Published in final edited form as:

Thromb Res. 2009 July ; 124(3): 259–261. doi:10.1016/j.thromres.2008.12.030.

Circulating Tissue Factor Procoagulant Activity is Elevated in Stable Moderate to Severe Chronic Obstructive Pulmonary Disease

Vijender Reddy Vaidyula³, Gerard J. Criner², Carla Grabianowski², and A. Koneti Rao^{1,3}

¹Division of Hematology and Thromboembolic Diseases, Temple University School of Medicine, Philadelphia, PA 19140

²Division of Pulmonary and Critical Care Medicine, Temple University School of Medicine, Philadelphia, PA 19140

³Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA 19140

Abstract

Introduction—Chronic obstructive pulmonary disease (COPD) patients have increased risk for cardiovascular mortality and venous thromboembolism. Tissue factor (TF) is the physiological initiating mechanism for blood coagulation and is pro-inflammatory.

Methods—We have studied circulating blood-borne TF-procoagulant activity (TF-PCA), plasma coagulation factors (F) VIIa and FVIII, and thrombin-antithrombin (TAT) complexes in 11 stable, moderate-severe COPD patients, 10 free of exacerbation for > 3 weeks.

Results—TF-PCA was increased in COPD patients (52.3 ± 5.6 U/ml, (SE)) compared to control subjects (20.7 ± 1.5 , $n = 45$, $p < 0.0001$). TAT levels were increased (COPD patients: 2.99 ± 0.65 ug/l; control subjects: 1.31 ± 0.13 , $n = 53$, $p < 0.0001$), indicating enhanced thrombin generation. Plasma FVIIa (the activated form of FVII) was higher in COPD (83 ± 11 mU/ml; controls, 64 ± 5 mU/ml, $n = 20$) but did not reach statistical significance. Plasma FVIIc and FVIII were not increased. TF-PCA levels were inversely related to plasma FVIIa ($r = -0.80$, $p = 0.003$) and FVIIc ($r = -0.76$, $p = 0.007$).

Conclusions—Blood-borne TF-PCA is elevated and constitutes a prothrombotic and proinflammatory state in stable but moderate-severe COPD, and may contribute to the increased risk for vascular events.

Keywords

Chronic Obstructive Pulmonary Disease; Tissue Factor; Factor VII; Factor VIII; Thrombotic Disease

Introduction

Chronic obstructive pulmonary disease (COPD) is a debilitating disease that affects between 10 and 24 million adults in the U.S. Chronic inflammation is a central component of COPD pathogenesis [1]. COPD patients are at 2-3 times greater risk for cardiovascular mortality, which accounts for ~ 50% of all COPD deaths [2]. Atherosclerosis and thrombosis constitute

the major mechanisms leading to the acute cardiovascular events [3]. Tillie-Leblond et al [4] have recently reported a 25% prevalence of pulmonary embolism in 197 consecutive COPD patients with severe exacerbation of unknown origin. In a cohort study of over 23,000 patients, Curkendall and colleagues [5] reported an increased risk of acute myocardial infarction, stroke and pulmonary embolism in COPD.

Tissue factor (TF), a membrane-bound protein, is the primary physiological mechanism of initiation of blood coagulation [6,7] and has proinflammatory effects [8]. Binding of native coagulation factor VII (FVII) to TF converts FVII to the activated form, FVIIa. The resulting TF-FVIIa complex activates factors IX and X to factors IXa and Xa, respectively, leading to the formation of the prothrombinase complex and thrombin generation. TF present in the adventitia of blood vessels and atherosclerotic plaques has been long recognized to initiate coagulation and thrombus formation when the vessel wall is injured or plaques fissured [6,7]. It has been recently shown that there is a circulating pool of TF associated with hematopoietic cells and microparticles which plays a role in thrombosis [6,7,9-11] and is elevated in diseases associated with thrombosis and premature atherosclerosis. Because of the high prevalence of atherothrombosis and venous thrombosis in COPD, we examined circulating tissue factor procoagulant activity (TF-PCA) and FVIIa in stable COPD patients who are free of exacerbation.

Methods

Patients

We studied 11 stable but moderate to severe COPD patients (mean age 64 ± 2 years, mean $\pm$ SE; 4 males, 7 females) with forced-expiratory volume (FEV_1) of $42 \pm 8\%$ and >20 pack years of smoking. At the time of study, 10 were free of COPD exacerbation for over 3 weeks and 6 patients had no COPD exacerbation in over 3 months. Patients had smoked 74 ± 14 pack-years; none were current smokers. These patients were recruited from the outpatient setting. Control subjects were healthy adult individuals recruited from the Temple University Health Sciences Center.

TF-PCA was measured by the method of Key et al. [10,12] as described by us previously [12] in whole blood cell lysates with a two-stage clotting assay using recombinant FVIIa, Factor X, and normal human plasma containing phospholipid vesicles. This assay measures both cell membrane-bound and microparticle-associated TF activity. The following parameters were measured in plasma harvested by centrifugation (2500 g, 20 min) from blood collected into one-tenth volume of 3.8% sodium citrate, as described by us previously [12]. Plasma factors VIII (FVIII) and VII (FVIIc) coagulant activities were measured with a clotting assay using FVIII and FVII deficient plasmas (George King Biomedical, Overland Park, KS) [12]. A part ($\sim 1\%$) of the Factor VII in plasma circulates in an activated form (FVIIa). Factor VIIa activity was measured by a commercially available clotting assay (Diagnostica Stago Inc., Parsippany, NJ) using recombinant soluble TF. The FVIIc clotting assay measures the activity of both the zymogen and the activated forms of FVII. To assess thrombin generation thrombin-antithrombin (TAT) complexes were measured in plasma using ELISA (Enzygnost; Dade Behring, Marburg, Germany). These studies were performed after approval by our institutional human subjects review board and all subjects gave their informed consent. The data were analyzed using the two-way student t test.

Results

Circulating TF-PCA levels were increased over two fold in COPD (52.3 ± 5.6 U/ml, $n=11$, mean $\pm$ SE) compared to healthy subjects (20.7 ± 1.5 U/ml, $n = 45$, mean age 41 ± 2 y; 35 males, 10 females; $p<0.0001$) (Fig. 1). These TF-PCA levels in patients were higher even when

compared to control subjects >50 years age (28.2 ± 3.0 U/ml; $p < 0.001$; 55 ± 2 years, $n = 12$). We observed no relationship between age and TF-PCA in our control subjects. TAT levels were increased in COPD patients (2.99 ± 0.65 ug/l; controls: 1.31 ± 0.13 , $n = 53$, $p < 0.0001$), indicating enhanced thrombin generation (Fig. 1). Plasma FVIIa (the activated form of FVII) was higher in COPD patients (83 ± 11 mU/ml; controls, 64 ± 5 mU/ml, $n = 20$; $p = 0.09$) but did not reach statistical significance (Fig. 1). Plasma FVIIc (0.89 ± 0.09 U/ml, $n = 11$ vs 0.93 ± 0.03 U/ml, $n = 22$) and FVIII levels (COPD: 0.78 ± 0.06 vs. controls: 0.84 ± 0.04 U/ml, NS) were not different from those in control subjects. Membrane-bound TF binds to FVIIc and FVIIa and in our previous studies in healthy individuals subjected to combined hyperglycemia and hyperinsulinemia we observed a striking rise in TF-PCA with a decline in FVII [12]. We therefore assessed the relationship between TF-PCA and FVII in COPD patients. There was an inverse relationship between TF-PCA and FVIIa ($r = -0.80$, $p = 0.003$) (Fig. 1) and FVIIc ($r = -0.76$, $p = 0.007$). There was no relationship between TF-PCA and TAT levels ($r = 0.19$, $p = \text{NS}$).

Discussion

Tissue factor is present in circulating blood and hematopoietic-cell associated TF is important in thrombogenesis [6,7,9-11]. Elevated circulating TF has been reported in several disorders associated with enhanced thrombotic tendency, including patients with cardiovascular disease [13], sickle cell disease [10], hyperlipidemia [14], disseminated intravascular coagulation [15], and diabetes mellitus [12,14,16,17]. We have previously shown that TF-PCA levels are elevated by hyperglycemia and hyperinsulinemia in healthy subjects [12]. Our current study shows that patients with stable but moderate to severe COPD have elevated circulating TF-PCA.

Several lines of evidence indicate an important role of blood-borne TF in thrombus formation and the significance of our findings. Circulating TF is biologically active in converting factor X to Xa [9]. TF-bearing membrane microparticles are highly procoagulant and propagate further thrombus growth [6,9,11]. Even where thrombus initiation is mediated by vessel wall TF, the propagation of the initial thrombus is dependent on the recruitment of blood-borne TF [6,7,9,11]. Enhanced thrombus formation has been observed when blood from patients with diabetes mellitus and elevated circulating TF levels is perfused over a collagen-coated surface [14]. In patients undergoing angioplasty or stenting, elevated whole blood TF-PCA has predicted restenosis [13]. These observations suggest that the presence of elevated circulating TF-PCA may contribute to enhanced thrombotic tendency and cardiovascular events, which are a major cause of mortality in COPD. TF plays a role in venous thrombosis as well [18], and this is particularly relevant in the context of the high prevalence of pulmonary embolism in COPD patients [4]. Elevated leukocyte TF mRNA has been observed in patients with venous thromboembolism [19], and reduced plasma TF levels (due to TF gene polymorphisms) are associated with lower risk of venous thrombosis [20].

Thrombin-antithrombin complexes were elevated in our COPD patients suggesting an ongoing thrombin generation even in the quiescent stages of COPD.

Elevated plasma FVIIa has been linked to increase in cardiovascular events in some studies [21]. In COPD patients plasma FVIIa was higher but did not achieve statistical significance, possibly due to a small sample size. However, we found an inverse relationship between TF-PCA and both FVIIa and FVIIc levels. We attribute this to a clearance of FVIIa/FVIIc from plasma, secondary to binding of FVII to enhanced membrane-bound TF, its principal ligand. This inverse relationship is in line with similar findings in our studies in healthy subjects with hyperglycemia-hyperinsulinemia-induced elevation of TF-PCA [12].

Overall, our studies suggest that the elevated blood-borne TF-PCA in patients with stable but at least moderately severe COPD constitutes a prothrombotic and proinflammatory state and may contribute to the increased risk for vascular events, both arterial and venous, in COPD. Because TF is a proinflammatory molecule [8] it may also accentuate the ongoing systemic inflammation in COPD [22]. Further studies are warranted in COPD patients to understand the relationships between the coagulation changes, the inflammatory component, and predisposition to vascular events, as well as impact of therapeutic intervention with antithrombotic agents on vascular events.

Acknowledgments

The authors thank Jay Gunawardana for his assistance with the statistical analyses.

Funded in part by Pennsylvania Dept of Health RFA 02-07-20 (GC) and NIH R01 DK58895 (AKR).

References

1. Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med* 2000;343(4):269–80. [PubMed: 10911010]
2. Sin DD. Chronic obstructive pulmonary disease as a risk factor for cardiovascular morbidity and mortality. *Proc Am Thorac Soc* 2005;2:8–11. [PubMed: 16113462]
3. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352(16):1685–95. [PubMed: 15843671]
4. Tillie-Leblond I, Marquette CH, Perez T, Scherpereel A, Zanetti C, Tonnel AB, Remy-Jardin M. Pulmonary embolism in patients with unexplained exacerbation of chronic obstructive pulmonary disease: prevalence and risk factors. *Ann Intern Med* 2006;144(6):390–6. [PubMed: 16549851]
5. Curkendall SM, DeLuise C, Jones JK, Lanes S, Stang MR, Goehring E Jr, She D. Cardiovascular disease in patients with chronic obstructive pulmonary disease, Saskatchewan Canada cardiovascular disease in COPD patients. *Ann Epidemiol* 2006;16(1):63–70. [PubMed: 16039877]
6. Rauch U, Nemerson Y. Tissue factor, the blood, and the arterial wall. *Trends Cardiovasc Med* 2000;10(4):139–43. [PubMed: 11239792]
7. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol* 2004;24(6):1015–22. [PubMed: 15117736]
8. Bokarewa MI, Morrissey JH, Tarkowski A. Tissue factor as a proinflammatory agent. *Arthritis Res* 2002;4(3):190–5. [PubMed: 12010569]
9. Giesen PL, Rauch U, Bohrmann B, Kling D, Roque M, Fallon JT, Badimon JJ, Himber J, Riederer MA, Nemerson Y. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci U S A* 1999;96(5):2311–5. [PubMed: 10051638]
10. Key NS, Slungaard A, Dandele L, Nelson SC, Moertel C, Styles LA, Kuypers FA, Bach RR. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. *Blood* 1998;91(11):4216–23. [PubMed: 9596669]
11. Chou J, Mackman N, Merrill-Skoloff G, Pedersen B, Furie BC, Furie B. Hematopoietic cell-derived microparticle tissue factor contributes to fibrin formation during thrombus propagation. *Blood* 2004;104(10):3190–7. [PubMed: 15280200]
12. Vaidyula VR, Rao AK, Mozzoli M, Homko C, Cheung P, Boden G. Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. *Diabetes* 2006;55(1):202–8. [PubMed: 16380494]
13. Tutar E, Ozcan M, Kilickap M, Gulec S, Aras O, Pamir G, Oral D, Dandele L, Key NS. Elevated whole-blood tissue factor procoagulant activity as a marker of restenosis after percutaneous transluminal coronary angioplasty and stent implantation. *Circulation* 2003;108(13):1581–4. [PubMed: 12975255]
14. Sambola A, Osende J, Hathcock J, Degen M, Nemerson Y, Fuster V, Crandall J, Badimon JJ. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation* 2003;107(7):973–7. [PubMed: 12600909]

15. Asakura H, Kamikubo Y, Goto A, Shiratori Y, Yamazaki M, Jokaji H, Saito M, Uotani C, Kumabashiri I, Morishita E, Aoshima K, Nakamura S, Matsuda T. Role of tissue factor in disseminated intravascular coagulation. *Thromb Res* 1995;80(3):217–24. [PubMed: 8578548]
16. Abdel-Hafiz E, Vaidyula VR, Bagga S, London FS, Boden G, Rao AK. Elevated whole blood tissue factor procoagulant activity in diabetes mellitus. Vitamin E inhibits glucose induced tissue factor activity in vitro. *Blood* 2002;100:496a.
17. Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. *J Clin Endocrinol Metab* 2007;92(11):4352–8. [PubMed: 17785358]
18. Himer J, Wohlgensinger C, Roux S, Damico LA, Fallon JT, Kirchhofer D, Nemerson Y, Riederer MA. Inhibition of tissue factor limits the growth of venous thrombus in the rabbit. *J Thromb Haemost* 2003;1(5):889–95. [PubMed: 12871353]
19. Kamikura Y, Wada H, Nobori T, Kobayashi T, Sase T, Nishikawa M, Ishikura K, Yamada N, Abe Y, Nishioka J, Nakano T, Shiku H. Elevated levels of leukocyte tissue factor mRNA in patients with venous thromboembolism. *Thromb Res* 2005;116(4):307–12. [PubMed: 16038715]
20. Arnaud E, Barbalat V, Nicaud V, Cambien F, Evans A, Morrison C, Arveiler D, Luc G, Ruidavets JB, Emmerich J, Fiessinger JN, Aiach M. Polymorphisms in the 5' regulatory region of the tissue factor gene and the risk of myocardial infarction and venous thromboembolism: the ECTIM and PATHROS studies. *Etude Cas-Temoins de l'Infarctus du Myocarde. Paris Thrombosis case-control Study. Arterioscler Thromb Vasc Biol* 2000;20(3):892–8. [PubMed: 10712418]
21. Morrissey, JH.; Mutch, NJ. Tissue factor structure and function. In: Colman, RW.; Marder, VJ.; Clowes, AW.; George, JN.; Goldhaber, SZ., editors. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*. Lippincott Williams & Wilkins; Philadelphia: 2006.
22. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax* 2004;59(7): 574–80. [PubMed: 15223864]

Figure 1. Circulating TF-PCA (A), thrombin-antithrombin complexes (B) and plasma factor VIIa (C) in COPD patients and control subjects. Relationship between circulating TF-PCA and plasma levels of FVIIa (D).