The CFHR3 value in the positive group was lower than in the negative group at all time points.

Table 13. Pair Test of Different Times of CFHR3

(I) time	(J) time	Mean difference (I-J)	Standard error	*P*	95% CI	
					The lower limit	The higher limit
the 1st day	The 7th day	55.066	69.310	.434	-86.910	197.041
the 1st day	The 14th day	124.955	40.799	.005*	41.383	208.528
the 7th day	The 14th day	69.890	54.831	.213	-42.427	182.206

note: *P* values were compared between the positive group and negative group (*$P < .05$ vs. negative group)

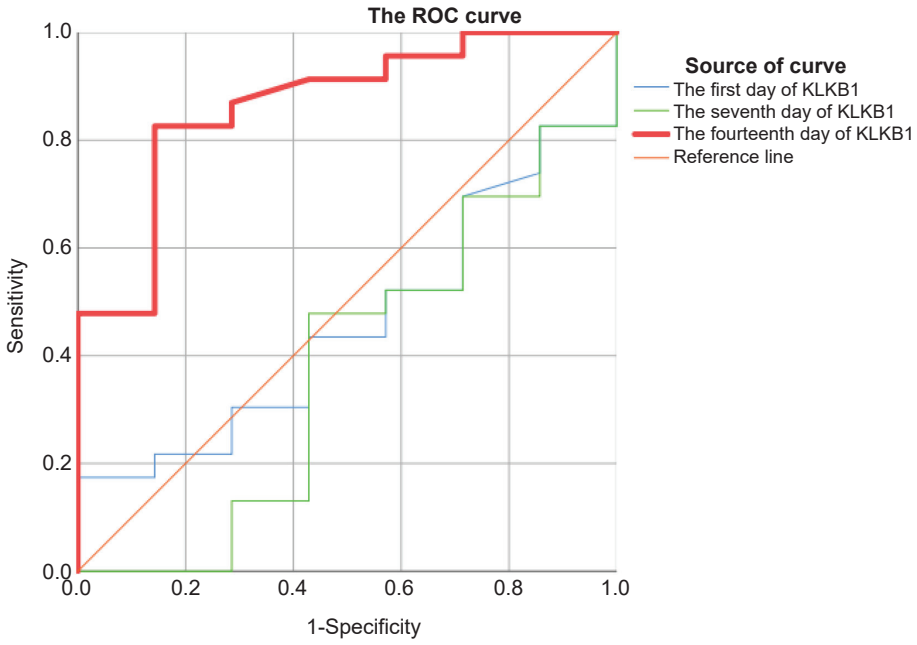

Figure 8. ROC Curve of KLKB1 [The area under the curve (AUC) and sensitivity and specificity of the receiver operating characteristic curve (ROC) were used to evaluate the diagnostic value of KLKB1 in thrombus formation after CVC catheterization. The analytical results indicated that the diagnostic value of KLKB1 on the first day and the fourteenth day was low.]

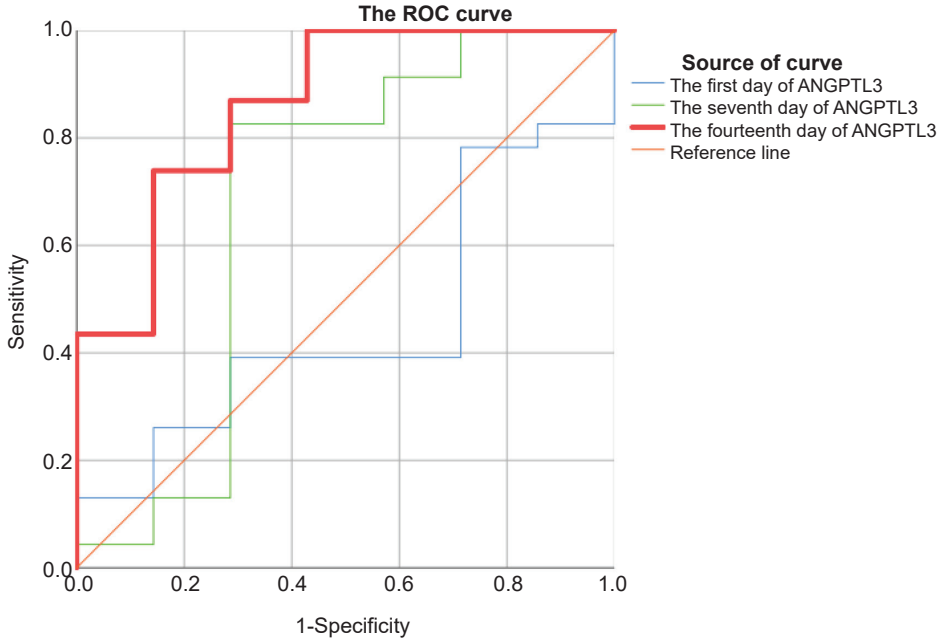

Figure 9. ROC Curve of ANGPTL3 [The analytical results indicated that the diagnostic value of ANGPTL3 on the seventh day was low, while, on the fourteenth day was high.]

to thrombosis.

In our study, TMT proteomics was used to observe the changes in serum protein levels in the thrombus-positive and thrombus-negative groups after CVC insertion, and it was realized that specific proteins may be involved in the regulation of certain metabolic and signaling pathways, contributing to the formation of thrombus after CVC insertion. Four proteins with significant differences were verified in our study, including the human plasma kallikrein B1 (KLKB1), human complement component 1S (C1S), complement factor H-related protein 3 (CFHR3) and angiopoietin-like protein 3 (ANGPTL3). KEGG enrichment analysis may be used as a potential biomarker for thrombosis after CVC insertion in ESKD patients. Verification results showed that KLKB1 and ANGPTL3 in the thrombus-positive group were significantly different from those in the thrombus-negative group on day 14 after CVC insertion, which may be used as potential biomarkers for the diagnosis of thrombosis formation after CVC insertion in ESKD patients, while C1S and CFHR3 were not verified.

Previous studies have confirmed that single nucleotide polymorphisms in F11, F12, selectin P(SELP) and KLKB1 are associated with venous thrombosis and can be detected at the level of RNA expression in whole blood.[22-23] Some studies have shown that KLKB1 exon rs3087505 may be associated with thrombosis, and this Single nucleotide polymorphism (SNP) is associated with two F11 SNPs (rs2036914, Rs3756008) and is associated with thromboembolism.[24-25] In this study, we found that the expression of KLKB1 was upregulated by TMT proteomics, and confirmed by ELISA that showed the expression of KLKB1 was significantly increased in the thrombus-positive group on day 14 compared with the thrombus-negative group after CVC insertion, which was the same as the result of TMT. ROC curve analysis also showed that KLKB1 had a high diagnostic value on day 14 for the diagnosis of thrombus positive group.

The main function of ANGPTL3 is to regulate lipid metabolism, which is mainly expressed in the liver and can also be detected in renal podocytes, but the expression is very low, and its role in podocyte function has not been fully clarified. [26-27] Earlier studies show that ANGPTL3 is strongly associated with renal damage, involved in lipid metabolism and glomerular podocyte damage, and is a key molecule in the development of nephrotic range proteinuria.[28-29] In this study, we found that the expression of ANGPTL3 was down-regulated in thrombus-positive group. ELISA method was used to verify that the expression level of ANGPTL3 in the thrombus-positive group was significantly

decreased on day 14 compared with the thrombus-negative group after CVC insertion, which was the same as the result of TMT. ROC curve analysis also showed that ANGPTL3 had high diagnostic value on day 14 for the diagnosis of thrombosis.

Previous studies have shown that age, sex, smoking, diabetes mellitus, body mass index (BMI), fibrinogen, blood lipid level, hypoproteinemia and other risk factors such as C-reactive protein, P-selectin, are related to CRT formation.[30] Our study found that univariate analysis of CRT formation was related to diabetes mellitus history and PLT count, while no statistical difference was found in multivariate analysis, indicating that certain false-positive results can exist in univariate analysis, and diabetes mellitus and PLT count may be affected by age and other factors such as smoking and underlying diseases. In this study, the ALB, FIB, blood lipids, sex and smoking history did not show any statistically significant difference between the thrombus- positive the thrombus-negative groups. However, due to the small sample size of this study, further studies with larger sample size and studies on the structure and function of proteins are needed to confirm whether KLKB1 and ANGPTL3 are related to blood hypercoagulability after CVC insertion and whether they are potential biomarkers of thrombosis after CVC insertion.

CONCLUSION

After central vein catheterization, the coagulative state of the whole body and the local area near the catheter changes dynamically. Continuous monitoring and in-depth understanding of these changes helps to have a deeper understanding of thrombosis. Using TMT proteomics, we found that KLKB1 and ANGPTL3 change in thrombosis earlier than ultrasound, which may be able to predict thrombosis earlier. We thus speculate that KLKB1 and ANGPTL3 proteins change earlier than ultrasound, which may be able to predict thrombus formation earlier than ultrasound. This may provide an earlier basis for clinical intervention and a longer time window for treatment, which may decline the incidence of thrombosis and improve the prognosis of ESKD patients.

LIMITATIONS OF THE STUDY

There are still some limitations in this study. At first, the study might not consider metabolic pathways that induce interference as it just concentrated on changes in serum protein level. Based on Proteomics and Metabolomics, more potential specific biomarkers can be identified via analysis of serum, urine, stool, and saliva. Secondly, the major limitation was its small sample size. So, larger sample sizes and multi-center studies are recommended for more analysis and verification. Meanwhile, the situation of CVC thrombi in patients after stable dialysis cannot be observed due to the short observation time and requirement on CVC placement period.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Kalantar-Zadeh K, Jafar TH, Nitsch D, et al. Chronic kidney disease [J]. Lancet. 2021 Aug 28;398(10302):786-802.
2. Zhang L, Wu HL, Yu HF, et al. Time spent outside of the hospital, CKD progression, and mortality: a prospective cohort study[J]. Int Urol Nephrol. 2021 Aug;53(8):1659-1663.
3. Yang C, Wang H, Zhao X, et al. CKD in China: Evolving Spectrum and Public Health Implications[J]. Am J Kidney Dis. 2020 Aug;76(2):258-264.
4. Hussein WF, Ahmed G, Browne LD, et al. Evolution of Vascular Access Use among Incident Patients during the First Year on Hemodialysis: A National Cohort Study[J]. Kidney360. 2021 Apr 22;2(6):955-965.
5. Poinen K, Quinn RR, Clarke A, et al. Complications From Tunneled Hemodialysis Catheters: A Canadian Observational Cohort Study[J]. Am J Kidney Dis. 2019 Apr;73(4):467-475.
6. Almeida BM, Moreno DH, Vasconcelos V, et al. Interventions for treating catheter-related bloodstream infections in people receiving maintenance haemodialysis[J]. Cochrane Database Syst Rev. 2022 Apr

1;4(4):CD013554.

7. Adwaney A, Lim C, Blakey S, et al. Central Venous Stenosis, Access Outcome and Survival in Patients undergoing Maintenance Hemodialysis[J]. Clin J Am Soc Nephrol. 2019 Mar 7;14(3):378-384.
8. Desai SS. Two-Year Outcomes of Early Cannulation Arteriovenous Grafts for End-Stage Renal Disease[J]. Ann Vasc Surg. 2019 Aug;59:158-166.
9. Wang Y, Lin S, Jiang P, et al. Focal Adhesion Kinase Inhibitor Inhibits the Oxidative Damage Induced by Central Venous Catheter via Abolishing Focal Adhesion Kinase-Protein Kinase B Pathway Activation[J]. Biomed Res Int. 2021 Mar 1;2021:6685493.
10. Sutherland DW Jr, Blanks ZD, Zhang X, et al. Relationship Between Central Venous Catheter Protein Adsorption and Water Infused Surface Protection Mechanisms[J]. Artif Organs. 2018 Nov;42(11):E369-E379.
11. Dubin RF, Rhee EP. Proteomics and Metabolomics in Kidney Disease, including Insights into Etiology, Treatment, and Prevention[J]. Clin J Am Soc Nephrol. 2020 Mar 6;15(3):404-411.
12. Glazyrin Y E, Veprintsev D V, Ler I A, et al.Proteomics-Based Machine Learning Approach as an Alternative to Conventional Biomarkers for Differential Diagnosis of Chronic Kidney Diseases[J].International Journal of Molecular Sciences, 2020, 21(13):4802.
13. van de Logt AE, Fresquet M, Wetzels JF, et al. The anti-PLA2R antibody in membranous nephropathy: what we know and what remains a decade after its discovery[J]. Kidney Int. 2019 Dec;96(6):1292-1302.
14. Moulder R, Bhosale SD, Goodlett DR, et al. Analysis of the plasma proteome using iTRAQ and TMT-based Isobaric labeling[J]. Mass Spectrom Rev. 2018 Sep;37(5):583-606.
15. Zheng W, Xu S. Analysis of Differential Expression Proteins of Paclitaxel-Treated Lung Adenocarcinoma Cell A549 Using Tandem Mass Tag-Based Quantitative Proteomics[J].Onco Targets Ther.2020,13:10297-10313
16. Dai J, Yu X, Han Y, et al.TMT-labeling Proteomics of Papillary Thyroid Carcinoma Reveal Invasive Biomarkers[J].J Cancer.2020,11(20):6122-6132
17. Sun S, Zhang H, Wang Y, et al.Proteomic Analysis of Human Esophageal Cancer Using Tandem Mass Tag Quantifications[J].Biomed Res Int.2020,20(20):5849323
18. Cao H, Zhu X, Chen X, et al.Quantitative proteomic analysis to identify differentially expressed proteins in the persistent atrial fibrillation using TMT coupled with nano-LC-MS/MS[J].Am J Transl Res.2020,12(9):5032-5047
19. Xing L, Xue Y, Yang Y, et al.TMT-Based Quantitative Proteomic Analysis Identification of Integrin Alpha 3 and Integrin Alpha 5 as Novel Biomarkers in Pathogenesis of Acute Aortic Dissection[J].Biomed Res Int.2020,20:1068402
20. Li K, Chen Z, Zhang Y, et al.Applications of iTRAQ and TMT Labeling Techniques to the Study of Neurodegenerative Diseases[J].Curr Protein Pept Sci.2020,21(12):1202-1217
21. Sun J, Jiang T, Gu F, et al.TMT-Based Proteomic Analysis of Plasma from Children with Rolandic Epilepsy[J].Dis Markers.2020,20:8840482
22. Pagliari M T , Boscarino M , Cairo A ,et al.ADAMTS13 activity, high VWF and FVIII levels in the pathogenesis of deep vein thrombosis[J].Thrombosis Research: An International Jou
23. rnal on Vascular Obstruction, Hemorrhage and Hemostasis, 2021(197-):197. Identification of four hub genes in venous thromboembolism via weighted gene coexpression network analysis[J].BMC Cardiovascular Disorders, 2021, 21(1):1-12.
24. De H , Van H , Lotta L A , et al. Targeted sequencing to identify novel genetic risk factors for deep vein thrombosis: a study of 734 genes[J]. Journal of Thrombosis and Haemostasis. 2018 Dec;16(12):2432-2441.
25. Leskelä J, Toppila I, Härma MA, et al. Genetic Profile of Endotoxemia Reveals an Association With Thromboembolism and Stroke[J]. J Am Heart Assoc. 2021 Nov 2;10(21):e022482.
26. Reeskamp LF, Tromp TR, Huijgen R, et al.Statin therapy reduces plasma angiopoietin-like 3 (ANGPTL3) concentrations in hypercholesterolemic patients via reduced liver X receptor (LXR) activation[J]. Atherosclerosis.2020,315:68-75.
27. Yma B , Jla C , Hlb D , et al. Podocyte protection by Angptl3 knockout via inhibiting ROS/GRP78 pathway in LPS-induced acute kidney injury[J]. International Immunopharmacology. 2022 Apr;105:108549.
28. Gao X, Suo Y, Zhang M, et al.Angiopoietin-like protein 3 markedly enhanced in the hyperlipidemia related proteinuria[J].Lipids Health Dis.2019,18(1):116.
29. Lv Q , Han X , Ni J ,et al.Anti-ANGPTL3-FLD monoclonal antibody treatment ameliorates podocyte lesions through attenuating mitochondrial damage[J].Cell Death & Disease[2023-07-11].DOI:10.1038/s41419-022-05313-7.
30. Mohazzab A , Khavanin Zadeh M , Dehesh P ,et al.Investigation of risk factors for tunneled hemodialysis catheters dysfunction: competing risk analysis of a tertiary center data[J].BMC Nephrology, 2022, 23(1):1-7. DOI:10.1186/s12882-022-02927-z.

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