Heart Vessels (2012) 27:295–299
DOI 10.1007/s00380-011-0142-4

ORIGINAL ARTICLE

Soluble CD40 ligand levels in acute pulmonary embolism: a prospective, randomized, controlled study

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Received: 19 January 2011 / Accepted: 25 March 2011 / Published online: 14 April 2011

Abstract CD40 ligand is a thromboinflammatory molecule that predicts cardiovascular events. Platelets constitute the major source of soluble CD40 ligands (sCD40L), which has been shown to influence platelet activation. The main aim of this study was to evaluate sCD40L levels in patients with acute pulmonary embolism (PE). Sixty-five PE patients (32 males, mean age 58 ± 12 years) and 29 healthy controls (15 males, mean age 56 ± 14 years) were enrolled in the study. sCD40L levels were evaluated at the enrollment by ELISA method. Multislice detected pulmonary computed tomography was performed on all patients with a suspected diagnosis of PE. In addition, echocardiography was performed to evaluate right ventricular (RV) dysfunction. There was no statistically significant difference between the two groups regarding demographic features. sCD40L levels were significantly higher in acute PE group compared to healthy controls (5.3 ng/ml and 1.4 ng/ml, respectively; $p < 0.001$). sCD40L levels of patients with and without RV dysfunction were similar. Correlation analysis between echocardiographic findings and sCD40L levels did not show significant difference. The present study demonstrated a role of sCD40L in pathogenesis of PE for the first time. Further studies are needed to clarify a predictive and prognostic value of sCD40L levels in acute PE patients.

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Keywords Pulmonary embolism · Soluble CD40 ligand · Thrombosis · Biomarker

Introduction

Acute pulmonary embolism (PE) is a life-threatening disorder and the overall mortality in patients with acute PE remains high despite of modern diagnostic and reperfusion strategies. Early diagnosis of PE is of utmost importance and there are several diagnostic tests (laboratory and imaging) to diagnose the disease. Although the pathogenesis of PE was well described, there is no ideal biomarker that can predict the first PE attack. On the other side, most of these biomarkers were identified in prognosis and prediction of atherothrombosis development. CD40 ligand (CD40L), a transmembrane protein structurally related to TNF-α, was originally identified on $CD4^+$ T cells, but has recently been found on activated platelet [1–3]. Recently, several studies have suggested that CD40–CD40 ligand interactions play an important role in pathogenesis of atherosclerosis, inflammation, and thrombus formation [4, 5]. Circulating soluble CD40L (sCD40L) is believed to derive predominantly from activated platelets and, hence, may reflect platelet activation [6]. High plasma concentrations of sCD40L were proposed to be associated with increased vascular risk [7–9]. There are several studies investigating the role of CD40L in various circumstances such as acute coronary syndromes [10], ischemic cerebrovascular accidents [11], peripheral artery diseases [12], heart failure [13, 14], and atrial fibrillation (AF) [15]. Pulmonary arterial hypertension (PAH) is a complex disorder and both thrombus activation and inflammation seem to play an important role in pathogenesis [16]. Damas et al. [17] found increased plasma levels of sCD40L in patients with

primary and secondary PAH compared to control subjects and suggested a role for sCD40L in the pathogenesis of PAH.

The role of CD/CD40L system in venous thrombus formation has not been investigated before and there are no reported studies in the literature. Theoretically, because of the procoagulant effect of sCD40L, it seems that this marker may play a role in the pathogenesis of venous thromboembolism (VTE). The main aim of the present study is to show the significance of the level of sCD40L in patients with acute PE and identify a relation with both demographic and echocardiographic features.

Materials and methods

Study population

This is a descriptive and cross-sectional study that was conducted in patients with acute PE and healthy volunteers. Between January 2010 and July 2010, details of all patients with newly diagnosed PE according to European Society of Cardiology guidelines [18] were recorded. Sixty-five PE patients (32 males, mean age 58 ± 12 years) and 29 healthy age-matched controls were included in this study. All patients gave their consent and oral agreement to participate in this study. The study was approved by the ethical committee of Selcuk University, Meram School of Medicine in December, 2009. Demographic features and comorbid conditions of the patients were also examined. The exclusion criteria were as follows: new onset of coronary heart disease (acute coronary syndrome), patients with congestive heart failure, history of valvular heart disease, cerebrovascular events, evidence of AF or paroxysmal AF history, and any known peripheral artery disease. All patients were followed-up during the hospital stay.

Diagnosis of pulmonary embolism

All patients in the suspected PE diagnosis underwent multislice detected pulmonary computed tomography (CT) (Siemens Somatom-Sensation 64 CT scanner) on admission to the emergency department. Two experienced radiologists interpreted the tomographic imaging. PE patients were divided into two groups according to the thrombus localization in the pulmonary artery: those in the main pulmonary artery and those in the segmental pulmonary arteries.

Echocardiography

In addition, transthoracic echocardiography was performed to evaluate right ventricular dysfunction. Examinations were performed with a Philips EnVisor C HD ultrasound machine (Royal Philips Electronics, Bothell, WA, USA) with a 2.5-MHz transducer. The LV and RV ejection fractions (EF) were assessed by the modified biplane Simpson method [19]. Cardiac dimensions were measured according to the recommendations of the American Society of Echocardiography (ASE) by M-mode and two-dimensional echo [19]. Assessment of pulmonary artery pressure (PAP) was provided from the tricuspid flow by Doppler echocardiography. Inferior vena cava (IVC) collapse index was measured by M-mode echocardiography from the subcostal view and the following equation was used to determine this index: [IVC (end-expiratory) − IVC (end-inspiratory)]/IVC (end-expiratory).

Blood sampling and collection protocol

Twenty-one patients of the control groups were biochemically analyzed because of insufficient kit samples. After diagnosing PE with CT, blood samples (5 cc) were drawn from the peripheral venous blood and collected to the tubes. The tubes were centrifuged immediately at 3,000 × g for 10 min. The separated serum samples were stored at −80°C. Serum sCD40L levels were quantified with eBioscience kits (BenderMed Systems, Austria) using a specific enzyme-linked immunosorbent assay (ELISA). Each serum sample was diluted 1:2 with sample diluent into the each well. The lower limit of detection for the sCD40L assay was 0.06 ng/ml, and the intra-assay and inter-assay coefficients of variation were 4.0 and 6.8%, respectively.

Statistical analysis

Normal distribution of data (parametric or non-parametric) was evaluated with Kolmogorov–Smirnov test. Comparison of parametric variables was performed with Student's t test. Mann–Whitney U test was utilized to compare non-parametric variables. Comparison of groups regarding demographic features and comorbidities were made by χ^2 test. The association of echocardiographic measurements with sCD40L levels was assessed by the Spearman rank correlation test. All values are reported as mean ± 1SD. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using the SPSS 15.0 statistical package (SPSS Inc., Chicago, IL, USA).

Power analysis was performed by using a Minitab 16 packet program. Sample volume was calculated as 25 for each group to determine the difference of 2 mg/dl with 80% power. However, one packet of commercial forms of ELISA kits for the CD40L included 96 kits and therefore, study sample was enlarged in both groups, especially in the PE group. At the end of study, the power was calculated as 92% with 95% confidence interval (CI) because of the enlarged sample size.

Table 1 Demographic and echocardiographic features of the study population

	PE, $n = 65$	Controls, $n = 29$	p value
Age (years)	58.4	55.8	NS
Gender (M/F) (*n*)	32/33	14/15	NS
ACS (%)	6.1	10.3	NS
HT (%)	27.6	31	NS
DM (%)	15.3	17.2	NS
Etiology and comorbidities			
Smoking (%)	21.5	17.2	NS
Malignancy (%)	13.8	3.4	NS
CVA (%)	9.2	10.3	NS
Pregnancy (%)	3	3.4	NS
Immobilization (%)	50.7	0	<0.01
Surgery (%)	27.6	2.4	<0.01
Echocardiographic measurements			
RV EF (%)	46.2	60.5	<0.01
RV stroke volume (ml)	24.3	25.2	NS
PAP (mmHg)	45.0	24.7	<0.01
IVC collapse index	0.36	0.62	<0.01
RV/LV ratio	0.84	0.64	<0.01

PE pulmonary embolism, *M* male, *F* female, *ACS* acute coronary syndrome, *HT* hypertension, *DM* diabetes mellitus, *CVA* cerebrovascular accident, *RV EF* right ventricle ejection fraction, *PAP* pulmonary arterial pressure, *IVC* inferior vena cava, *LV* left ventricle, *NS* non-significant

Main results

The baseline characteristics of the groups are listed in Table 1. There were no significant differences between groups regarding age, gender, and co-morbidity status. Immobilization and a history of surgery were significantly higher in PE group (Table 1).

Patients with PE showed significantly raised levels of sCD40L compared to healthy controls (5.3 ± 0.9 vs. 1.4 ± 0.5 ng/ml, $p < 0.01$) (Fig. 1). Evaluation of patients according to thrombus localization by MDCT showed non-significant difference between groups (Fig. 2). In addition, sCD40L levels of patients with and without right ventricular dysfunction were similar between groups (PE vs. controls; 5.9 ± 0.6 and 5.9 ± 0.4 ng/ml, $p = 0.98$, respectively) (Fig. 3) and there was no significant correlation between RV dysfunction and plasma sCD440L levels. Four patients died during the follow-up period in the hospital. The mean value of sCD40L of the patients who died in the hospital was 4.2 ± 0.8 ng/ml. There was no relation between in-hospital stay duration and sCD40L levels.

TTE evaluation of groups showed significantly impaired RV functions in PE group compared to controls. Especially RV ejection fraction (EF), pulmonary systolic arterial pressure (PAP), and RV/LV ratio were significantly higher in patients with PE compared to healthy volunteers (Table 1).

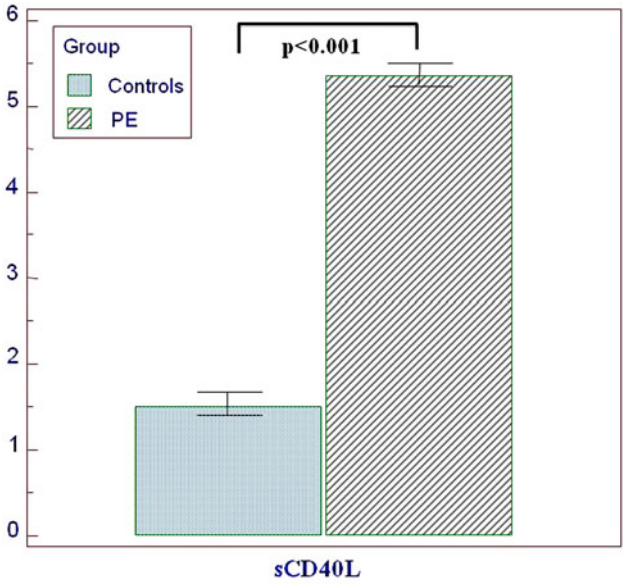

Fig. 1 Mean values of soluble CD40 ligand (sCD40L) in patients with pulmonary embolism (PE) and healthy controls

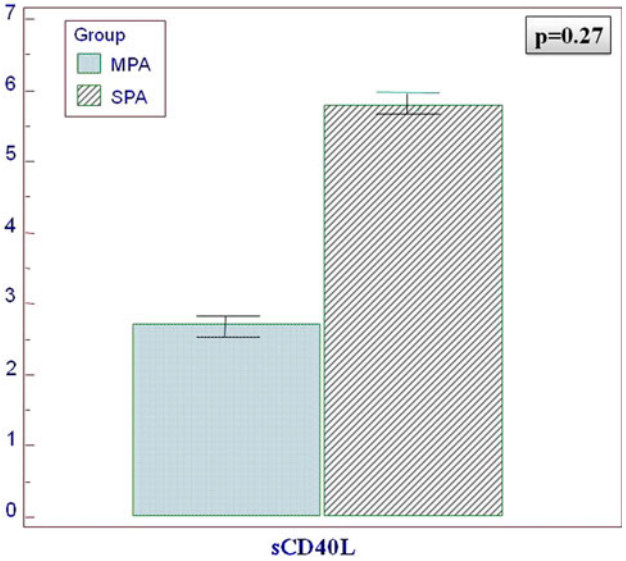

Fig. 2 The figure depicts a comparison of plasma sCD40L levels in patients with thrombus in the main pulmonary artery (MPA) and segmental or sub-segmental pulmonary arteries (SPA)

Discussion

To the best of our knowledge, this study is the first to provide important information regarding increased sCD40L levels in acute PE. There is only one study in the literature reported by Damas et al. [17] that showed a role

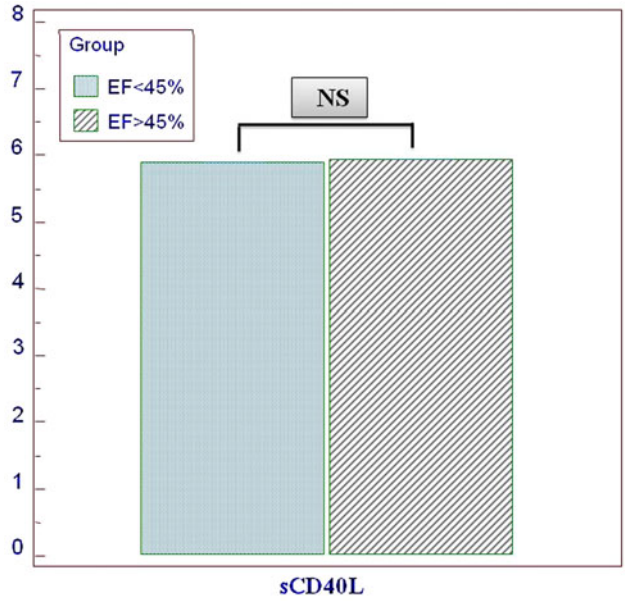

Fig. 3 The sCD40L levels of patients with and without right ventricular (RV) dysfunction were similar between groups. RV dysfunction was defined as ejection fraction (EF) under 45% (RVEF < 45%)

of sCD40L in the pathogenesis of thrombosis in patients with PAH. Their study population was mostly included primary and secondary PAH patients and only eight patients with chronic thromboembolic pulmonary hypertension (CTEP) were enrolled. They found that sCD40L was similar between CTEP and controls. However, in the present study, plasma levels of sCD40L were significantly higher in patients with acute PE compared to healthy controls. The main difference between our study and the study of Damas et al. [17] was the study population and the size of the study population. Our study population consisted of acute PE cases in contrast to the previous study. In addition, sCD40L was not associated with severity of disease and there was no significant correlation between echocardiographic parameters and plasma sCD40L levels.

Acute PE is a life-threatening cardiovascular condition. Despite the improvements in diagnosis and treatment of PE, the overall mortality of the disease still remains high, i.e., between 7 and 11% [20, 21]. Knowing the predisposing factors in acute PE and recognizing the pathogenesis of the disease becomes very important.

sCD40L as a marker taking part in inflammation process was also investigated in atherothrombotic procedures and in pathogenesis of arterial thrombus formation. Previous reports showed a role of sCD40L in different circumstances. Raised levels of sCD40L have been reported in patients with acute coronary syndromes [7, 22–24] and diabetes mellitus [25]. Cipollone et al. [8] found that increased plasma levels of sCD40L are associated with late restenosis after percutaneous transluminal coronary angioplasty. Besides, raised sCD40L levels were found in acute cerebral ischemia [11], peripheral artery disease [12], acute and chronic heart failure [13, 14], and pulmonary arterial hypertension [17]. Interestingly, plasma sCD40L may prospectively predict stroke in AF and provide a useful marker to identify patients at high thromboembolic risk with non-valvular AF [15].

There is a lack of evidence regarding the role of sCD40L in patients with VTE. Theoretically, sCD40L may play a role in pathogenesis of hypercoagulability, endothelial damage, and venous stasis. Possibly, sCD40L may explain the association between arterial and venous thrombosis. Although we may predict high-risk patients prior to VTE development, there is no cheap and simple non-invasive biomarker for predicting the recent group. In the present study, although sCD40L was higher in the PE groups, we could not demonstrate an association between the current biomarker and disease severity. Also, sCD40L was not correlated with thrombus localization. Similarly, sCD40L was not correlated with both conventional and tissue Doppler echocardiographic parameters. These findings suggest that sCD40L appears to be a part of physiopathologic procedures. Nevertheless, sCD40L is a factor taking a part in the coagulation process more than the result of the processes.

Limitations

The present study has some limitations. Because of the study design, most of the patients in the PE groups were hemodynamically stable and we could not examine patients at high risk due to poor clinical course. Because of the small study sample, interaction between sCD40L and success of pharmacologic therapy modality remains unclear. This issue is crucial, especially regarding the efficiency of heparin in PE patients with highly elevated sCD40L. This study may be accepted as a pilot study in the PE.

In conclusion, in the present study, we demonstrated the role of sCD40L in thrombosis in patients with acute PE. On the other hand, are the raised plasma levels of sCD40L a result of the thrombotic processes or a predictor of the recent process? This issue is still unclear and further randomized trials are needed.

References

1. Hollenbaugh D, Grosmaire LS, Kullas CD, Chalupny NJ, Braesch-Andersen S, Noelle RJ, Stamenkovic I, Ledbetter JA,

Aruffo A (1992) The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 11(12):4313–4321
2. Grewal IS, Flavell RA (1997) The CD40 ligand. At the center of the immune universe? Immunol Res 16(1):59–70
3. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, Kroczek RA (1998) CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 391(6667):591–594
4. Mach F, Schonbeck U, Libby P (1998) CD40 signaling in vascular cells: a key role in atherosclerosis? Atherosclerosis 137(Suppl): S89–S95
5. Phipps RP (2000) Atherosclerosis: the emerging role of inflammation and the CD40–CD40 ligand system. Proc Natl Acad Sci USA 97(13):6930–6932
6. Henn V, Steinbach S, Buchner K, Presek P, Kroczek RA (2001) The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. Blood 98(4):1047–1054
7. Aukrust P, Muller F, Ueland T, Berget T, Aaser E, Brunsvig A, Solum NO, Forfang K, Frøland SS, Gullestad L (1999) Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina. Possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. Circulation 100(6):614–620
8. Cipollone F, Ferri C, Desideri G, Paloscia L, Materazzo G, Mascellanti M, Fazia M, Iezzi A, Cuccurullo C, Pini B, Bucci M, Santucci A (2003) Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty. Circulation 108(22):2776–2782
9. Gokulakrishnan K, Deepa R, Mohan V, Gross MD (2006) Soluble P-selectin and CD40L levels in subjects with prediabetes, diabetes mellitus, and metabolic syndrome—the Chennai Urban Rural Epidemiology Study. Metabolism 55(2):237–242
10. Garlichs CD, Eskafi S, Raaz D, Schmidt A, Ludwig J, Herrmann M, Klinghammer L, Daniel WG, Schmeisser A (2001) Patients with acute coronary syndromes express enhanced CD40 ligand/CD154 on platelets. Heart 86(6):649–655
11. Garlichs CD, Kozina S, Fateh-Moghadam S, Handschu R, Tomandl B, Stumpf C, Eskafi S, Raaz D, Schmeisser A, Yilmaz A (2003) Upregulation of CD40–CD40 ligand (CD154) in patients with acute cerebral ischemia. Stroke 34(6):1412–1418
12. Blann AD, Tan KT, Tayebjee MH, Davagnanam I, Moss M, Lip GY (2005) Soluble CD40L in peripheral artery disease. Relationship with disease severity, platelet markers and the effects of angioplasty. Thromb Haemost 93(3):578–583
13. Stumpf C, Lehner C, Eskafi S, Raaz D, Yilmaz A, Ropers S, Schmeisser A, Ludwig J, Daniel WG, Garlichs CD (2003) Enhanced levels of CD154 (CD40 ligand) on platelets in patients with chronic heart failure. Eur J Heart Fail 5(5):629–637
14. Ueland T, Aukrust P, Yndestad A, Otterdal K, Froland SS, Dickstein K, Kjekshus J, Gullestad L, Damås JK (2005) Soluble CD40 ligand in acute and chronic heart failure. Eur Heart J 26(11):1101–1107
15. Duygu H, Barisik V, Kurt H, Turk U, Ercan E, Kose S (2008) Prognostic value of plasma soluble CD40 ligand in patients with chronic non-valvular atrial fibrillation. Europace 10(2):210–214
16. Tuder RM, Voelkel NF (1998) Pulmonary hypertension and inflammation. J Lab Clin Med 132(1):16–24
17. Damas JK, Otterdal K, Yndestad A, Aass H, Solum NO, Froland SS, Simonsen S, Aukrust P, Andreassen AK (2004) Soluble CD40 ligand in pulmonary arterial hypertension: possible pathogenic role of the interaction between platelets and endothelial cells. Circulation 110(8):999–1005
18. Torbicki A, Perrier A, Konstantinides S, Agnelli G, Galie N, Pruszczyk P, Bengel F, Brady AJ, Ferreira D, Janssens U, Klepetko W, Mayer E, Remy-Jardin M, Bassand JP, ESC Committee for Practice Guidelines (CPG) (2008) Guidelines on the diagnosis and management of acute pulmonary embolism: the Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur Heart J 29(18):2276–2315
19. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I (1989) Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 2(5):358–367
20. Stein PD, Kayali F, Olson RE (2004) Estimated case fatality rate of pulmonary embolism, 1979 to 1998. Am J Cardiol 93(9):1197–1199
21. Goldhaber SZ, Visani L, De Rosa M (1999) Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet 353(9162): 1386–1389
22. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML, CAPTURE Study Investigators (2003) Soluble CD40 ligand in acute coronary syndromes. N Engl J Med 348(12):1104–1111
23. Nannizzi-Alaimo L, Rubenstein MH, Alves VL, Leong GY, Phillips DR, Gold HK (2002) Cardiopulmonary bypass induces release of soluble CD40 ligand. Circulation 105(24):2849–2854
24. Setianto BY, Hartopo AB, Gharini PP, Anggrahini DW, Irawan B (2010) Circulating soluble CD40 ligand mediates the interaction between neutrophils and platelets in acute coronary syndrome. Heart Vessels 25(4):282–287
25. Marx N, Imhof A, Froehlich J, Siam L, Ittner J, Wierse G, Schmidt A, Maerz W, Hombach V, Koenig W (2003) Effect of rosiglitazone treatment on soluble CD40L in patients with type 2 diabetes and coronary artery disease. Circulation 107(15):1954–1957