Research Paper

Increased Expressions of Integrin Subunit β1, β2 and β3 in Patients with Acute Infection

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Abstract

Objective: Our previous studies have shown that integrin subunits β1, β2 and β3 were the core proteins of venous thrombi and potential useful biomarker of venous thromboembolism (VTE). Patients with acute infection have a high risk of VTE. In this study we explored that is there any relevance between core proteins and acute infection.

Methods: A total of 230 patients (112 females) with clinically proven acute infection in the emergency unit were recruited into this study, meanwhile 230 patients without acute infection matched in sex and age were recruited as control group. Flow cytometry was done to measure the expressions of blood integrin β1, β2, β3 and cellular immunity (CD3, CD4, CD8, CD4/CD8, CD16CD56 and CD19). The association degree between increased core proteins and acute infection was analyzed by calculating the relative risk (RR).

Results: The expression of integrin β1, β2 and β3 was markedly increased in patients with acute infection (P=0.000, 0.000 and 0.015, respectively). The relative risk ratio (RR) of increased integrin β1, β2 and β3 in acute infection patients was 1.424 (95%CI: 1.156-1.755, P=0.001), 1.535 (95%CI: 1.263-1.865, P=0.000) and 1.20 (95%CI: 0.947-1.521, P=0.148), respectively. Combined integrin β1, β2 and β3 analysis showed that the relative risk ratio (RR) of increased in patients with acute infection was 2.962 (95%CI: 1.621-5.410, P=0.001), and this relative risk (RR) rise to 3.176 (95%CI: 1.730-5.829, P=0.000) in patients with respiratory tract infection (RTI).

Conclusion: As the core proteins of venous thrombi, integrinβ1, β2 and β3 were markedly increased expression in patients with acute infection, which maybe explain the increased risk of VTE in acute infection patients. A weakened immune system could be the basic condition of VTE occurrence.

Key words: core protein, integrinβ1, integrinβ2, integrinβ3, venous thromboembolism, acute infection

Introduction

Venous thromboembolism (VTE) is a common disease, including pulmonary embolism (PE) and deep venous thrombosis (DVT). PE has become a global medical health care problem due to the high morbidity, mortality and misdiagnosis rate [1, 2]. Guideline of the American College of Chest Physi-

cians has put forward various risk factors of acquired VTE, including surgery, trauma, infection, tumor, aging, pregnancy, long-bedding and immobilization, etc. [3]. Acute infection is commonly faced in clinical practice, and there is a 2-3 times increased incidence of VTE in patients with community-acquired or hos-

pital-acquired infection [4-6].

Acute venous thrombosis is red thrombus, which is composed of red blood cells, platelets, white blood cells and plasma proteins [7]. In 2011, we reported that the main component of red thrombus in acute PE patients was fibrinogen, rather than fibrin, with only a small quantity of cellular cytoskeletal and plasma proteins [8]. Fibrinogenic thrombus is dissolvable, which can explain why delayed thrombolytic therapy is effective for acute and subacute VTE and thrombi are autolytic in some VTE patients. However, the action mechanism of fibrinogen in thrombosis remains unclear. We hypothesized that, due to the binding of fibrinogens (ligands) and activated receptors on surfaces of various leukocytes, platelets and lymphocytes, the thrombus protein network is constructed and red thrombus forms, with erythrocytes and plasma components filled in the spaces. In our previous studies [7, 9], genomics analysis, proteomics analysis and bioinformatics analysis of acute venous thrombi of PE patients confirmed that integrin β1, β2 and β3 were the core proteins of acute venous thrombi. Activated integrin β3 was involved in the accumulation of platelet, activated integrin β2 and β3 bound to fibrinogens and the biofilter-like grid structure of thrombi formed [7]. When this structure was fully filled with red blood cells, red thrombus formed.

Integrins are cell adhesion receptors, they play important roles in interaction between cell and extracellular matrix, and in cell-cell interactions [10]. Integrins are heterodimers consisting of non-covalently linked α and β transmembrane glycoprotein subunits. They consist of at least 18 α and 8 β subunits, producing 24 different heterodimers [11]. β1 subunit is expressed mainly on surface of lymphocytes. β2 subunit is distributed on surfaces of neutrophils and monocytes. β3 subunit is observed on platelets.

Integrinβ1, β2 and β3 subunits are core proteins and potential biomarkers of VTE [12]. Acute infection is a common risk factor of VTE. Is there any relevance between core proteins of acute venous thrombi-- integrin β1, β2 and β3 and acute infection? To answer the question, we catched a case-control study, the differential expression of integrin β1 and β2 and β3 was compared between acute infection group and non-infection group, the relative risk of increased expression of integrin β1 and β2 and β3 in acute infection was acquired, and their clinical importance was also investigated.

Materials and methods

Study population

A total of 230 inpatients with acute infection diagnosed from April 2011 to April 2012 in the emergency unit were recruited into this study, including 118 males and 112 females, aged 23-93 years, with a mean age of 72.53 years old. The classification of acute infection was according to previously reported[13], including 197 cases of respiratory tract infections (pneumonia and bronchitis), 19 cases of urinary tract infection, 19 cases of skin and soft tissue infection, 7 cases of abdominal infection (liver and gallbladder and gastrointestinal tract) and 8 cases of sepsis without clear foci. Among them, 18 cases were complicated with two kinds of infections. All infected patients were diagnosed in our hospital. Meanwhile, 230 age and gender matched inpatients without infection served as control group, including 114 males and 116 females, aged 21-98 years (mean 70.31 years). Patients with cancer, autoimmune disease or patients taking immunosuppressive drugs were excluded. Patients with clinical symptomatic thrombus were also excluded. This study was approved by the Ethics Committee of Affiliated Tongji Hospital of Tongji University, and informed consent was obtained before study.

Blood collection and measurements

Detailed clinical data were collected from each acute infection patient and control patient on admission. Blood routine test, hsCRP and D-Dimer were detected. HsCRP was detected by immune scatter turbidimetry, using Siemens BNII specific protein and auxiliary reagent. D-Dimer was detected by Latex enhanced immune turbidimetric turbidity method, using SYSMEX CA1500 automatic blood coagulation analyzer. Fasting venous blood (2 ml) was collected from the cubical vein in the morning and anti-coagulated with EDTA. After mixing, flow cytometry was done within two hours.

Monoclonal antibodies against integrin β1 (CD29), β2 (CD18) and β3 (CD61) (BD company) were used to detect the integrin β1, β2 and β3, respectively. Integrin β1 and integrin β2 were tagged by IgG1-PE, and integrin β3 was tagged by IgG2-PE. Three tag monoclonal antibodies (BECKMAN-COULTER) were used for CD3, CD4 and CD8 detection (PC5 labeled for CD3, FITC labeled for CD4, and PE labeled for CD8). In brief, 100 µl of EDTA treated blood was added to each tube. Then, 20 µl of mouse IgG1-PC5, IgG1-FITC or IgG1-PE was added (20 µl of IgG2-PE was mixed with CD29), followed by addition of corresponding fluorescence antibodies (20 µl). Following vortexing, incubation was done in dark for 30 min at room temperature. Then, 500 µl of hemolysin (BECKMAN-COULTER) was added, followed by incubation at 37°C for 30 min. Following washing, 500 µl of sheath fluid was added to each tube, and then de-

tected by flow cytometry (EPICS XL-4; BECKMAN-COULTER). The PMT voltage, fluorescence compensation and sensitivity of standard fluorescent microspheres (EPICS XL-4; BECKMAN-COULTER) were used to adjust the flow cytometer and a total of 10000 cells were counted for each tube. The corresponding cell population in the scatterplot of isotype controls was used to set the gate, and the proportion of positive cells was determined in each quadrant (%). SYSTEM-II software was used to process the data obtained after flow cytometry.

Statistical analysis

SPSS18.0 statistical software was used for statistical analysis. Normality test was performed for all measurement data using the Kolmogorov-Smirnov test, with $P > 0.05$ as normal distribution. Data of normal distribution were expressed as means $\pm$ SD and were compared with student's t-test between groups. Corrected t-test was applied when heterogeneity of variance. Non-normal data were expressed as median P_{50} and interquartile range (P_{25}-P_{75}), and group comparison was analyzed using nonparametric test (Mann-Whitney U test). Categorical data were compared using chi-square test. The association degree between two categorical variables was analyzed by calculating the relative risk (RR). $P < 0.05$ was considered statistically significant for all tests.

Results

Patients' characteristics

A total of 230 patients with acute infection and 230 patients without acute infection matched in age and sex were enrolled into this study. Among 230 patients with acute infection, 197(85.7%) were diagnosed with respiratory tract infections (RTI), 19(8.3%) were diagnosed with urinary tract infection (UTI), 19(8.3%) were diagnosed with skin infection, 7(3.0%) were diagnosed with intra-abdominal infection and 8(3.5%) were diagnosed with septicaemia. Patients' demographics, type of infection and comorbidities are shown in **Table 1**.

Elevated plasma D-Dimer and hsCRP levels in patients with acute infection

The median levels of D-Dimer and hsCRP were all significantly higher in patients with acute infection when compared with patients without acute infection (P=0.000 and 0.000) (**Table 2**). There was also significant difference between RTI patients and the controls (P=0.000 and 0.000) (**Table 3**).

Disordered cellular immunity in patients with acute infection

Among CD3, CD4, CD8, CD4/CD8, CD16CD56

and CD19 levels, significant differences of CD16CD56 and CD19 were found between patients with acute infection (all acute infection P=0.008, P=0.018; RTI P=0.004, P=0.013) and the controls. CD16CD56 markedly increased in acute infection patients, while CD19 reduced (**Table 2, Table 3**).

Table 1. The baseline characteristics of 230 patients with acute infection and controls

	Acute infection (%) N=230	Controls (%) N=230	P value
Mean age (SD)	72.53(16.81)	70.31(12.61)	0.110
Gender, male	118(51.3)	114(49.6)	0.780
Acute infection			
respiratory tract infection (RTI)	197(85.7)		
urinary tract infection (UTI)	19(8.3)		
skin infection	19(8.3)		
intra-abdominal infection	7(3.0)		
septicaemia	8(3.5)		
Comorbidities			
CAD	104(51.2)	114(49.6)	0.349
hypertension	102(44.3)	84(41.4)	0.106
CI	63(27.4)	53(23.0)	0.284
DM	48(20.9)	42(18.3)	0.557
COPD	34(14.8)	22(9.6)	0.116

Note: Ages are shown with mean (SD); categorical data are shown with the number and percentage of the sample group. Ages were compared by student's t test. The frequency of categorical data was compared with the chi-square test. CAD, coronary artery disease; CI, cerebrovascular infarction; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease.

Table 2. Expression of cellular immunity, hsCRP and D-Dimer in patients with acute infection and controls

	Acute infection N=230	Controls N=230	P value
CD3 (%)	63.46 (12.28)	64.93 (12.40)	0.203
CD4 (%)	36.82 (11.55)	37.29 (10.96)	0.654
CD8 (%)	23.02 (9.01)	22.16 (8.11)	0.287
CD4CD8 (%)	1.80 (1.10-2.70)	1.80 (1.40-2.70)	0.376
CD16CD56 (%)	14.95 (9.18-20.68)	9.90 (5.48-17.20)	0.008
CD19 (%)	7.8 (4.20-11.33)	10.1 (6.33-15.23)	0.018
D-Dimer (mg/l)	0.28 (0.11-0.49)	0.09 (0.05-0.25)	0.000
hsCRP (mg/l)	28.85 (10.70-55.80)	3.10 (0.98-14.70)	0.000

Note: CD3, CD4 and CD8 were shown with mean (SD) and compared by student's t test. CD4/CD8, CD16CD56, CD19, D-Dimer and hsCRP were shown with median (p25th-p75th) and compared by Mann–Whitney U test.

Table 3. Expression of cellular immunity, hsCRP and D-Dimer in patients with RTI and controls

	RTI N=197	Controls N=230	P value
CD3 (%)	63.87 (11.42)	64.93 (12.40)	0.362
CD4 (%)	36.99 (11.11)	37.29 (10.96)	0.781
CD8 (%)	23.38 (9.18)	22.16 (8.11)	0.148
CD4CD8 (%)	1.70 (1.10-2.40)	1.80 (1.40-2.70)	0.311
CD16CD56 (%)	13.10 (9.10-19.00)	9.90 (5.48-17.20)	0.004
CD19 (%)	8.30 (4.99-12.40)	10.1 (6.33-15.23)	0.013
D-Dimer (mg/l)	0.29 (0.12-0.55)	0.09 (0.05-0.25)	0.000
hsCRP (mg/l)	27.45 (9.63-55.73)	3.10 (0.98-14.70)	0.000

Note: CD3, CD4 and CD8 were shown with mean (SD) and compared by student's t test. CD4/CD8, CD16CD56, CD19, D-Dimer and hsCRP were shown with median (p25th-p75th) and compared by Mann–Whitney U test. RTI, respiratory tract infection

Increased relative risk of integrins expression in patients with acute infection

When compared with the control group, the expression of integrin β1, β2 and β3 markedly increased in the acute infection group (P=0.000, 0.000 and 0.015, respectively) (**Table 4**). The relative risk ratio (RR) of increased integrin β1, β2 and β3 in acute infection patients was 1.424 (95%CI: 1.156-1.755, P=0.001), 1.535 (95%CI: 1.263-1.865, P=0.000) and 1.20 (95%CI: 0.947-1.521, P=0.148), respectively (**Table 6**). Combined integrin β1, β2 and β3 analysis showed (integrin β1, β2 and β3 increased at the same time means rise, otherwise normal) the relative risk ratio (RR) of increased in acute infection patients was 2.962 (95%CI: 1.621-5.410, P=0.001) (**Table 6**).

Table 4. Expression of integrin β1, β2 and β3 in patients with acute infection and controls

	Acute infection (%)	Controls (%)	P value
	N=230	N=230	
integrin β1	10.60(7.60-15.50)	8.80(6.50-11.85)	0.000
integrin β2	92.00(88.40-96.40)	90.40(86.70-93.85)	0.000
integrin β3	9.60(7.60-12.30)	9.00(7.45-11.05)	0.015

Note: Integrin β1, β2 and β3 were shown with median (p25th-p75th) and compared by Mann-Whitney U test.

Table 5. Expression of integrin β1, β2 and β3 in patients with RTI and controls

	RTI (%)	Controls (%)	P value
	N=197	N=230	
integrin β1	10.70(7.80-15.60)	8.80(6.50-12.00)	0.000
integrin β2	92.00(88.40-96.50)	90.40(86.75-94.15)	0.000
integrin β3	9.70(7.60-12.40)	9.10(7.50-10.85)	0.013

Note: Integrin β1, β2 and β3 were shown with median (p25th-p75th) and compared by Mann-Whitney U test.

Table 6. Relative risk of increased expression of integrin β1, β2 and β3 in patients with acute infection

	Acute infection	Controls	RR	95%CI	P value
	above/ normal	above/ normal			
integrinβ1	120/108	85/145	1.424	1.156-1.755	0.001
integrinβ2	133/90	89/140	1.535	1.263-1.865	0.000
integrinβ3	94/134	79/151	1.20	0.947-1.521	0.148
Combination of integrinβ1,β2 and β3	38/189	13/217	2.962	1.621-5.410	0.000

When compared with the controls, the expression of integrin β1, β2 and β3 also markedly increased in the RTI group (P=0.000, 0.000 and 0.013, respectively) (**Table 5**). The relative risk ratio (RR) of increased integrin β1, β2 and β3 in patients with RTI was 1.457 (95%CI: 1.177-1.803, P=0.001), 1.563 (95%CI: 1.281-1.906, P=0.000) and 1.254 (95%CI: 0.986-1.596, P=0.072), respectively (**Table 7**). Combined integrin

β1, β2 and β3 analysis showed (integrin β1, β2 and β3 increased at the same time means rise, otherwise normal) the relative risk ratio (RR) of increased in patients with RTI was 3.176 (95%CI: 1.730-5.829, P=0.000) (**Table 7**).

Table 7. Relative risk of increased expression of integrin β1, β2 and β3 in patients with RTI

	RTI	Controls	RR	95%CI	P value
	above/normal	above/normal			
integrinβ1	105/90	85/145	1.457	1.177-1.803	0.001
integrinβ2	116/75	89/140	1.563	1.281-1.906	0.000
integrinβ3	84/111	79/151	1.254	0.986-1.596	0.072
Combination of integrinβ1,β2 and β3	35/160	13/217	3.176	1.730-5.829	0.000

Note: RTI, respiratory tract infection

Discussion

Acute infection and the associated systemic inflammation may increase the risk of VTE [14, 15], but the elaborate mechanism is not clear. Our previous study [16] has showed that symptomatic venous thromboembolism is a disease related to infection and immune dysfunction. This study we found that integrin β1, β2 and β3 markedly increased in patients with acute infection. The relative risk (RR) of increased integrin β1, β2 and β3 in acute infection patients was 1.424, 1.535 and 1.20 respectively. Combined integrin β1, β2 and β3 analysis showed the relative risk (RR) of increased in acute infection patients was 2.962. While considered respiratory tract infection (RTI) alone, the relative risk rises to 3.176. Integrin β1, β2 and β3 subunits are core proteins and potential biomarkers of VTE in our previous studies [7, 9, 12]. The results in this study maybe explain the increased risk of VTE in acute infection patients.

Acute infection is a risk factor of thrombotic diseases [17-20]. In 2006, Smith et al reported [4] that the risk for DVT increased by 1.91 folds within 2 weeks to 6 months after acute respiratory tract infection. Similar finding was also noted in patients after urinary infection. Recently, in two large case-control studies [5,6], results also demonstrated that acute infection increased the risk for VTE by 2~3 folds after adjustment of other risk factors of VTE, and this risk was the highest within 2 weeks after acute infection. Infections may induce thromboembolism by a number of mechanisms, while increased activity of inflammation during acute infection may be the key determination.

In a recent clinical guideline on VTE prophylaxis in hospitalized medical patients, the American College of Physicians stated that a decision to initiate prophylactic heparin therapy should be based on an

individualized assessment of the risk for VTE and bleeding, and that current evidence does not support the use of any specific VTE risk assessment tool [21]. Our study indicate that it might be advantageous to include new plasma markers—integrin β1, β2 and β3 subunits in any future VTE risk assessment for use in medical inpatients.

In addition, our results revealed the acute infection patients had a tendency in disorder cellular immunity. Our previous studies [22, 23] also showed VTE patients had association with compromised cellular immunity. These findings suggest acute infection patients with compromised cellular immunity have an increased risk for VTE. A weakened immune system could be the basic condition of VTE occurrence. When immune system cannot timely and effectively remove intravenous antigen of heterotypic cells, platelets and white blood cells activated and bound to fibrinogens to form the biofilter-like grid structure of thrombi in which red blood cells filled, forming red thrombi. The disease process was from the body's defense to venous thrombosis. We speculates that in immunocompromised conditions, intravenous cytokines or toxins may activate β subunit configuration change, combine with ligand--fibrinogen. Chemokines attract neutrophils and monocytes to participate in the local inflammatory response. Further research on precise mechanisms need to be done.

A limitation of our study is that our sample size is relatively small. In all patients with acute infection, respiratory tract infection accounts for most cases. While patients with urinary tract infection and skin infection were less included in the group, we couldn't find significant differences between these two kinds of infection and controls. Another limitation of this study is that we haven't got microbial verification in all patients with infection, since there were studies showed that Gram-positive bacteria including S. aureus may have an exceptionally high propensity for inducing thrombosis [24, 25]. A study includes a large sample size and microbiological verification of infection should be done in future to further validate our conclusion.

Acknowledgements

Conflict of interest

None.

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