Tromboembolija u 14-godišnjeg dječaka – prikaz bolesnika

Thromboembolism in a 14-year-old boy – case report

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Sažetak

Uvod: Opisuje se slučaj venske tromboembolije (VTE) u 14-godišnjeg dječaka u kojega se razvila duboka venska tromboza desne noge, a potom i plućna embolija komplicirana plućnim infarktom.

Cilj: Prikazati rezultate laboratorijske dijagnostike koji upućuju na mogući uzrok nastanka VTE u ovog bolesnika.

Materijali i metode: Laboratorijska obrada VTE uključila je određivanje antifosfolipidnih antitijela: lupus antikoagulanta (LAC) i antikardiolipinskih antitijela (ACA) klase IgG i IgM, inhibitora zgrušavanja (antitrombin, protein C, protein S) i fibrinolize (inhibitor aktivatora plazminogena-1 (PAI-1)), određivanje rezistencije na aktivirani protein C (APCR) te molekularnu dijagnostiku čimbenika trombofilije: genotipizacija polimorfizama u genu za PAI-1 i metilentetrahidrofolat reduktazu (MTHFR), mutacije G20210A faktora II zgrušavanja (protrombina) i mutacije G1691A faktora V zgrušavanja (faktor V Leiden).

Rezultati: U bolesnika su utvrđena antifosfolipidna antitijela: lupus antikoagulant (omjeri LAC 3,0 i 2,76) i antikardiolipinska antitijela klase IgG (104,7 i 103,7 GPL-U/mL) u dva nezavisna mjerenja u razmaku od devet tjedana. Ovi rezultati uz kliničku sliku ukazuju na prisutnost antifosfolipidnog sindroma. Molekularnom dijagnostikom utvrđen je polimorfizam gena za PAI-1 (genotip 4G/5G), što je u skladu s izmjerenom povećanom koncentracijom PAI-1 u plazmi (4,9 IU/mL).

Zaključak: Laboratorijskom dijagnostikom trombofilije u opisanog bolesnika utvrđen je antifosfolipidni sindrom kao čimbenik rizika za nastanak VTE. Iako se polimorfizam PAI-1 ne smatra neovisnim čimbenikom rizika za nastanak VTE, moguće je da u bolesnika s utvrđenim čimbenikom trombofilije kao što je antifosfolipidni sindrom i ovaj polimorfizam ima ulogu u nastanku VTE. Infekcija i mirovanje koji su prethodili trombozi mogli bi biti dodatni čimbenici rizika za nastanak VTE u ovog bolesnika. Prikazani slučaj govori u prilog dosadašnjim saznanjima da je pojava VTE najčešće rezultat međudjelovanja genetskih i stečenih čimbenika rizika.

Ključne riječi: venska tromboembolija, antifosfolipidni sindrom, polimorfizam PAI-1

Abstract

Introduction: A case of venous thromboembolism (VTE) in a 14-year-old boy who developed deep venous thrombosis of the right leg followed by pulmonary embolism complicated with pulmonary infarction is described in this article.

Aim: To present results of laboratory diagnosis suggesting the possible cause of VTE occurrence in this patient.

Materials and methods: Laboratory diagnosis of VTE included determination of antiphospholipid antibodies: lupus anticoagulant (LAC) and anticardiolipin antibodies (ACA) IgG and IgM classes, inhibitors of coagulation (antithrombin, protein C, protein S) and fibrinolysis (plasminogen activator inhibitor-1 (PAI-1)), determination of activated protein C resistance (APCR) and molecular diagnosis of thrombophilic factors: genotyping polymorphisms in the gene for PAI-1 and methylenetetrahydrofolate reductase (MTHFR), mutation G20210A for coagulation factor II (prothrombin) and mutation G1691A for coagulation factor V (factor V Leiden).

Results: The following antiphospholipid antibodies were demonstrated in the patient: lupus anticoagulant (LAC ratios 3.0 and 2.76) and anticardiolipin antibodies of IgG class (104.7 and 103.7 GPL-U/mL) on two independent measurements nine weeks apart. These results, along with clinical presentation, suggested the presence of antiphospholipid syndrome. Molecular diagnosis confirmed polymorphism in the gene for PAI-1 (genotype 4G/5G), which was in accordance with elevated concentration of PAI-1 measured in plasma (4.9 IU/mL).

Conclusion: In this patient, laboratory diagnosis of thrombophilia revealed antiphospholipid syndrome as a risk factor for VTE. Although PAI-1 polymorphism is not considered as an independent risk factor for VTE, it is possible that in patients with established thrombophilic factor such as antiphospholipid syndrome this polymorphism may play a role in VTE occurrence. Infection and prolonged bed rest that preceded thrombosis could have been additional risk factors for VTE occurrence in this patient. The reported case supports the current concepts according to which VTE occurrence most frequently results from interaction of genetic and acquired risk factors.

Key words: venous thromboembolism, antiphospholipid syndrome, PAI-1 polymorphism

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Uvod

Pojava venske tromboembolije (VTE) znatno je rjeđa u djece u odnosu na odraslu populaciju. Procjenjuje se da je pojavnost bolesti u djece oko 1:10 u odnosu na odrasle (1). Ispitivanja VTE u dječjoj dobi pokazala su da je u većine djece s dubokom venskom trombozom (DVT) i plućnom embolijom (PE) u podlozi genetska trombofilija (2,3,4,5). Dodatni čimbenici rizika (infekcija, imobilizacija, trauma, operativni zahvat, maligne bolesti), u kombinaciji s nasljednim čimbenicima trombofilije značajno povećavaju rizik nastanka VTE. Svaki slučaj VTE u dječjoj dobi zahtijeva temeljit klinički pristup i sveobuhvatnu laboratorijsku dijagnostiku koja će ukazati radi li se o izoliranom događaju, genetskoj trombofiliji ili kombinaciji genetskih i stečenih čimbenika rizika za nastanak ove bolesti.

Materijali i metode

Sve koagulacijske pretrage načinjene su na analizatoru Behring Coagulation System (BCS, Dade Behring, GmbH, Austria) s izvornim komercijalnim reagencijama. Određivanje rezistencije na aktivirani protein C (APCR) izvedeno je koagulacijskom metodom uporabom reagensa ProC® Global, a lupus antikoagulanta (LAC) uporabom reagencija LA1/LA2 iste tvrtke. Pretraga antikardiolipinskih antitijela (ACA) klasa IgG i IgM učinjena je enzimimunokemijskom metodom (ELISA, Orgentec Diagnostika, GmbH, Njemačka), kao i antitijela na dvostruku uzvojnicu DNA (anti-dsDNA) (ELISA, Pharmacia Diagnostics, Švedska). Određivanje mutacije G20210A faktora II i mutacije G1691A faktora V zgrušavanja (faktor V Leiden) načinjeno je analizom krivulje taljenja na uređaju Roche LightCycler® uporabom komercijalnih kitova (PCR-LightCycler Mutation Detection Kits, Roche, Njemačka). Polimorfizmi C667T u genu za metilentetrahidrofolat reduktazu (MTHFR) i 4G/5G u genu za inhibitor aktivatora plazminogena-1 (PAI-1) ispitani su metodom PCR-RFLP.

Prikaz bolesnika

Dječak u dobi od 14 godina došao je u hitnu ambulantu Klinike za pedijatriju zbog bolova i otoka desne potkoljenice, gdje je pregledan i zaprimljen na istu Kliniku. Unatrag mjesec dana dječak je bio ambulantno liječen antibioticima zbog infekta. Dva dana prije hospitalizacije uz bolove u desnoj nozi nastalo je otvrdnuće i oteklina desne potkoljenice. Zbog sumnje na DVT prvog dana hospitalizacije hitno je učinjen dupleks dopler dubokih vena desne noge, kojim je i dokazana DVT potkoljeničnih vena i vene popliteje. Uvedena je terapija niskomolekularnim heparinom (Fragmin®, 5000 IJ, s.c.). Petog dana boravka bolesnik je osjetio zaduhu i bolove u lijevoj strani prsišta. Magnetskom spiralnom kompjutoriziranom tomografijom toraksa dijagnosticirana je PE komplicirana plućnim infarktom, zbog čega je bolesnik iste noći premješten u koronarnu jedinicu Klinike za unutarnje bolesti. Uvedena

Introduction

The incidence of venous thromboembolism (VTE) is considerably lower in children than in adults. It is estimated that the incidence of VTE in children is one thenth of that in adults (1). Investigations of VTE in childhood have shown that most children with deep venous thrombosis (DVT) and pulmonary embolism (PE) have underlying hereditary thrombophilia (2-5). Additional risk factors (infection, immo bilization, trauma, surgery, malignancy) in combination with genetic factors of thrombophilia significantly increase the risk of VTE occurrence. Each case of VTE in childhood requires thorough clinical approach and complete laboratory diagnosis that will suggest whether it represents an isolated event, genetic thrombophilia or a combination of genetic and acquired risk factors for the occurrence of this disease.

Materials and methods

All coagulation tests were performed on a Behring Coagulation System analyzer (BCS, Dade Behring, GmbH, Austria) with commercially available reagents. Activated protein C resistance (APCR) was determined by the coagulation-based method using ProC® Global reagent, and lupus anticoagulant (LAC) by use of LA1/LA2 reagents from the same company. Enzyme immunoassay was used to analyze anticardiolipin antibodies (ACA) of IgG and IgM classes (ELISA, Orgentec Diagnostika, GmbH, Germany) and antibodies to double-stranded DNA (anti-dsDNA) (ELISA, Pharmacia Diagnostics, Sweden). Detection of mutations for factor II (G20210A) and factor V (G1691A, Factor V Leiden) was performed by melting curve analysis using commercially available kits (PCR-LightCycler Mutation Detection Kits, Roche, Germany) on a Roche LightCycler® apparatus. The polymorphisms C667T in the gene for methylenetetrahydrofolate reductase (MTHFR) and 4G/5G in the gene for plasminogen activator inhibitor-1 (PAI-1) were determined using PCR-RFLP method.

Case report

A 14-year-old boy presented to emergency pediatric clinic of the University Department of Pediatrics for the right lower extremity pain and swelling, where he underwent medical examination and was admitted to the Department. For a month before, the boy was on outpatient antibiotic treatment for infection. Two days before hospitalization, in addition to pain in the right leg, rigidness and swelling of the right leg occurred. Because of suspect DVT, duplex doppler of deep veins was obtained on day 1 of hospitalization to prove DVT of the leg veins and popliteal vein. Therapy with low-molecular-weight heparin (Fragmin®, 5000 IU, s.c.) was introduced. On day 5, the patient felt dyspnea and pain in the left thorax. The diagnosis of PE complicated with pulmonary infarction was made by magnetic spiral computerized tomography of

je terapija nefrakcioniranim heparinom (s početnom dozom od 25.000 IJ/24 h, i.v.). U bolesnika je postupno postignut dobar klinički odgovor uz ciljne vrijednosti aktiviranog parcijalnog tromboplastinskog vremena (APTV) od 1,9 do 2,7. Nakon dva dana u terapiju je uz nefrakcionirani heparin uveden i oralni antikoagulans (Marivarin®). Određivanje APTV i protrombinskog vremena (PV) rađeno je svakodnevno prema protokolu za praćenje dvojne antikoagulantne terapije VTE. Nakon osam dana liječenje je nastavljeno na Klinici za pedijatriju nefrakcioniranim heparinom i Marivarinom®. Nefrakcionirani heparin isključen je nakon 11 dana i ponovno je uz Marivarin® uveden niskomolekularni heparin (Fragmin®, 5000 IJ, s.c.). Ova terapija trajala je mjesec dana. Liječenje je nastavljeno samo Marivarinom® i provodi se i nakon otpuštanja bolesnika iz bolnice do danas uz redovitu ambulantnu kontrolu PV (udjel, INR) u hematološkoj ambulanti Klinike za pedijatriju.

Iz osobne i obiteljske anamneze doznajemo da je bolesnik dijete iz prve trudnoće i prvog poroda. Nakon ove trudnoće majka je imala spontani pobačaj. Dječak ima urednu perinatalnu anamnezu te uredan rast i razvoj. U obiteljskoj anamnezi baka po ocu preboljela je moždani udar, a djed i baka po majci su srčani bolesnici. Majka boluje od šećerne bolesti. Nije poznata pojava DVT u obitelji u mlađoj dobi.

Rezultati

Rezultati svih laboratorijskih pretraga prikazani su u tablici 1. prema vremenskom slijedu učinjenih pretraga. Nakon dolaska u hitnu ambulantu Klinike za pedijatriju učinjene su opće koagulacijske i hematološke pretrage koje pokazuju lagano produženo APTV (1,26), povećanu koncentraciju fibrinogena (5,2 g/L) i snižen broj trombocita ($106x10^9$/L) (tablica 1., stupac A). Zbog sumnje na DVT istog dana određena je i koncentracija D-dimera koja je bila povišena (1,3 mg/L). Rezultati proširenih laboratorijskih pretraga koje su uključile i ispitivanje čimbenika trombofilije, učinjenih prvoga dana hospitalizacije neposredno nakon postavljene dijagnoze DVT prikazani su u tablici 1., stupac B. Broj trombocita ostao je snižen ($90x10^9$/L). Aktivnosti faktora FII do FXII bile su unutar referentnog intervala, a aktivnost faktora XIII bila je snižena (50,6%). Određivanjem aktivnosti inhibitora sustava zgrušavanja: antitrombina (AT), proteina C (PC) i proteina S (PS) dobivena je snižena aktivnost PS (50,1%), uz aktivnosti AT i PC unutar referentnog intervala. Testom ProCGlobal dobiven je granični rezultat za rezistenciju na aktivirani protein C (omjer APCR 0,81). Molekularnom dijagnostikom isključena je mutacija G1691A faktora V zgrušavanja (faktor V Leiden) kao jedan od mogućih čimbenika koji mogu dovesti do APC rezistencije. Molekularnom dijagnostikom ostalih nasljednih čimbenika trombofilije isključena je mutacija G20210A u genu za faktor II zgrušavanja (protrombin) i

the thorax and that night the patient was transferred to coronary unit of the University Department of Medicine. Therapy with unfractionated heparin was introduced (at an initial dose of 25,000 IU/24 h, i.v.). Gradual good clinical response was achieved with target values of activated partial thromboplastin time (APTT) from 1.9 to 2.7. After two days, an oral anticoagulant (Marivarin®) was introduced in therapy, along with unfractionated heparin. APTT and prothrombin time (PT) were determined daily according to the protocol for monitoring of double anticoagulant therapy for VTE. After eight days of hospital stay, the treatment was continued at University Department of Pediatrics with unfractionated heparin and Marivarin®. Unfractionated heparin was excluded after eleven days and low-molecular-weight heparin (Fragmin®, 5000 IU, s.c.) was introduced again with Marivarin®. This therapy was continued for one month. Upon discharge from the hospital, the treatment has been continued with Marivarin® alone until now, with regular outpatient control of PT (portion, INR) at hematology clinic of the University Department of Pediatrics.

The patient's personal and family history revealed him to be the first-born child from the first pregnancy. After this pregnancy, the patient's mother had a spontaneous abortion. The boy had normal perinatal and growth history. In his family history, his paternal grandmother suffered cerebrovascular insult, and his mother's parents were cardiovascular patients. The patient's mother suffered from diabetes mellitus. There was no family history of DVT in young relatives.

Results

Results of all laboratory analyses according to time points are presented in Table 1. Common hematology and coagulation analyses performed upon the patient's admission to emergency pediatric clinic showed a slightly prolonged APTT (1.26), elevated fibrinogen concentration (5.2 g/L) and decreased platelet count ($106x10^9$/L) (Table 1, column A). Because of suspect DVT, the concentration of D-dimer was determined on the same day and was found to be increased (1.3 mg/L). Results of extended laboratory analyses that included studies of trombophilic factors, performed on hospitalization day 1, immediately after the diagnosis of DVT had been established, are presented in Table 1, column B. Platelet count remained low ($90x10^9$/L). The activities of coagulation factors FII to FXII were within the reference ranges, and the activity of FXIII was decreased (50.6%). Determination of the activities of the coagulation inhibitors antithrombin (AT), protein C (PC) and protein S (PS) showed a decreased activity of PS (50.1%), while the activities of AT and PC were within the reference ranges. The ProCGlobal coagulation-based assay for APCR yielded a borderline value (APCR ratio 0.81). Molecular diagnosis excluded G1691A mutation for coagulation

Tablica 1. Rezultati laboratorijskih pretraga načinjenih nakon dolaska u hitnu ambulantu (A), prvoga dana hospitalizacije neposredno nakon postavljene dijagnoze DVT uz početak terapije niskomolekularnim heparinom (B), petoga dana hospitalizacije kada je nastupila PE i započeta terapija nefrakcioniranim heparinom (C) i devet tjedana od dolaska u hitnu ambulantu, uz liječenje Marivarinom (liječenje niskomolekularnim heparinom isključeno) (D)

Table 1. Results of laboratory analyses performed upon admission to emergency pediatric clinic (A), on hospitalization day 1, immediately after the diagnosis of DVT had been established and therapy with low-molecular-weight heparin initiated (B), on hospitalization day 5 when PE developed and therapy with unfractionated heparin was initiated (C), and nine weeks of admission to emergency pediatric clinic, on Marivarin® therapy (heparin therapy excluded) (D)

Analysis	A	B	C	D	Reference range
CRP (mg/L)	21.1	-	-	-	0.1-2.8
ESR (mm/3,6 ks)	38	-	-	-	2-21
Leukocytes (x10^9/L)	12.6	10.6	-	-	4.4-11.6
Platelets (x10^9/L)	106	90	-	206	178-420
Erythrocytes (x10^{12}/L)	4.26	4.20	-	-	4.43-5.88
Hemoglobin (g/L)	128	118	-	-	129-166
Hematocrit (L/L)	0.360	0.340	-	-	0.390-0.487
APTT (ratio)	1.26	1.26	1.48	1.35	0.80-1.20
PT (portion)	0.77	0.80	0.78	0.56	≥0.70
PT (INR)	-	-	-	1.29	2.0-3.5
TT (s)	13	16	-	-	14-21
Fibrinogen (g/L)	5.2	5.3	8.6	-	1.8-3.5
Euglobulin fibrinolysis test (min)	180	210	-	-	150-210
D-dimer (mg/L)	1.3*	1.2	1.9	-	<0.3
AT (%)	-	92.0	-	-	70-120
PC (%)	-	93.7	-	-	70-140
PS (%)	-	50.1	-	-	70-123
FII (%)	-	78.9	-	-	70-120
FV (%)	-	84.0	-	-	70-140
FVII (%)	-	73.4	-	-	70-120
FVIII (%)	-	117.2	-	-	70-150
FIX (%)	-	110.6	-	-	70-120
FX (%)	-	78.0	-	-	70-120
FXI (%)	-	97.9	-	-	70-120
FXII (%)	-	81.3	-	-	70-150
FXIII (%)	-	50.6	-	-	70-140
PLG (%)	-	89.5	-	-	70-150
PAI-1 (IU/mL)	-	4.9	-	-	0.3-3.5
APCR (ratio)	-	0.81	-	-	0.80-1.10
LAC (ratio)	-	3.0	-	2.76	≤1.2
ACA-IgG (GPL-U/mL)	-	104.7	-	103.7	<10
ACA-IgM (MPL-U/mL)	-	5.1	-	2.1	<7
Anti-dsDNA (IU/mL)	-	16.3	-	-	<35
Factor V Leiden	-	G/G (wild type)	-	-	G/G (wild type)
PAI-1 polymorphism 4G/5G	-	4G/5G (heterozygous)	-	-	5G/5G (wild type)
FII mutation G20210A	-	G/G (wild type)	-	-	G/G(wild type)
MTHFR polymorphism C667T	-	C/C (wild type)	-	-	C/C (wild type)

* pretraga učinjena istoga dana iz novog uzorka 6 sati nakon prvog uzorkovanja
* analyses performed on the same day from a new sample, six hours of the first sampling

polimorfizam C667T u genu za MTHFR, a utvrđen je polimorfizam (genotip 4G/5G) u genu za PAI-1. Koncentracija PAI-1 u plazmi bila je povišena (4,9 IU/mL).
U bolesnika su dokazana antifosfolipidna antitijela: LAC i značajno povišen titar ACA klase IgG. S obzirom na to da su pretrage kojima se određuju antifosfolipidna antitijela izvedene nakon uvođenja terapije niskomolekularnim heparinom, iste su ponovljene nakon devet tjedana i isključivanja heparina iz terapijskog postupka, a uz terapiju Marivarinom® pri čemu su vrijednosti i dalje bile povišene (tablica 1., stupac D).
Nakon postavljene dijagnoze PE koja je uslijedila petoga dana hospitalizacije koncentracije D-dimera i fibrinogena bile su u porastu (tablica 1., stupac C).

Rasprava

Prikazom ovoga bolesnika iznijeli smo slijed kliničke i laboratorijske dijagnostike od trenutka dolaska u hitnu pedijatrijsku ambulantu, prijma na odjel i postavljanja dijagnoze te utvrđivanja mogućeg uzroka nastanka VTE.
Poznato je da laboratorijsku dijagnostiku čimbenika trombofilije nije poželjno izvoditi u akutnoj fazi bolesti, kao niti dok je bolesnik na antikoagulantnoj terapiji (5,6). U ovom slučaju to nije bilo moguće budući da je bolesnik od prvoga dana hospitalizacije do danas na antikoagulantnoj terapiji, a zbog utvrđenih čimbenika rizika kandidat je za dugotrajnu terapiju. Laboratorijske pretrage koje uključuju ispitivanje nasljednih i stečenih čimbenika trombofilije učinjene su prvoga dana hospitalizacije, neposredno nakon postavljanja dijagnoze DVT i započete terapije niskomolekularnim heparinom. Određivanje antifosfolipidnih antitijela, koje je ukazalo na prisutnost antifosfolipidnog sindroma (engl. *antiphospholipid syndrome,* APLS) u bolesnika, na koji rezultat može utjecati heparinska terapija, ponovljene su nakon ukidanja terapije niskomolekularnim i nefrakcioniranim heparinom.
U akutnoj fazi tromboze bolesnik je imao snižen broj trombocita. Prolazno sniženi broj trombocita u osoba s DVT često je rezultat povećane potrošnje tijekom akutne faze tromboze (6). Tijekom liječenja bolesnika broj trombocita povisio se na vrijednosti unutar referentnog intervala (175-234x10^9/L, podatci nisu prikazani). Snižena aktivnost faktora XIII koja je bila prisutna u akutnoj fazi bolesti rezultat je potrošnje u procesu aktivacije sustava zgrušavanja (6). Koncentracija D-dimera u početku kliničke sumnje na DVT bila je povišena (1,3 mg/L), a s razvojem PE uslijedio je daljnji porast (1,9 mg/L). Ovi rezultati govore u prilog kliničkoj značajnosti određivanja koncentracije D-dimera u dijagnostici DVT i PE (6,7).
U bolesnika su dokazana antifosfolipidna antitijela: LAC i ACA klase IgG u dva nezavisna mjerenja u razmaku od devet tjedana. Ovi rezultati, uz kliničku sliku VTE, ukazuju na prisutnost APLS u ovog bolesnika. Najčešća klinička manifestacija APLS je DVT donjih ekstremiteta, a u oko

factor V (factor V Leiden) as the most common possible cause of APCR. Molecular diagnosis of other hereditary thrombophilic factors excluded G20210A mutation in the gene for coagulation factor II (prothrombin) and C667T polymorphism in the gene for MTHFR, while demonstrating polymorphism for PAI-1 (genotype 4G/5G). Plasma concentration of PAI-1 was increased (4.9 IU/mL).
Antiphospholipid antibodies, LAC and significantly elevated titer of ACA-IgG, were demonstrated in the patient. Since the analyses of antiphospholipid antibodies (LAC, ACA IgG and IgM) were performed when therapy with low-molecular-weight heparin had already been introduced, the same tests were repeated after nine weeks when heparin was excluded from therapy protocol, and the patient was on therapy with Marivarin® still remained elevated (Table 1, column D).
After the diagnosis of PE was established on day 5 of his hospital stay, the concentrations of D-dimer and fibrinogen (8.6 g/L) showed further increase (Table 1, column C).

Discussion

Presenting this patient we report on the sequence of clinical and laboratory diagnosis from the patient's presenting to the emergency pediatric clinic, admission to the ward, establishment of the diagnosis, and identification of the possible cause of VTE.
It is known that laboratory diagnosis of thrombophilia is not recommended to perform in the acute stage of disease or during anticoagulant therapy (5,6). In this case, it was not possible since the patient was maintained on anticoagulant therapy from the first day of hospitalization until now, and because of the established trombophilic factors he is a candidate for long-term therapy. Laboratory analyses that included investigation of hereditary and acquired thrombophilic factors were performed on hospitalization day 1, immediately after the diagnosis of DVT had been made and therapy with low-molecular-weight heparin initiated. Determination of antiphospholipid antibodies that suggested the presence of antiphospholipid syndrome (APLS) in the patient and the results of which can be influenced by heparin therapy, was repeated after therapy with unfractionated and low-molecular-weight heparin had been excluded.
In the acute stage of thrombosis, the patient had a decreased platelet count. Transiently decreased platelet count in patients with DVT is often the result of the increased platelet consumption during the acute stage of thrombosis (6). During treatment, platelet count reached values within the reference range (175-234x10^9/L, data not presented). The decreased activity of factor XIII in the acute stage of disease is the result of consumption in the process of coagulation activation (6). On initial clinical suspicion of DVT, the concentration of D-dimer was elevated (1.3 mg/L), with further increase when PE developed (1.9 mg/L). These

polovice slučajeva komplicira ju PE (8). U osoba s APLS tromboza može nastati spontano ili u prisutnosti drugog čimbenika rizika, nasljednog ili stečenog. Dijagnoza APLS postavlja se kada je zadovoljen jedan od kliničkih kriterija (venska ili arterijska tromboza, spontani pobačaj) i jedan od laboratorijskih kriterija (povećan titar ACA klase IgG i/ili IgM, prisutnost LAC u dva nezavisna određivanja u razmaku od šest ili više tjedana) (9). APLS je najčešći uzrok stečene trombofilije, a povezanost sindroma s učestalom pojavom VTE pokazana je u mnogim studijama (10-16).

Molekularnom dijagnostikom nasljednih čimbenika trombofilije utvrđen je polimorfizam PAI-1 (genotip 4G/5G), uz uredan nalaz ostalih molekularnih biljega. Iako se polimorfizam PAI-1 ne smatra neovisnim čimbenikom rizika za nastanak VTE, neki literaturni podatci upućuju na dodatnu ulogu ovoga polimorfizma u nastanku VTE u osoba koje su nosioci nasljednog ili stečenog čimbenika trombofilije (17-20). PAI-1 fiziološki je regulator aktivnosti fibrinolitičkog sustava, koji vezanjem na tkivni aktivator plazminogena inhibira aktivaciju plazminogena u plazmin te tako inhibira fibrinolitičku aktivnost. U osoba koje su nosioci 4G/5G polimorfizma u genu za PAI-1, povišena koncentracija PAI-1 u plazmi djeluje protrombotički upravo zbog poremećaja u fibrinolitičkom sustavu (6,20,21). Povećana koncentracija PAI-1 (4,9 IU/mL) u plazmi bolesnika u skladu je s utvrđenim 4G/5G genotipom za PAI-1 te govori u prilog tome da bi polimorfizam PAI-1, uz APLS, mogao imati dodatnu ulogu u nastanku VTE u našeg bolesnika.

Koagulacijskom metodom određena APCR ukazuje na graničnu vrijednost rezistencije na APC (omjer APCR 0,81). Molekularnom dijagnostikom isključena je mutacija G1691A faktora V zgrušavanja (faktor V Leiden), koja je u 90% slučajeva uzrok rezistencije na APC. Budući da se radi o bolesniku s antifosfolipidnim antitijelima LAC i ACA, moguće objašnjenje graničnog omjera APCR je interferencija antifosfolipidnih antitijela u sustavu aktiviranog proteina C (5,8).

Određivanjem aktivnosti inhibitora sustava zgrušavanja utvrđena je snižena aktivnost PS (50,1%). Međutim, interpretacija vrijednosti PS tijekom akutne bolesti je otežana. Oko 60% PS u cirkulaciji vezano je na C4b-vezujući protein (C4b-BP, engl. *C4b-binding protein*), dok je 40% PS u slobodnom obliku i djeluje kao sučimbenik APC u inaktivaciji faktora zgrušavanja Va i VIIIa. C4b-BP je reaktant akutne faze koncentracija kojega u plazmi značajno raste tijekom akutne faze bolesti, te se povećava i udio PS vezanog na C4b-BP, a smanjuje se udio slobodnog PS. Izmjerena smanjena aktivnost PS mogla bi stoga biti rezultat porasta koncentracije C4b-BP u infekciji koja je bila prisutna više tjedana prije nastanka tromboze, kao i porasta koncentracije C4b-BP u akutnoj fazi tromboze (5,6,22). Na temelju mjerenja aktivnosti PS u akutnoj fazi tromboze ne može se utvrditi radi li se o nasljednom nedostatku PS ili stečenom nedostatku uzrokovanom infekcijom i trombo-

results suggest clinical significance of D-dimer determination in the diagnosis of DVT and PE (6,7).

Antiphospholipid antibodies, LAC and ACA IgG, were demonstrated on two independent measurements nine weeks apart. These results, along with clinical presentation of VTE, suggest the presence of APLS in this patient. The most common clinical manifestation of APLS is DVT of lower extremities, complicated with PE in one half of patients (8). In subjects with APLS, thrombosis may occur spontaneously or in the presence of other risk factors, genetic or acquired. The diagnosis of APLS is established if at least one clinical criterion (venous or arterial thrombosis, spontaneous abortion) and one laboratory criterion (increased ACA IgG and/or IgM, presence of LAC on two independent measurements at least six weeks apart) are met (9). APLS is the most common cause of acquired thrombophilia and the syndrome association with frequent occurrence of VTE has been demonstrated in a number of studies (10-16).

Molecular diagnosis of hereditary thrombophilic factors confirmed PAI-1 polymorphism (genotype 4G/5G), with regular reports for other molecular markers. Although PAI-1 polymorphism is not considered as an independent risk factor for VTE, some literature data suggest an additional role of this polymorphism in VTE occurrence in subjects who are carriers of genetic or acquired thrombophilic factors (17-20). PAI-1 is a physiological regulator of the fibrinolytic system, whose binding to tissue plasminogen activator inhibits activation of plasminogen into plasmin and thus inhibits fibrinolytic activity. In subjects with 4G/5G polymorphism in PAI-1 gene, elevated plasma concentration of PAI-1 acts as a prothrombotic factor because of disorder in the fibrinolityc system (6,20,21). The increased plasma concentration of PAI-1 (4,9 IU/mL) is in accordance with established 4G/5G genotype for PAI-1 and suggests that PAI-1 polymorphism, besides APLS, may have played an additional role in VTE occurrence in our patient.

Coagulation-based assay for APCR suggested the borderline resistance to APC (APCR ratio 0.81). Molecular diagnosis excluded G1691A mutation for coagulation factor V (factor V Leiden), that is in 90% of cases the cause of resistance to APC. Because it is the case of a patient with the antiphospholipid antibodies LAC and ACA, the possible explanation for decreased APCR is an interference of antiphospholipid antibodies in the activated protein C system (5,8).

Determination of activities for the coagulation inhibitors antithrombin (AT), protein C (PC) and protein S (PS) showed a decreased activity of PS (50.1%), with the activities of AT and PC within the reference range. However, interpretation of PS values during acute disease is difficult. About 60% of PS in the circulation is bound to C4b-binding protein (C4b-BP), while 40% of PS is found in the free form and acts as a cofactor of APC in the inactivation of factors Va and VIIIa. C4b-BP is an acute phase reactant, whose plasma concentration significantly increases duri-

zom. Dijagnostika nasljednog nedostatka PS zahtijeva određivanje aktivnosti i koncentracije slobodnog oblika PS u plazmi nakon završenog liječenja DVT.

U zaključku se može reći da je laboratorijskom dijagnostikom trombofilije u opisanog bolesnika utvrđen APLS kao čimbenik rizika za nastanak VTE. Dodatno, iako se polimorfizam PAI-1 ne smatra neovisnim čimbenikom trombofilije, moguće je da u bolesnika s utvrđenim čimbenikom trombofilije kao što je APLS, ovaj polimorfizam može imati dodatnu ulogu u nastanku VTE. Infekcija i mirovanje koji su prethodili trombozi mogli bi biti dodatni čimbenici rizika za nastanak VTE u ovog bolesnika. Prikazani slučaj govori u prilog dosadašnjim saznanjima da je pojava VTE najčešće rezultat međudjelovanja genetskih i stečenih čimbenika rizika.

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ng the acute stage of disease, thus the portion of bound PS increases and the portion of free PS decreases. Therefore, a decreased activity of PS measured in our patient could have been the result of elevated C4b-BP concentration in the infection that had been present several weeks before DVT as well as the result of elevated C4b-BP concentration in the acute stage of thrombosis (5,6,22). On the basis PS activity measurement in the acute stage of thrombosis it is not possible to establish whether there is a hereditary deficiency of PS or acquired deficiency caused by infection and thrombosis. The diagnosis of hereditary deficiency of PS requires determination of the activity and concentration of free PS in plasma upon completion of treatment for DVT.

In conclusion, laboratory diagnosis of thrombophilia in the described patient confirmed APLS as a risk factor for VTE. Additionally, although PAI-1 polymorphism is not considered as an independent thrombophilic factor, it is possible that in patients with established thrombophilic factors such as APLS this polymorphism may play an additional role in VTE occurrence. Infection and prolonged bed rest that preceded thrombosis could have been additional risk factors for VTE occurrence in this patient. The reported case supports the current concept according to which VTE most commonly results from interaction of genetic and acquired risk factors.

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