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Short Communication

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The Assessment of Anticoagulative System: Two Natural Anticoagulants Protein C and Antithrombin III

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Abstract

Protein C and antithrombin III, two natural anticoagulants, play an important role in the regulation of hemostatic balance. Activated protein C (APC) and protein S complex inactivate the activated factor Va and VIIIa. Moreover, excess protein S can drive cancer cell proliferation and cell survival through oncogenic receptor Axl. Antithrombin III (ATIII) and thrombin form an inactive complex in a 1:1 molar ratio. The binding of heparin to ATIII induce conformational changes which facilitate the binding of thrombin. ATIII also inactivate factor IXa, Xa, XIa and XIIa at slow rates. At present, regarding the assay method for routine work, protein C antigen was determined by electroimmunoassay. The clotting assay was used for detecting ATIII activity (ATIII:C), ATIII antigen(ATIII:Ag) was measured using immunoassay (EIA). Assessment of protein C and ATIII has been monitoring congenital protein C deficiency and ATIII deficiency. In my detection of 20 liver cirrhosis, the results showed markedly decreased protein C antigen (PC:Ag 0.5501 vs 1.0578u/ml) and antithrombin III (ATIII:Ag 21.8 vs 39.8mg/dl, ATIII:C 40.25 vs 105.04%) respectively. The PC and ATIII assays are helpful to monitoring the liver disease and might play a predictable marker.

Keywords: *Protein C, Antithrombin III, Assay methods, Congenital deficiency, Liver diseases*

1. Introduction

During the past decades a number of new anticoagulative proteins have been identified that play an important role in the regulation of hemostatic balance (Stenflo, 1976). These proteins included Antithrombin III (ATIII), protein C and its inhibitor, protein S, and thrombomodulin. In except for ATIII, the others form the components of the protein C pathway.

The cDNA codes for human protein C protein which consists of a preproleader sequence of 42 amino acids, a light chain region of 155 amino acids, a connecting dipeptide of lys-Arg, and a heavy chain region of 262 amino acids (Foster and Davie, 1984; Foster *et al.*, 1985). During intracellular processing, the preproleader sequence removed, protein C is secreted as a mature protein of 419 amino acids.

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Protein C (PC), a vitamin K-dependent plasma protein, and its inhibitor of activated protein C (APCI) are part of a major regulatory system of hemostasis. Activated protein C destroys the activity of activated factor V and VIII. Protein S as cofactor of activated protein C (APC) is due to the inability of APC to prolong the APTT or the Va-induced clotting time in protein S deficient plasma (Bertina *et al*, 1985). APC and protein S complex could inactivate factor Va and VIIIa more rapidly under the condition of calcium and phospholipids than did APC alone (Suzuki *et al*., 1983; Lawrence *et al*., 1985). More recent, it has been found that protein S drives cancer cell proliferation and cell survival via oncogenic receptor Axl (Abboud-Jarrous *et al*., 2017; Lee *et al*., 2019; Song *et al*., 2020; Nuzzo *et al*., 2019; Wimmel *et al*., 1999; Zhu *et al*., 2020). Aberrant activation of RTKs often leads to malignant transformation, whereas PI3K / Axl is required for this oncogenic receptor signaling (Utermark *et al*., 2012). At present, the oncogenic receptors and their target antibodies (Abs) (Yang *et al*., 2014) strategies have been widely extended to CD44 oncogenic receptor of Hyaluronic Acid(HA) (He *et al*., 2013), RAGE oncogenic receptor (Radia *et al*., 2013; Xu *et al*., 2013; Wang *et al*., 2015; Zhang *et al*., 2016), the oncogenic receptor platelet—derived growth factor receptor-β (PDGFRβ) or PDGFRa linked to the pathogenesis of myeloproliferative neoplasm or chronic leukemia (Zhu, 2017, 2018; Esposito *et al*., 2018),cytokine IL-3 / oncogenic receptor IL3R, IL-6 / IL6R- STAT3-ADAR1 (P150) oncogenic transcription factors (Teoh *et al*., 2020), oncogenic IL7R (Mansour *et al*., 2015), IL-11 / IL-11 gp130 receptor pro-oncogenic signaling (Zhu, 2018; Ernst *et al*.,2008, Ernst and Putoczki, 2014; Merchant, 2008) and oncogenic receptor IL17rb (Huang *et al*., 2017; Poultsidi *et al*., 2018). Targeting against a deregulated dominant oncogenic receptor such as the oncogenic Estrogen Receptor (ER) pathway (tamoxifen) (Green and Chambon, 1986; Chin *et al*., 2014; Singh and Kumar, 2005; Elangovan *et al*., 2011; Zinger *et al*., 2019; Ludwik *et al*., 2018; Veeraraghavan *et al*., 2014; Tilli *et al*., 2003; Davis, 2012; Yue *et al*., 2013; Hickey *et al*., 2021; Marx *et al*., 2007; Zhu, 2017, 2018), and blocking oncogenic receptor HER3 / HER2 agent trastuzumab is enough to slow tumor progression (Zhu, 2017, 2018; Marx *et al*., 2007; De Bacco *et al*., 2004; Moody *et al*., 2015; del Mar Maldonado *et al*., 2020; Duarte *et al*., 2017; Jenke *et al*., 2021; Staerk *et al*., 2011; Wilmes *et al*., 2020).

In clinics, decreased plasma PC levels can predispose an individual to thrombotic disease, whereas decreased levels of APCI can cause hemorrhages. Patients with recurrent thromboembolic episodes of unknown etiology would be monitoring for the assessment of protein C deficiency. Congenital protein C deficiencies were reported at earliest by Griffin *et al.* (1981), and by Bertina *et al.* (1982), Broekmans *et al*. (1983). Hereditary protein S deficiency was identified in three Dutch families by Broekmans *et al*. (1983). At present, protein C antigen was determined by electroimmuno- assay as described elsewhere.

The term “antithrombin” was first postulated by Morawitz (1905). Antithrombin III (ATIII) is an a2-globulin with ellipsoid form. The single polypeptide chain contains 425 amino acids. Heparin belongs to the glycosaminoglycans (GAGs) , which are sulphated carbohydrates widely distributed in human (Danishefsky *et al*.,1978). The binding of heparin to ATIII induce conformational changes which facilitate the binding of thrombin. Upon the formation of a stable thrombin-ATIII complex, heparin is released and joins another AT molecule (Abildgaard, 1969; Rosenberg *et al*., 1973; Li *et al*., 1976; Machovich *et al*., 1975). The data suggested that lysine was required for heparin binding. Another intact serine at the active site of thrombin was also essential for reaction with ATIII (Abildgaard, 1969). Thrombin and ATIII form an inactive complex in a 1:1 molar ratio. ATIII also inactivates factor IXa, Xa, XIa and XIIa at slow rates (Abildgaard, 1969; Rosenberg *et al*., 1973). In clinical aspects, regarding the assay method for routine work, the clotting assays for determining ATIII activity (ATIII:C) is to be complicated. ATIII antigen (ATIII:Ag) was measured using immunoassays (EIA). The method is simple, cheap and require about 20 h, but cannot detect all congenital ATIII deficiencies. Egeberg O and Abildgaard U (Egeberg, 1965; Abildgaard, 1970) reported the results that mean ATIII activity (ATIII:C) were about half the normal in the classical ATIII deficiency and the affected family members.

In cirrhosis of the liver, the protein C antigen and the ATIII concentration are often low, and was positively correlation with serum albumin levels (Zhu *et al*., 2020; Griffin *et al*., 1982; Mannucci *et al*., 1982; Mannucci *et al*., 1973; Nagy *et al*., 1976). In biliary tract occlusion and primary biliary cirrhosis, normal PC and ATIII concentrations were found. These data indicated that two natural anticoagulants (PC and ATIII) deficiencies occur the dysregulation of hepatic PC and ATIII synthesis, and might play a central role as a predictor index in liver diseases.

In following Table 1, in our group, determination of protein C and antithrombin III was carried out in 20 liver cirrhosis. The results showed markedly decreased protein C antigen (PC:Ag 0.5501 vs 1.0578u / ml) and antithrombin III (ATIII:Ag 21.8 vs 39.8mg / dl, ATIII:C 40.25 vs 105.04%) respectively.

Table 1: Plasma Protein C and Antithrombin III Levels in 20 Liver Cirrhos

Diseases	No.	PC:Ag(u/ml)	ATIII:Ag(mg/dl)	ATIII:C (%)
Liver cirrhosis	20	0.5501 +/- 0.2536*	21.80 +/- 16.99*	40.25 +/- 30.89*
Hepatitis B liver cirrhosis	14	0.5066 +/- 0.2514*	20.84 +/- 14.93*	36.04 +/- 25.87*
Normal control group	50	1.0578 +/- 0.1886	39.8 +/- 6.54	105.04 +/-15.81

Note: *$p < 0.001$.

In recent many topics were focused on coagulation and malignancy. Some haemorrhagic and thromboembolic complications are frequent in patients with malignancy. Acute leukemias varied in PC and ATIII level (Zhu *et al.*, 1989, 2020; Griffin *et al.*, 1982; Mannucci *et al.*, 1982). The decreased PC concentration was frequently found in individuals with M5 subtype and hyper- leukocytic acute leukemias. Plasma PC:Ag and ATIII:Ag level had no significant lower in some patients with Acute Promyelocytic Leukemia (APL) complicated by DIC, which suggested the coagulopathy in APL might be due to mechanisms different from other forms of DIC such as infectious disease(e.g. septic shock) (Zhu *et al.*, 1989, 2020; Griffin *et al.*, 1982). Many authors (Donati *et al.*, 1981) described that a procoagulant activity was in term of 'cancer coagulative factor', 'cancer cell procoagulant activities', 'leukemic cell mediated procoagulant activities'(Zhu *et al.*, 1989), 'Cancer Procoagulant A (CPA)' which was presented in malignant cells. The most prevalences are that low PC and ATIII levels were detected both in clinical infectious and experimental DIC, and the term 'consumption coagulopathy' characterized this PC and ATIII decrease (Fourrier *et al.*, 1992). Thus, the PC and ATIII assays are helpful to monitoring the disease progress and act as an important biomarker or predictable marker.

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