Ischemic Stroke with Anticoagulant Protein C Deficiency

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Abstract

Ischemic stroke is a circulatory disorder in brain is caused by reduced or stopped blood flow in some areas of brain. It can be caused by thrombotic lesions resulting from atherosclerosis and by emboli that have detached from the lesions atherosclerosis in the heart or cerebral arteries. Ischemic stroke category consists of emboli and lacunae. Characteristic profile clinical manifestations of acute embolic stroke are sudden onset with maximal neurologic deficits. Embolic often originate from heart or from ulcerative carotid plaques. Atrial fibrillation is a predisposing factor for cardiac thrombus, associated with six times of risk increase in strokes. The patient had a diagnosis with a clinical description of a history of post cerebrovascular disorder non hemorrhagic (CVD-NH/ischemic stroke) with hypercoagulable state from an outpatient clinic on April 13th, 2016 for examination antithrombin III (AT III), protein C, protein S, ACA IgG, ACA IgM, fibrinogen, D-dimer, and INR.

Keywords: *Brain, Embolic, Ischemic, Neurologic, Stroke.*

A. INTRODUCTION

1. Ischemic Stroke

Ischemic stroke is a circulatory disorder in brain is caused by reduced or stopped blood flow in some areas of brain. It can be caused by thrombotic lesions resulting from atherosclerosis and by emboli that have detached from the lesions atherosclerosis in the heart or cerebral arteries. Ischemic stroke category consists of emboli and lacunae. Characteristic profile clinical manifestations of acute embolic stroke are sudden onset with maximal neurologic deficits. Emboli often originate from the heart or from ulcerative carotid plaques. Atrial fibrillation is a predisposing factor for cardiac thrombus, associated with six times of the risk increase in strokes. Cardiac embolism is highly correlated with great vessel ischemia. Using warfarin is recommended to prevent stroke if atrial fibrillation is found unless there are contraindication.

Thrombotic infarction, including lacunae infarction, frequently presents as a transient ischemic attack (TIA) and can last several hours to several days. TIA is correlated with stenosis carotids. The prognosis for TIA varies, with up to 33% of patients with TIA having persistent strokes in 5 years. The incidence of stroke after TIA is 10-20% in the first 12 months and 5% every subsequent year. Lacunar infarctions are usually deep, ischemic vessel diameters small <10 mm and the number of cases ranges from 10-25% of all ischemic strokes. Often found in patients with a history of hypertension, diabetes, hypercholesterolemia with disease atherosclerosis, and smoking habits. The pathophysiology is thought to be multi factorial and these include small vessel lipohyalinosis and fibrinoid degeneration, decreased penetrating arterial perfusion, and atheromatous occlusion or embolism.

Diseases associated with ischemic stroke, namely hereditary and acquired hypercoagulable conditions, as shown in Table 1:

Table 1. The Strength of the Relationship Between Coagulopathy and Arterial Stroke

Coagulopathy	Arterial stroke risk
Sickle cell disease	Strong
Antiphospholipid antibody syndromes	Strong
Hyperhomocysteinemia	Currently
Activated protein C resistance	Light
Prothrombin gene mutation	Light
S protein deficiency	Light
C protein deficiency	Seldom
Anthrombin deficiency III	Seldom

Source: Caplan & Liebeskind (2016).

Among risk factors for thrombosis, only a few are simultaneously associated with thrombosis arterial and venous thrombosis. Hypercholesterolemia is a risk factor for arterial disease coronary artery disease but not a risk factor for venous thrombosis or pulmonary embolism. Otherwise disorder natural anticoagulants such as antithrombin, protein C and protein S are important risk factors for venous thrombosis, but their association with arterial thrombosis is unclear.

Based on recent attention has been paid to hyperhomocysteinemia (hHcy) as the risk factor thromboembolic disease has increased. There are 2 important things that differentiate hHcy from another thrombosis risk factor is that hHcy is associated with an increased risk of either arterial and venous thrombosis; and these abnormalities are easily corrected with therapy which are relatively cheap and safe such as limiting methionine intake, betaine supplementation, acid folate, vitamins B12 and B6 (Setiabudy, 2012).

Homocysteine is an amino acid that has a thiol group on its molecule, not used to form proteins and is an intermediate product of acid metabolism the essential amino methionine in cells. Methionine and Hcy metabolism are regulated in a cycle (picture) where there is a transfer reaction of the methyl group needed in the process synthesis of DNA, RNA, protein methylation, synthesis of neurotransmitters, phospholipids and hormones (Setiabudy, 2012).

Methionine derived from the diet is activated by ATP through the transmethylation pathway, becoming S-adenosyl methionine (SAM) which is converted by the methyl transferase enzyme to S-adenosyl homocysteine (SAH). Furthermore, SAH is converted to Hcy by SAH hydrolase.

Hcy metabolism occurs through enzymatic pathways that require vitamins as cofactors. There are 2 pathways of remethylation from Hcy to methionine. The first is catalyzed by methionine synthase (MS) with cobalamin as a cofactor and a methyl group derived from 5-methyltetrahydrofolate derived from the reduction of 5,10 methyl-tetra-hydrofolate by methylene-tetra-hydrofolate reductase (MTHFR). On the other hand, which occurs primarily in the liver, betaine is a methyl donor and the reaction is catalyzed by betaine-homocysteine methyltransferase (BHMT). If the remethylation pathway is saturated, or if the system requires it. Hcy will enter the transulfuration pathway. In the transulfuration pathway, Hcy is converted to cystathionine by cystathionine-ÿ-synthase (CBS) with pyridoxal 5-phosphate cofactor. Next, cystation converted to cystationisase (C) into cysteine which is then combined with glutathione or further metabolized to sulfate and excreted in the urine.

In plasma Hcy exists in 4 forms, most of which (70-80%) are bound to protein is mainly albumin, the rest (20-30%) is in the form of Hcy dimers or combined with other thiols such as cysteine and about 1-2% are in the free sulfhydryl form. In under normal conditions, plasma total homocysteine (tHcy) levels range from 5 – 15 μM [2].

Hyperhomocysteinemia is increased in tHcy > 15 μM. these conditions can be classified on light, medium and heavy with respective levels range, 16-30 μM, 31-100 μM and > 100 μM, increased in blood tHcy levels > 50 μM will be accompanied by an increase Cystine excretion in the urine is known as homocystinuria. The determining factor in tHcy. Plasma factors include physiological factors, generic and acquired disorders. Severe hyperhomocysteinemia usually results from a rare genetic disorder, but mild hHcy is common found in the general population. The most common cause of hHcy is nutrient-folate deficiency and vitamin B12, after which kidney disorders and other causes, namely certain drugs as well polymorphism of the gene encoding MTHFR enzyme.

Severe hyperhomocysteinemia is usually due to homozygous CBS deficiency. In the general population, the prevalence of this disorder is 1:200,000 to 1:335,000. manifestation Clinically, the classic syndrome of homocystinuria is characterized by mental retardation, ectopic lens, skeletal abnormalities, premature viscular disease and thromboembolism around 5-10% of cases of severe hHcy are caused by inherited defects in the consequent remethylation pathway homozygous MTHFR deficiency. This disorder is characterized by impaired neurologic function, psychomotor delay, seizures, peripheral neuropathy, premature vascular disease and thromboembolism.

Mild and moderate hyperhomocysteinemia is found in subjects with genetic disorders either acquired or a combination of both. Genetic disorders associated with hHcy moderate is heterozygous CBS or MTHFR deficiency with 50% enzyme activity. Another genetic disorder characterized by the presence of a thermolabile MTHFR mutation due to substitution C becomes T at nucleotide 677.

In Caucasians, prevalence of the C677T mutation varies between 5-20%. Not all subjects with this disorder will have total tHcy levels which increases. This indicates the influence of other factors such as MTHFR mutations if accompanied by low folic acid levels, tHcy levels will increase acquired hyperhomocysteinemia is seen in deficiencies of folate, cobalamin, and pyridoxine is an important cofactor in Hcy metabolism. Vitamin deficiency is caused especially in elderly people. Drugs that interfere with folic acid metabolism such as metrotextate and anticonvulsants or those that interfere with vitamin B6 such as theophylline, isoniazid, cycloserine, hydralazine, penicillamine, and procarbazine can also cause tHcy increases. Another factor that affects Hcy levels is the type of age, smoking habits, drinking lots of coffee and lack of exercise. Hcy levels are higher in men than women, also increases with age. The rate is higher in blacks than whites. Coffee contains a lot chlorogenic, as well as which can decrease plasma folate levels resulting in remethylation pathways bother. Smokers found lower pyridoxine levels than non-smokers. Hyperhomocysteinemia is also associated with decreased kidney function. Hypertension, hypercholesterolemia and hypothyroidism.

Until now mechanism of thrombosis in hHcy has not been fully understood. The result of in vitro studies show that Hcy can damage the endothelium so that its properties change from antithrombotic to prothrombotic. Homocysteine will undergo autoxidation into Hcy disulfide and Hcy thiolactone which will produce reactive oxygen species (ROS). This ROS is a substance that is cytotoxic to endothelial cells so that production of nitric oxide (NO) decreases. Hcy effects on endothelial cells include increasing expression of tissue factor and factor Va as well as decreased thrombomodulin, protein C activity and heparin activity. It has also been reported inhibition of fibrinolysis and increase Lp(a) binding. Homocysteine also stimulates production and increases expression monocyte chemoattractor protein-1 (MCP-1) and interleukin 8 (IL-8). MCP-1 got induces migration of monocytes into the intima of the arterial wall while IL-8 increased migration of T lymphocytes. increased excretion of thrombox metabolites indicates that platelet activation has occurred. Effects of Hcy on blood vessels include smooth muscle cells proliferate, vascular reactivity decreases because NO synthesis is impaired. However, it is not yet clear which disorder is primarily responsible for vascular disease and thrombosis (Setiabudy, 2012).

In 1969 McCully first reported the presence of severe atherosclerosis patients with homocystinuria and then hypothesized that there is a pathogenesis link between hHcy and atheogenesis. In 1976 Wilcken and Wilcken first showed an increase in cysteine-Hcy disulfide levels after loading methionine in coronary heart disease patients. Then it was reported that there was an increase Plasma Hcy before and after methionine loading in cardiac disease patients coronary artery disease, cerebrovascular disease and peripheral arterial occlusive disease. Meta analysis of 27 case-control studies showed that the odds ratio as an estimate of risk relative hHcy in subjects was 1.7 for coronary heart disease, 2.5 for cerebrovascular disease, and 6.8 for peripheral arterial occlusive disease.

In 1994, Falcon found a moderately high prevalence of hHcy in patients venous thrombosis. In 1995 it was reported that there was a relationship between hHcy and thrombosis vein in a study of patients with recurrent venous thrombosis. Based on 3 prospective studies of Hcy as a predictor of venous thrombosis risk yielded results negative. Research on the prevalence of the C677T MTHFR mutation in patients with Venous thrombosis gives inconsistent results. In 2 studies it seems C677T is a risk factor for venous thrombosis, but other studies have failed to demonstrate this increased prevalence of C677T MTHFR in patients compared to controls (Setiabudy, 2012).

Hcy measurement can be done by various methods. Usual measurements performed by chromatographic or immunological methods. Examination by method immunology is more widely used, usually with the fluorescence polarization immunoassay (FPIA) method don't need to fast to check tHcy, but it's best not to eat 6-12 hours before blood collection. The material for examination is plasma EDTA/heparin, but it can also be draw of the examination material in form of serum. The tHcy level in serum is 10% higher than in plasma (Setiabudy, 2012).

Plasma/serum must be separated within 30-60 minutes, if not separated immediately, tHcy levels increased 10%/hour at room temperature because Hcy synthesis continues in erythrocytes. Prior to centrifugation, the test material can only be stored for up to 6 hours at 2-8 °C. tHcy levels in plasma/serum stabilize for several days on temperature of 20°C, stable for 2 weeks on 2-8°C and stable for 8 months on temperature 20°C (Setiabudy, 2012).

Until now, there has been no standardization for tHcy measurements, so the result of measurements from 2 laboratories should be carefully compared, unless using the same method and calibrator. The measurement results should be interpreted accordingly with age, sex and the method used.

The patient with tHcy level that is still within the reference range, if not available changes in health status, re-monitoring can be done after 3-5 years. Then, the result of two tHcy measurements carried out on certain time intervals are said to be significantly different if there is a difference of ÿ 20% (Setiabudy, 2012).

Patients with tHcy levels that are within the threshold (20-30% below the upper referral), if there are no symptoms, it is recommended to change lifestyle and be examined return to normal after 6-12 months. If accompanied by symptoms, the patient must Evaluate vitamin B levels and look for clinical signs of other B vitamin deficiencies. Patients with a risk of experiencing vitamin B deficiency are advised to do these measuring tHcy levels regularly every 3-5 years, or when symptoms develop. Measurement tHcy levels in neonates, 3-5 days after birth can describe the status cobalamin of baby.

Monitoring of tHcy levels in hHcy patients treated with vitamins, carried out after 14 days and after 3 months of therapy, then every 1-3 years.

2. Thrombophilia

Thrombophilia is a condition that tends to increase the formation of intravascular thrombus in both arteries and veins. The last disorder is a long time, sufferers experience repeated thrombosis Assessment of thrombophilia based on clinical history, examination physical and laboratory examination. The clinical manifestations of thrombophilia can be seen in table 2:

Table 2. Clinical manifestations of thrombophilia

Strong supporting conditions:
§ Venous thromboembolism: superficial or deep vein thrombosis (DVT), pulmonary embolism (PE)
Thrombosis at rare sites of venous circulation (eg, cerebral, hepatic, mesenteric, and renal veins; possibly arm, portal, and ovarian veins; not retinal arteries or veins)
§ *Warfarin-induced skin necrosis* §
Fulminant purpura (neonate/adult) § Recurrent
fetal loss
Weak supporting conditions:

Source: Green (2003), Pruthi & Heit (2009).

This disorder is actually a predisposing factor for thrombosis, therefore Laboratory tests carried out aim to look for the etiology of occurrence thrombosis, whether classified as congenital or acquired thrombophilia Currently, there is no single test that can screen for congenital thrombophilia, as shown in table 3:

Table 3. Congenital thrombophilia

Supports strong: ÿ
Abnormal procoagulant proteins:
ÿ APC-R (FVL)
ÿ Prothrombin G20210A
ÿ Variant dysfibrinogenemia
Abnormal anticoagulant proteins:
ÿ Antithrombin deficiency
ÿ Protein C deficiency ÿ
Protein S deficiency
ÿ Others:
ÿ Homocysteine
Support:
ÿ Increased procoagulant proteins: FII, FVIII, FIX, FXI and fibrinogenFactor
XIII polymorphism

Based on the resulting effects, genetic mutations can be divided into 2 groups, namely those that cause decreased function such as anticoagulant deficiency naturally and which results in increased function such as the prothrombin 20210A allele which causes an increase in prothrombin levels.

Some of acquired thrombophilic disorders associated with stroke are quite widespread such as shown in table 4.

Table 4. Acquired Conditions Associated with Ischemic Stroke and Thrombophilia

Anti-phospholipid syndrome (APS)
Polycythemia vera, Essential thrombocythemia
Vasculitis, Systemic lupus erythematosus
Thrombotic microangiopathy *Sickle cell disease*,
Thalassemia Drugs (ergot alkaloids, cocaine, etc.)
Atrial fibrillation, Hypertensive
Cardiomyopathy, Diabetes, Hyperlipidemia
* Including *thrombotic thrombocytopenic purpura* (TTP) and *heparin-induced thrombocytopenia*

Polycythemia, thrombocythemia, macroglobulinemia, cryoglobulinemia are result in cerebral infarction. Cerebral arteritis occurs in patients with lupus erythematosus and polyarteritis nodosa. Main features of thrombotic thrombocytopenic purpura (TTP) is small blood vessel occlusion in the brain. Patients with hemoglobinopathies, sickle cell disease and their variants are frequent manifestations of stroke at a young age, so do cocaine and amphetamine addicts. Consequent stroke known oral contraceptives. Patients with atrial fibrillation, cardiomyopathy and endocarditis often lead to cerebral embolism. Hypertension, diabetes and hyperlipidemia is associated with atherosclerosis and stroke. Anti-phospholipid syndrome is an important cause of thrombophilia with neurological disorders (Green, 2003; Sutanto & Setiabudy, 2007; Pruthi & Heit, 2009; Aucutt-Walter, Jewels & Huang, 2009; Watson & Robertson, 2010).

Laboratory tests are grouped into general diagnostic tests, special coagulation tests, and additional tests if the abnormality is known as predisposing factors for thrombosis, as seen in table 5:

Table 5. Laboratory Evaluation of Suspected Congenital and Acquired Thrombophilia

General diagnostic examination:

Complete hematology with peripheral blood picture ÿ
PT before administration of warfarin
ÿ APTT (use of thromboplastin which is relatively sensitive in the presence of Lupus anticoagulant)
ÿ Serum creatinine
ÿ Liver enzymes (SGOT, SGPT, GGT, CHE)

Special coagulation tests and DNA:

• Tests based on protein:
ÿ APC-R ratio with molecular assay (DNA) for factor V R506Q mutation (Leiden)
ÿ Anticoagulant proteins (Protein C, protein S, and Antithrombin) ÿ
Lupus anticoagulant panel
Antikardiolipin and anti-beta2 glycoprotein 1 antibodies (IgG and IgM)
ÿ DIC screening and fibrinolysis (fibrinogen, soluble fibrin monomer complex, and D-dimer)
ÿ TT with *reflexive reptile tiare* (to detect effects of heparin or DTI) and screening dysfibrinogenemia
• Molecular (DNA) examination:
ÿ Genotyping of prothrombin G20210A mutation (direct genomic DNA mutation examination)
• Additional special examinations:
- Homocysteine (basal)

Additional examinations based on clinical suspicion and/or historical findings and examination results:

Source: Pruthi & Heit (2009)

In generally, specific coagulation tests in thrombophilia consist of the detection of clot-based endpoint (eg, lupus anticoagulant, protein S activity) and chromogenic (eg: protein C activity, antithrombin), or variants of the enzyme linked immunosorbent assays (ELISA). Ideally, an examination of activity and levels is carried out to classify type of deficiency.

Several preanalytic conditions affect the results of this examination, such as anticoagulants, acute thrombosis, liver disease, race, etc., so that in interpreting the results it is necessary to pay attention to these conditions. Factors affecting coagulation tests consists of:

a. Acute thrombotic effect

   During episodes of acute thrombosis, antithrombin, protein C and protein S may decrease temporary. Thus, the examination must be repeated, otherwise a misdiagnosis will occur considered a congenital deficiency.

b. Anticoagulant effect

   Heparin therapy will decrease the false antithrombin. Although on lupus reagent anticoagulants (such as: dilute russel viper venom time (DRVVT and Staclot APTT) contains heparin neutralizer up to 1 U/mL, but there is an excess of heparin result in false positives, which may result in prolongation of prophylactic therapy or administration secondary prophylaxis. False positive lupus anticoagulant results should be investigated repeated after heparin was discontinued.

c. Vitamin K antagonist therapy

   Protein C and S will be low on vitamin K antagonists (such as: warfarin, because proteins C and S are vitamin K dependent proteins). In addition, vitamin antagonist therapy K causes a prolonged lupus anticoagulant outcome in DRVVT. Direct thrombin inhibitors (DTIs eg: argatroban, lepirudin, bivalirudin). Because the majority of anticoagulant activity tests rely on thrombin formation to reach the endpoint, namely clot formation, the presence of DTI elongation occurs clot formation. This results in a false positive lupus anticoagulant test or protein C and S are falsely decreased. In this case the examination by chromogenic method is an option.

d. Effects of liver disease

The majority of anticoagulant and procoagulant proteins are produced in the liver. In advanced liver disease, both levels decreased.

Additional examinations to detect disorders known to predispose to thrombosis Venous embolism should be sought if clinically indicated. Flowcytometry for CD55 and CD59 is indicated in patients with intravascular hemolysis with or without pancytopenia to detect paroxysmal nocturnal hemoglobinuria (PNH). ADAMTS-13 check in patients with microangiopathic hemolytic anemia, thrombocytopenia with or without symptoms Neurologic signs, fever, and renal insufficiency seen in thrombotic thrombocytopenic purpura (TTP). In patients given heparin, check for heparin antibodies induced thrombocytopenia (HIT) should be assessed using a functional assay (examination of serotonin release, heparin-dependent platelet aggregation) or ELISA. Patients w/erythrocytosis, thrombocytosis, or portal or mesenteric vein thrombosis evaluated for myeloproliferative disorder (MPD) by screening for JAK-2 mutations V617F uses peripheral blood specimens as well as bone marrow aspiration/biopsy. At the moment, Routine examination for malignancy is difficult, what can be done is by age screening appropriate cancer in the presence of symptoms/signs (Green, 2003; Pruthi, & Heit, 2009; Aucutt-Walter, Jewels & Huang, 2009; Watson & Robertson, 2010; Schick, 2010).

Protein C has short half-life, around 6 hours. Will rapidly decline by giving warfarin. Factor II levels decrease more slowly, about 4-5 days. For reducing the risk of warfarin-induced skin necrosis or progressive thrombosis, warfarin should be used initiated only after heparinization therapy, with a low initial dose (such as 2 mg) later raised slowly. The effect of warfarin on protein S levels is around 4-6 weeks. It is recommended that abnormal results should be confirmed by re-examination.

3. Protein Anticoagulan System Deficiency C

In the period between the 1960s and 1980s, the role of natural anticoagulants in mechanism of prevention of venous thrombosis has been strengthened by the discovery of an association between deficiency of antithrombin (AT), protein C and protein S with venous thrombosis. deficiency Protein C or S homozygotes can get result in severe thrombotic manifestations, such as: fulminant neonatal purpura or warfarin induced skin necrosis. In generally, individual Heterozygotes with natural anticoagulant deficiency will experience thrombosis with age younger than 40 years, often without precipitating environmental factors and sometimes in unusual places such as in the cerebral sinuses, abdominal veins or deep veins in the arm. These individuals also tend to have recurrent and frequent thrombosis family history of thrombosis (Setiabudy, 2007; Kottke-Marchant & Comp, 2002).

Protein C anticoagulant system consists of protein C, thrombomodulin and protein S. Protein C is a glycoprotein with a molecular weight of 62000 kD, synthesized in the liver and include in vitamin K dependent protein because it requires vitamin K for the carboxylation process. The structure of protein C consists of heavy chains and light chains connected by bonds disulfide. In the light chain there is a carboxyglutamate gamma group that functions for attached to the surface of phospholipids by means of calcium ions on heavy chain. There is the amino acid serine which is the active part. In the blood, protein C circulates in inactive form and levels range from 3-5 μg/mL with a half-life of 6-8 o'clock. Protein C will be activated into active protein C (APC) by thrombin with assistance thrombomodulin. The ability of thrombin to activate protein C will increase 20 thousand times more efficiently with the help of thrombomodulin (Setiabudy & Widjahakim, 2007; Mosnier & Griffin, 2013).

Active protein C is a serine protease that functions to break down factor Va (FVa) and FVIIIa. To break down FVa and FVIIIa, active free protein S will act as APC cofactor. As seen in Figure 1. Apart from inactivating FVa and FVIIIa, APC also increases fibrinolysis by inhibiting the action of plasminogen activator inhibitor-3 (PAI-3). APC is also profibrinolytic, due to inactivation of FVa and FVIIIa, it means that thrombin formation is inhibited so that thrombin activation is activatable fibrinolytic inhibitors (TAFI) which are antifibrinolytics are also inhibited. APC action is inhibited by C1- inhibitors, alpha2-antitrypsin, alpha2-macroglobulin and alpha2-antiplasmin (Setiabudy & Widjahakim, 2007; Mosnier & Griffin, 2013; Brummel-Ziedins et al., 2014).

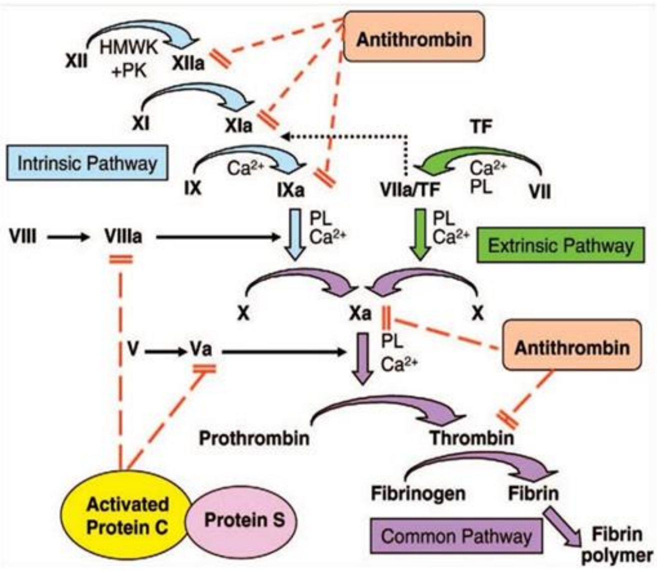

Figure 1. The Role of Natural Anticoagulants in the Coagulation Cascade

*Ca2+_ indicates calcium; HMWK, high-molecular-weight quininogen; PL, phospholipids; PK, prekallikrein; and TF, tissue factor.

Source: Rahemtullah & Van Cott (2007)

The first report of mutations in the protein C gene were published in 1987 by Romeo et al. With the discovery of the PCR technique, screening for gene disorders became easier so many mutations are found. Recent publications indicate that there were 190 mutations in the protein C gene. Congenital protein C deficiency is inherited in an autosomal dominant manner. Protein C deficiency can be divided into 2 groups, namely type 1 which is characterized by levels of and low protein C activity and type II which is characterized by normal levels but activity low. In individuals with protein C heterozygotes, the risk of thrombosis is 7-fold higher than individuals with normal protein C. Frequency of protein C deficiency in cases thrombosis reaches 6% while in a healthy population 0.3%. Thrombosis often occurs when protein C levels are 30-50% (Setiabudy, 2007; Mosnier & Griffin, 2013; Williams, 2016; Goldenberg & Manco-Johnson, 2008).

Protein S is a natural anticoagulant which is also classified as vitamin K dependent. In plasma 60% of protein S is bound to C4b binding protein and the rest is in the form active free. Protein S deficiency can be classified as type 1 or partial deficiency type II is impaired function and type III is characterized by low free protein S but Total protein S is normal. Protein S Heerlen caused pro460ser mutation cause free protein S is rather low probably because it is quickly cleared. thr103asn mutation causes type II protein S deficiency. Individuals with protein S deficiency are heterozygous have 6 or 10 times the risk of thrombosis compared with individuals who have normal S-protein. The frequency of protein S deficiency in cases of thrombosis ranges from 2-6%. It turns out that only about 15% of familial thrombophilia or about 5% of all patients. Venous thrombosis caused by a deficiency of antithrombin, protein C and protein S (Setiabudy, 2007).

Protein S can be checked for its levels and activity. In general, activity checks more useful than checking levels because in type II deficiency the levels are still normal, while the activity is low. However, for protein S it is recommended to check total and free protein S levels because the examination of protein S activity is influenced by APC-R (Setiabudy, 2007).

There are 2 types of protein C and protein S examinations, namely examination of levels by means of immunology and examination of activity by means of coagulation or chromogenic substrates. Arterial thrombosis occurs through 2 processes, namely atherosclerosis and thrombosis. Not yet known whether genetic mutations that risk factors for venous thrombosis are also a factor risk for arterial thrombosis. Patients with protein S deficiency tend to experience thrombosis from a young age. Zoller et al. reported increased risk arterial thrombosis in individuals with protein S deficiency heterozygous after age than 50 years (Setiabudy, 2007).

Vitamin K is the inactive form, except when it is in Vitamin K1 hydroquinone (KH2) is derived from the reduction of vitamin K1 by the reductase enzyme. The carboxylase enzyme together with KH2 will change the amino acid glutamate (Glu) to gamma carboxyglutamate (Gla) through the process of carboxylation on precursor factors coagulation factor "vitamin K dependent", such as the clotting factor precursor prothrombin, VII, IX, X. This carboxylation process is important for these clotting factors to function because the gamma carboxyglutamant group is needed to bind Ca ions and phospholipids in the blood clotting process.

According to Deykin & Verstraete, the main action of oral anticoagulants is inhibition the action of the epoxide reductase enzyme, so that the epoxide vitamin K changes to vitamin K disturbed so that there is an accumulation of precursors "vitamin K dependent factors" which are by Hemkar is referred to as “Protein Induced by Vitamin K Absence or Antagonists” (PIVKA). This happens due to the failure of PIVKA to convert into a clotting factor “vitamin K dependent factors”. According to Deykin, oral anticoagulants can also inhibit the conversion of vitamin K to KH2 (Wirawan, 2007; Williams, 2016).

Oral anticoagulants works to inhibit the activity of vitamin K, so it will decrease vitamin K-dependent protein activity, thereby reducing protein activity vitamin K dependent agents such as prothrombin, FVII, FIX and FX, as well as protein C and S. If DVT is caused by a deficiency of protein C or S, then administration oral anticoagulants can further decrease the activity of protein C and S, so it can occur skin necrosis (coumarin induced skin necrosis). Within 48 hours after the start of administration warfarin, protein C and S levels can drop to 40-60%, but will rise again until 70% after 2 weeks without warfarin. Therefore, to find the cause of DVT, examination of protein C and S should be done after several weeks of warfarin therapy. If the level of protein C or S are less than 60%, then there is a strong suspicion that they are congenital deficiency. Sometimes coumarin resistance occurs, even though the dose has been increased but the INR target was not reached. In this case it is necessary to think about giving drugs that block the action of coumarins such as barbiturates, phenytoin, cholestyramine, rifampicin, antacids, antihistamines or maybe the patient consumes lots of green leafy vegetables which contain lots of vitamin K such as broccoli.

B. RESULT AND DISCUSSION

A 73-years-old female patient, was brought to the laboratory from an outpatient clinic on April 13th, 2016 with a clinical description of a history of post cerebrovascular disorder non-hemorrhagic (CVD-NH/ischemic stroke) with hypercoagulable state, for examination antithrombin III (AT III), protein C, protein S, ACA IgG, ACA IgM, fibrinogen, D-dimer, and INR.

Laboratory Examination Results April 13th, 2016

Checking type	Results	Unit	Reference value
		Hemostasis	
Antithrombin III	97.60	%	79.40-112.00
C protein	71,40	%	70.00-140.00
S protein	**55.80***	%	58.60-126.00
ACA IgG	1.7	GPL	<15
ACA IgM	3,1	MPL	<12.5
Fibrinogen	349	mg/dL	213-450
D-dimer	137	ng/mL	<300
INR	2.55		2-3

1. **Before Additional Data**

Received inspection material Mrs. RR, 73 years old, from an outpatient clinic with clinical description of history of post CVD-NH (ischemic stroke) with hypercoagulability.

In general, cause of ischemic stroke in elderly patients is due to atherosclerosis, meanwhile hypercoagulable usually at a young age. From the request for laboratory examination, it shows that the clinician wants to find the cause hypercoagulation, should also request laboratory tests to look for factors risk of atherosclerosis, such as blood glucose status and lipid profile.

Broadly speaking, ischemic stroke category consists of emboli and lacunae. From the results of the MRI head on December 2nd, obtained multiple small cerebral infarctions, there was a lacunae ischemic stroke, but there was no head MRI data on February 10th, 2016 when the patient had an ischemic stroke. Clinical manifestations of stroke Ischemic lacunae often present as TIA. TIA patients may develop persistent strokes in 5 years.

The result of fibrinogen and D-dimer levels were within normal limits, indicating no mass acute thrombosis. Fibrinogen is an acute phase protein and D-dimer is a marker coagulation activation. These results indicate that the acute period has passed and there is no process clot lysis.

Requests for ACA IgG and IgM examinations in elderly patients are included in the criteria low or weak probability of anti-phospholipid syndrome (APS), usually requests these was preceded by an unexplained prolongation of the APTT results. On In this case there is no APTT data.

The result of INR 2.55 indicates the patient is on oral anticoagulant therapy and the target of administration achieved because it is within the target range of 2 to 3. The examination results showed that the activity of protein C and protein S tended to be low, with antithrombin results within the reference range. From the results of the examination concluded, there is a deficiency of protein C and protein S. Examination of protein C and protein S which carried out is an examination to assess its functional activity, so it cannot differentiate the type of protein C and protein S deficiency in these patients.

Deficiency of protein C and protein S in the patient is thought to be the result of a co-existing disorder obtained because the patient was 73 years old. In deficiency of protein C and protein S which caused by congenital abnormalities, symptoms usually appear at a younger age. Although Zoller et al. reported an increased risk of arterial thrombosis in individuals with heterozygous protein S deficiency after age 50 years is appropriate in these patients.

In addition, in congenital abnormalities there is usually a significant decrease in one of protein C or protein S alone. In these patients decreased activity of protein C and protein S tend to occur simultaneously, thus further strengthening the suspicion of the disorder as a result of the abnormality.

Acquired deficiencies of protein C and protein S can be seen in liver disease, DIC, acute thrombosis, vitamin K deficiency, or due to use of vitamin K antagonists. Because the results of patient's antithrombin examination are still within the reference value limit, so think about it protein C and protein S deficiency in patients due to vitamin K deficiency or due to effects vitamin K antagonist.

2. After Additional Data

Based on the diagnosis recorded on the medical resume, it is known that the patient has had CVD-NH (ischemic stroke) with hypercoagulability on 10 February 2016. From additional data, conventional risk factors for ischemic stroke such as hypertension, diabetes mellitus, dyslipidemia denied. The absence of hypertension can be excluded from the pressure examination blood obtained 130/80 mmHg included in the category within normal limits and from drug history given no antihypertensive found. To rule out the presence of diabetes mellitus and dyslipidemia there is no evidence of blood glucose level or lipid profile, even from history the drugs given were not antidiabetic and antilipidemic or anticholesterol.

On January 14th, 2016, the result of complete hematology examination were within limits normal, rule out predisposing factors for acquired thrombophilia due to hematologic abnormalities causes of ischemic stroke, such as polycythemia and thrombocythemia. From the drug history, one of them was given 'Simarc' or warfarin or oral anticoagulant drugs group of vitamin K antagonists. Administration of warfarin aims to prevent ischemic stroke repeated in patients. From previous laboratory tests, INR was found 2.55 is within the appropriate target range for warfarin use. Recommended Re-monitor INR every 4 to 8 weeks.

A history of taking oral anticoagulant warfarin in the patient supports protein C deficiency and protein S due to acquired abnormalities. Warfarin is a vitamin K antagonist with how to inhibit the action of the epoxide reductase enzyme, thereby interfering with the conversion of vitamins K epoxide into vitamin K. As a result, in examining the activity of protein C and protein S, decreased activity was found because protein C and protein S could not be converted to form functional.

In patients, protein C and protein S examinations are carried out while the patient is still consuming the oral anticoagulant warfarin. Warfarin should be discontinued for at least 2 weeks before examining protein C and protein S. However, the patient had a history of stroke ischemia with hypercoagulation so that the risk of recurrent thrombosis in these patients is high and it is difficult to stop warfarin therapy in patients.

C. CONCLUSION

An examination of protein C, protein S, and antithrombin was carried out on Mrs. RR, age 73 years, with clinical information post CVD-NH with hypercoagulation. From the result of examination found tend to be deficient in protein C and protein S, with antithrombin within the reference range. From the additional data obtained moderate patients taking oral anticoagulant warfarin at the time of examination resulting in protein deficiency C and protein S in the patient may be due to the use of warfarin.

ACKNOWLEDGEMENT

I am deeply indebted to my supervisor, Prof. Dr. dr. Rahajuningsih Dharma Setiabudhy, DSc. Sp. PK (K) for warm support, inspiration and thoughtful guidance. Special thanks to Mahar Mardjono National Brain Center Hospital for their continuos support and encouragement for my pursuit.

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