Zagazig University Medical Journal
www.zumj.journals.ekb.eg

Volume 29, Issue 1, January 2023, Page (159-166) Supplement Issue

Manuscript ID ZUMJ-2002-1740 (R2)

DOI **10.21608/zumj.2020.23937.1740**

ORIGINAL ARTICLE

Study of Clinical and Prothrombotic Gene Mutation as Predictors of Thromboembolism in Pediatric Cancer

Suzan Atia Elshwadfy [1]*, Mervat Hesham Abdallah[1], Mervat Mohammed Atfy[1], Amal Fawzy[2]

[1] *Pediatric department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

[2] *Clinical Biochemistry department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

***Corresponding author:**
Suzan Atia Elshwadfy
E-mail:
suzanelshwad@gmail.com

Submit Date 2020-02-25
Revise Date 2020-07-11
Accept Date 2020-08-20

ABSTRACT

Background: Thromboembolism (TE) is a well-recognized complication in children with cancer and associated with chronic morbidity**,** delay or modification of treatment, adverse events associated with anticoagulation and rarely mortality, so we aimed to study the relation between susceptibility to prothrombotic defects and pediatric cancers.

Methods: this case control study included 50 patients aged from 1-14 years categorized into two groups: 25 children with newly diagnosed cancer as case group and 25 completely healthy, age and sex matched children as control group. All subjects were tested for prothrombin gene mutation by Polymerase chain reaction (PCR) and evaluated for other clinical and laboratory risk factors for TE in children with cancer including ; age ,sex, consanguinity, blood group, cancer type ,chemotherapy received, central venous catheter (CVC) insertion ,past history of TE, family history of TE, clinical presentation , level of serum protein C(PC) ,serum protein S(PS), D-Dimer, Complete blood count, prothrombin time and International normalized ratio(INR) then all cases were followed for 3 months after starting chemotherapy.

Results: Out of 25 pediatric patients with cancer, 28% had heterozygous prothrombin gene mutation, while 16% of control group had the same mutation, Also, 4% of studied patients developed TE. There was non-significant difference between studied groups regarding prothrombin gene mutation and there was significant difference between them according to PS, PC, D-dimer, prothrombin time and platelets counts. There was non-significant relation between prothrombin gene mutation and either gender, age, family, past history of TE or cancer type and development of thromboembolism.

Conclusions: There was a relationship between the age, presence of family or past history of TE, non (o) blood group, exposure to steroids and asparginase, presence of CVC, prothrombin gene mutation as strong risk factors for TE and pediatric cancer.

Keywords: Thromboembolism; Prothrombin gene mutation; Pediatric cancer

INTRODUCTION

Historically, the French physician and scientist Armand Troussea (1801–1867) was the first scientist to report the association between malignancy and thromboembolism. He postulated that most patients with cachexia and thrombosis are suspected to have cancer. A few years after his observation he noted thrombophlebitis of his left upper arm and a few months later he diagnosed to have to gastric cancer. While Trousseau's original description was about venous thrombosis in patients with visceral cancer. The term ''Trousseau's syndrome'' has been broadened to be applied to any type of venous thromboembolism occurring in solid tumors and hematological cancer patients. [1] Most studies concerned with the epidemiology of thrombosis with cancer have focused on adult populations, with thromboembolic complications being the second leading cause of death in cancer patients. In comparison, there is little knowledge about the pathophysiology and epidemiology of thromboembolism in children with cancer. The general incidence of VTE in children ranges from 0.7 to 1.4 VTE/100,000 children and 53 VTE/100,000 hospital admissions**.** [2]

The etiology of VTE in children with cancer is multifactorial and includes genetic predisposition

(thrombophilia), disease-related factors, and treatment-related factors including use of central venous catheters (CVC), surgery, and chemotherapy**.** Also, most children with cancer have CVC (which is considered a common risk factor for VTE) for the administration of chemotherapy and other supportive care. Catheter-related bloodstream, other infections in immunocompromised patients and immobility during hospitalization or after surgical intervention are other prothrombotic risk factors for VTE that result in serious consequences, including death [3]. Certain prothrombotic defects increase the risk of thromboembolism in adults and children. These include Factor V Leiden (FVL), deficiency of natural anticoagulants (Protein C, Protein S and Antithrombin), and elevated levels of coagulation factors VIII, IX and XI. In addition, mutations of the prothrombin (PT) gene G20210A and methylene tetrahydrofolate reductase (MTHFR) C677T are common and mild risk factors for venous TE in general population [4]. So, we aimed to identify the relationship between susceptibility to prothrombotic defect and pediatric cancers.

METHODS

The present study was a case-control study that was conducted in the Pediatric, Medical Biochemistry and Molecular Biology Departments, Faculty of Medicine, Zagazig University and Pediatric department of Tanta Cancer Center in the period from October 2018 to September 2019. A total sample of 50 patients aged 1–14 years was categorized into two groups: 25 children with newly diagnosed cancer as the case group and 25 completely healthy age- and sex-matched children as the control group were included in the present study. Written Informed consent to engage in the study was received from the child parents. Approval for the research was received from the Departments of Pediatrics and Molecular biology, University Hospitals of Zagazig, following the approval of the Institutional Review Board (IRB). The research was carried out in compliance with the World Medical Association's Code of Ethics (Helsinki Decleration) for human-related studies.

Children aged 1–14 years, both sexes included, newly diagnosed as cancer patients and before starting chemotherapy, were included in this study. Patients less than 1-year-old or more than 14 years old, those with relapsed tumors, those who had previously received anticoagulant therapy or steroids, those with associated blood disease, those who had already begun their cancer treatment protocol, and those unwilling to give consent to participate in the study were all excluded from this study.All patients included in the study were subjected to the following*:* Full medical history taking, physical examination with stress on signs and symptoms of TE such as; leg pain or tenderness of the thigh or calf and swelling (edema), warm or cold to the touch, Unexplained tachypnea, chest pain, tachycardia. Laboratory tests including; Complete blood count (CBC), Prothrombin time and activity, INR, D-dimer level, Protein C level, Protein S level, Prothrombin gene mutation and blood grouping. Doppler ultrasound examination in suspected cases of deep vein thrombosis (DVT). Assessment of prothrombin gene mutation and serum protein C and S: Blood was collected from peripheral venous blood of each participant into one 3.2% Sodium citrate tube (~ 1.8 mL) and one K ethylene diamine tetra acetic acid (EDTA K2) tube (~1.8 mL) under complete aseptic condition from all participants and from patients before starting chemotherapy and within 30 min of collection, blood in 3.2% Sodium citrate tube was centrifuged to separate platelet poor plasma which was aliquoted and frozen at –70 °C until the time of assay. Citrated sample was used for measurement of PC, PS and D-dimer. EDTA sample was stored at -20 °C for genomic DNA extraction.

Quantities determination of protein S and protein C were performed using The AssayMax Human Enzyme-Linked Immunosorbent Assay (ELISA) kit to determine the level of serum level of PC and PS in the collected samples. [5-6]

PCR process was used for detection of prothrombin G20210A gene polymorphism [7].

All participants were followed for 3 months for detection of thromboembolism development by weekly clinical assessment and measurement of serum D-Dimer and imaging if suspecting thrombosis.

STATISTICAL ANALYSIS

All data are obtained, tabulated and analyzed statistically using version 22 of SPSS. Continuous quantitative variables, e.g. ages, were expressed as mean ± SD & range and categorical qualitative variables as absolute frequencies and relative percentages were expressed. By using the kolmogorv-Smirnov method, continuous data are tested for normality. Independent samples Student's t-test was used to compare the normally distributed two groups of results. Two classes of non-normally distributed data were compared with the Mann-Whitney test. Wilcoxon-sign rank test was used before and after the stimulation test to compare non-normally distributed results. The analysis of categorical data was made using the Chi-square test (μ2 test). All measurements are deemed statistically significant p-value < 0.05 and p-value < 0.05 were statistically non-significant (NS).

RESULTS

The study included 50 subjects aged from 1-14 years categorized into two groups: 25 children with newly diagnosed cancer as case group and 25 completely healthy, age and sex matched children as control group and the Mean age of our studied patients was 7 years (range: 7.72+_4.92) years and male to female ratio was 1:1.08 (13/ 12) (males were 52%) (table 1)

The commonest malignancy among cases was Acute lymphoblastic leukemia (36%) followed by Acute myeloid leukemia and Non Hodgkin lymphoma (28 % and 12%) respectively (figure 1). There is statistically significant difference between the studied groups regarding levels protein C, S, white blood cells, platelet count, D-dimer, prothrombin time, prothrombin activity and INR. There is non-significant difference between them regarding hemoglobin level, blood group or RH typing (table 2). There was statistically non-significant difference between the studied groups regarding gene mutation (table 3). Incidence of confirmed TE among newly diagnosed cancer patients was 4% (figure 2). There was statistically non-significant relation between the presence of gene mutation and development of thromboembolism. Presence of GA gene mutation increases risk of thromboembolism by about 9 folds (table 4).There was statistically non-significant relation between type of gene mutation and either gender, age, family, past history of TE or cancer type (table 5). Patient who was confirmed to have DVT had many risk factors of thrombosis as older age, diagnosis of acute leukemia, blood group A, elevated white blood cell count, elevated serum D-Dimer, prothrombin gene mutation, exposure to steroids and aspaginase in her treatment protocol (table S1).

Table (1); Distribution of the studied patients according to demographic data and type of cancer:

Age at diagnosis (years):	
Mean ± SD	7.72 ± 4.92
Median	7
Range	1 - 17
Gender	
Male n(%)	13 (52)
Female n(%)	12 (48)
Cancer type:n(%)	
ALL	9 (36)
AML	7 (28)
Germ cell Tumor	2(8)
HD	2 (8)
NHL	3 (12)
RMS	2 (8)

-ALL: Acute lymphoblastic leukemia. AML: Acute myeloblastic leukemia. - HD: Hodgkin disease. NHL: Non-Hodgkin lymphoma. -RMS: Rhabdomyosarcoma.

Table (2): Comparison between the studied groups regarding to specific lab for thrombophilia (protein c, protein s, D-Dimer):

Studied groups	Case group		Control group		Test	
					t/Z	p
Labaratory data	Mean ± SD		Mean ± SD			
Protein C activity %	96.96 ±20.83		84.28±20.65		2.162	0.036*
Protein S activity %	95.48±17.76		77.04±22.197		3.243	0.002*
	Median		Median			
D dimer (ug/ml)	3.01 ± 5.04	1.4	0.57 ± 0.36	0.4	-3.793	<0.001**

*p<0.05 is statistically significant t Independent sample t test
**p≤0.001 is statistically highly significant Z Mann Whitney test
N.B: I expressed d-dimer by median as data are not normally distributed in contrast to Protein C and S.

Table (3): Comparison between the studied groups regarding prothrombin gene mutation:

Studied groups	Case group	Control group	Test		OR (95% CI)
			X2	p	
Genetic mutation	N=25 (%)	N=25 (%)			
Gene:					
GA	7 (28)	4 (16)	1.049	0.592	2.04 (0.51-8.12)
GG	18 (72)	21 (84)		NS	
AA	0 (0)	0 (0)			
Alleles (50):					
A	7 (14)	4 (8)	Fisher	0.525	1.87 (0.51-6.85)
G	43 (86)	46 (92)		NS	

Table (4) Relation between prothrombin gene mutation and occurrence of thromboembolism among the studied patients:

Prothrombin gene mutation	GA	GG	X^2	p
TE	N=7 (%)	N=18 (%)		
No	5 (71.4)	16 (88.9)	2.797	**0.247**
Suspected	1 (14.3)	2 (11.1)		
Confirmed	1 (14.3)	0 (0)		
OR of GA in development of conf TE (95% CI)		8.54 (0.31 – 236.91)		

OR: Odd Ratio CI: Confidence interval.

Table (5): Relation between type of prothrombin gene mutation and demographic and clinical characteristics among the studied patients:

Prothrombin gene mutation	GA	GG	X^2	p
Demographic characteristics	**N=7 (%)**	**N=18 (%)**		
Gender:				
Male	4 (57.1)	9 (50)	Fisher	1
Female	3 (42.9)	9 (50)		
Family history of TE:	7 (100)	14 (77.8)	Fisher	

Prothrombin gene mutation Demographic characteristics	GA N=7 (%)		GG N=18 (%)		X^2	p
No	0 (0)		4 (22.2)			0.294
Yes						
Past history of TE:					Fisher	
No	7 (100)		18 (100)			1
Yes	0 (0)		0 (0)			
Cancer type:						
ALL	1 (14.3)		8 (44.4)		Fisher	0.28
AML	4 (57.1)		3 (16.7)		Fisher	0066
Germ cell T	0 (0)		2 (11.1)		Fisher	1
HD	2 (28.6)		0 (0)		Fisher	0.07
Lymphoma	0 (0)		3 (16.7)		Fisher	0.534
RMS	0 (0)		2 (11.1)		Fisher	1
	Mean ± SD	Median	Mean ± SD	Median	Z	p
Age (years):	8.71±4.99	8	7.35±4.96	7	-0.639	0.523

*p<0.05 is statistically significant, Z Mann Whitney test

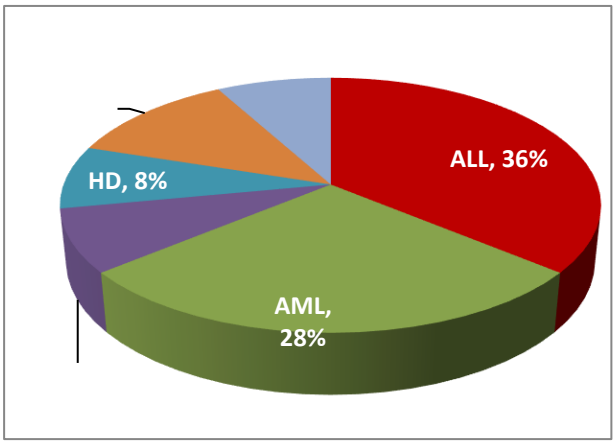

Fig (1): Pie chart showing distribution of the studied patients according to cancer type.
ALL: Acute lymphoblastic leukemia, AML: Acute myeloid leukemia, HD: Hodgkin disease, RMS: Rhabdomyosarcoma.

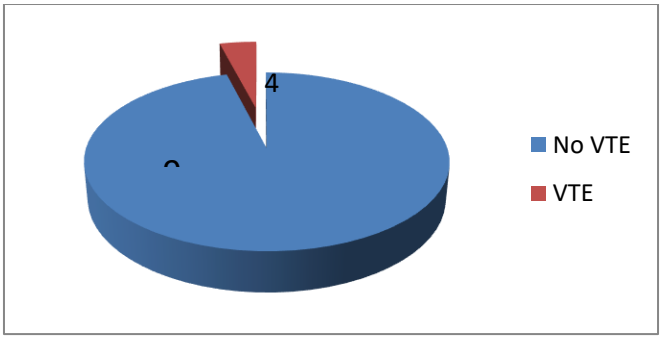

Fig (2): Pie chart showing distribution of the studied patients according to occurrence of TE. VTE: venous thromboembolism.

DISCUSSION

Thromboembolism (TE) is a well-recognized complication in children and adults with cancer [8]. As the survival rate of children with malignancies has increased to almost 80% over the last decade, it becomes more and more important to prevent mortality and morbidity of the disease and treatment-associated complications such as TE. Knowledge of the epidemiology, risk factors and recurrence rate of TE in children with various malignancy types is important to identify patients at risk, who might benefit from primary or prolonged secondary thromboprophylaxis [9].

The aim of our study was to assess the relationship between susceptibility to prothrombotic defect and pediatric cancers. The data obtained show that the mean age of our studied patients was 7 years (range: 7.72-4.92) years, which was consistent with the data obtained by Athale and colleagues, who found that the mean age was 6.4 years (range: 1-17). Also, the present study showed that Male to female ratio was 1:1.08 (13/ 12) (males were 52%) [4] and this is similar to Forbigger and colleagues where male sex was about 55 [10].

The present study showed that the commonest malignancy among studied patients was all cases (32%), followed by AML (28%), and NHL (12%) and this is comparable with the study of Choi and colleagues, who reported that brain tumours were the commonest type of malignancy among their patients, followed by ALL [11].

Also, the mean blood group of cases was (O) (40%) and non (O) blood group represent 60% of cases, and this was in agreement with Athale and colleagues who made a study where (O) blood group represent about 40.2% [4] One of non (O) blood group was confirmed to have DVT and this may encourage other previous studies in which non (O) blood group is considered risk factor for TE.

Our study showed that PS and PC deficiency in cases were 0/25 and this was in agreement with other studies as Alioglu and colleagues where PS and PC def. was 0/ 52 **[**12].

According to prothrombin gene mutation, our results showed a non-significant difference between the studied groups (p value =0.496) and none of all patients and controls were homozygous, but seven cases out of a total of 25 were heterozygous for prothrombin gene mutation (35%). This was higher than reported by Athale and colleagues where heterozygous gene mutation was 3.2% [4] and higher than results for Van Rooden and colleagues who reported that the prevalence of G20210A ranges between 0.6 and 2.6% in patients with cancer [13].

Also, according to exposure to Asparginase and steroids, our study revealed that about 9 patients (36%) were exposed to these risk factors in their treatment and only one case developed DVT.This was statistically non-significant, but it suggests their effect on developing VTE [14].

In the present study, only 3 cases had CVL (2 had CVL and one case had port-cath) and none of them developed TE. So it cannot be compared with other studies due to the low percentage of patients using CVC in our study. On the other hand, Choi and colleagues found CVC use alone was a risk factor for VTE in 12 patients [11].

Also, our study showed that there was no significant relationship detected between prothrombin gene mutation and family or past history of TE as there was no family or past history of TE in all seven cases of gene mutation in studied patients, but in the cross-sectional study, family history of VTE increased the relative risk (RR) of a child having inherited thrombophilia to 2.35 (95% CI, 1.1-5.2) [15].

Also, our results showed a non-significant relationship between prothrombin gene mutation and the development of thromboembolism as 2 patients of total 7 cases with prothrombin gene mutation were suspected to have TE and only one case was confirmed to have DVT. This data was in agreement with the study of Athale and colleagues, which reported that none of the genetic polymorphisms in isolation or in combination had an impact on the risk of development of sTE or on the time to develop TE [4] and was comparable with Tormene and colleagues, where 15 cases out of 143 cases of VTE had prothrombin gene mutation [16].

In our study, four of 25 cases suspected to have had VTE, one of them was confirmed to have DVT in left popliteal and deep calf vessels by Doppler, while the other three developed sepsis, DIC, elevated D-Dimer, swelling and redness at sites of cannula but deteriorated rapidly and died before imaging to confirm the occurrence of VTE. So the frequency of VTE among studied patients was 4% and this is higher than the incidence in another study where the incidence was (0.9%), Choi and colleagues, and higher than that in the general pediatric population (0.06 cases per 10,000 individuals) [17].Our study demonstrated that no significant association between the occurrence of TE and past or family history of TE, as no one of the suspected or confirmed cases of TE among all cases had a past or family history. This was similar to Onyeama and colleagues' study, who reported 2 cases with family history and no cases with past history of TE developed TE despite the presence of 12 cases with family history and 4 cases with a past history of TE included in his study [18].

Limitations of the study

Small sample size and short duration of the study do not clarify significant relation between

thrombophilia and pediatric cancer and between gene mutation and thromboembolism development. Also, not all pediatric cancer patients were included in the study so results cannot be generalized.

CONCLUSIONS

The present study revealed that there was a relationship between the age, presence of family or past history of TE, non (o) blood group, exposure to steroids and asparginase, presence of CVC, prothrombin gene mutation as strong risk factors for TE and pediatric cancer but not confirm their statistical significance.

Recommendations: Further Prospective studies with larger sample size and longer duration and including all or most cancer types are needed to validate our findings and to clarify the association between cancer and prothrombin gene mutation.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Funding sources: The authors have no funding to report.

REFERENCES

1- Matzdorff A ,Green D. Overview of Cancer and Thrombosis. Coagulation in cancer. Springer Scienc NY. 2009;Chapter6:83- 4.

2- Piovesan D, Attard C, Monagle P, Ignjatovic V. Epidemiology of venous thrombosis in children with cancer. Thromb Haemost. 2014;111(6):1015-21.

3- Ko RH, Thornburg CD. Venous thromboembolism in children with cancer and blood disorders. Front Pediatr. 2017;5:1-7.

4- Athale U, Laverdiere C, Nayiager T, Delva Y, Foster G, Thabane L, et al. Evaluation for inherited and acquired prothrombotic defects predisposing to symptomatic thromboembolism in children with acute lymphoblastic leukemia: a protocol for a prospective, observational, cohort study. BMC cancer. 2017;17(1),313.

5- Mosnier LO, Zlokovic B V., Griffin JH. The cytoprotective protein C pathway. Blood. 2007;109(8):3161-72.

6- Rezende S, Simmonds R , Lane D. Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S–C4b binding protein complex. Blood. 2004;103(4),1192-1.

7- Cooper P , Rezende S. An overview of methods for detection of factor V Leiden and the prothrombin G20210A mutations. Int J Lab Hematol. 2007;29(3),153-2.

8- Pelland-Marcotte M, Pole J, Kulkarni K, Athale U, Stammers D, Sabapathy C, et al Thromboembolism incidence and risk factors in children with cancer: a population-based cohort study. Thromb Haemost.2018; 118(09), 1646-55.

9- Klaassen I, Lauw M, Fiocco M, van der Sluis I, Pieters R, Middeldorp S,et al. Venous thromboembolism in a large cohort of children with acute lymphoblastic leukemia: Risk factors and effect on prognosis. RPTH.2019; 3(2), 234-41.

10- Forbrigger Z, Kuhle S, Brown M, Moorehead P, Digout C, Kulkarni K. The association of venous thromboembolism with survival in pediatric cancer patients: a population-based cohort study. Ann Hematol. 2018;97(10),1903-8.

11- Choi H., Kim H, Kang H, Lee J, Shin H, Park H, et al. Thromboembolism in children with cancer: a retrospective multicenter study in Korea. J Thromb Thromlys. 2019;47(4),558-5.

12- Alioglu B, Avci Z, Tokel K, Atac F, Ozbek N. Thrombosis in children with cardiac pathology: analysis of acquired and inherited risk factors. Blood Coagul Fibrinolysis. 2008;19(4), 294-304.

13- Van Rooden C, Rosendaal F, Meinders A, Van Oostayen J, Van Der Meer F ,Huisman M. The contribution of factor V Leiden and prothrombin G20210A mutation to the risk of central venous catheter-related thrombosis. Haematologica. 2004;89(2),201-6.

14- Appel I, Hop W, Van Kessel-Bakvis C, Stigter R, Pieters R. L-Asparaginase and the effect of age on coagulation and fibrinolysis in childhood acute lymphoblastic leukemia. J Thromb Haemost. 2008;100(08),330-7.

15- Ruud E, Holmstrøm H, Brosstad F, Wesenberg F. Diagnostic value of family histories of thrombosis to identify children with thrombophilia. Pediatr Hematol Oncol. 2005;22(6),453-62.

16- Tormene D, Simioni P, Prandoni P, Franz F, Zerbinati P, Tognin G, et al. The incidence of venous thromboembolism in thrombophilic children: a prospective cohort study. Blood. 2002; 100(7),2403-5.

17- Hong J, Lee J, Yhim H, Choi W, Bang S, Lee H, et al. Incidence of venous thromboembolism in Korea from 2009 to 2013. PloS one. 2018;13(1),e0191897.

18- Onyeama S, Hanson S, Dasgupta M, Baker K, Simpson P ,Punzalan R. Central Venous Catheter–associated Venous Thromboembolism in Children With Hematologic Malignancy. Pediatr Hematol Oncol. 2018;40(8),e519-e4.

To Cite:

Elshwadfy, S., Abdallah, M., Atfy, M., Fawzy, A., Study of Clinical and Prothrombotic Gene Mutation as Predictors of Thromboembolism in Pediatric Cancer. *Zagazig University Medical Journal*, 2023; (159-166): -.doi: 10.21608/zumj.2020.23937.1740

SUPPLEMENTARY FILES

Table (S1) criteria of patient with confirmed thromboembolism:

Parameter	Value (n=1)
Age:	14 years old
Gender	Female
Consanguinity	Negative
Age at diagnosis	14 years old
Cancer type	ALL
Blood group	A
Rh typing	Positive
Symptom/sign	Pallor Lymph node enlargement Tachycardia/palpitation Oral mucositis
Family history	Negative
Protein C activity %	95
Protein S activity %	99
WBCs $x10^3$/ uL	132
Hemoglobin g/dl	6.2
Platelet count $x10^3$/ uL	26
D dimer(ug/ml)	2.5
PT(seconds)	22
Prothrombin activity%	37%
INR	2.31
Protocol	Include (Asparaginase+steroid)
CVL insertion	No
Site	DVT in popliteal vein
Gene mutation	GA