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Decreased ADAMTS-13 level is related to inflammation factors and risk stratification of acute lymphoblastic leukemia patients

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Abstract

As a kind of metalloprotease of the ADAMTS family, ADAMTS-13 is crucial for maintaining the normal size of von Willebrand factor. Reduced ADAMTS-13 had been reported in patients with both localized and disseminated malignancies. However, the expression and potential role of ADAMTS-13 in hematological malignancies remain unclear. In this research, we measured and compared ADAMTS-13 levels in plasma of 35 acute lymphoblastic leukemia (ALL) patients and 30 healthy controls and found that ALL patients possessed lower level of ADAMTS-13 than controls. Correlations between ADAMTS-13 and inflammation factors were calculated and ADAMTS-13 was negatively correlated with C-reactive protein and interleukin-1β. ALL patients with infections had lower level of ADAMTS-13 than patients without infections. In addition, high-risk ALL patients possessed lower ADAMTS-13 than patients at low risk. To conclude, ADAMTS-13 level is decreased in the plasma of ALL patients and the level of ADAMTS-13 is related to plasma inflammation factors and risk stratification of ALL patients, which could contribute to better understanding of the clinical significance of ADAMTS-13.

Abbreviations: ADAMTS = A Disintegrin and Metalloproteinase with Thrombospondin motifs, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, CRP = C-reactive protein, FAB = French-American-British subtypes, IL = interleukin, ISTH = International Society on Thrombosis and Haemostasis, TNF = tumor necrosis factor; TTP = thrombotic thrombocytopenic purpura, vWF = von Willebrand factor.

Keywords: ADAMTS-13, ALL, CRP, IL-1β, inflammation, risk stratification

1. Introduction

von Willebrand factor (vWF) is 1 kind of circulating glycoprotein in the blood mediating the adhesion of platelets to damaged blood vessels.[1,2] Mature vWF is a multimeric protein, which is cleaved by ADAMTS-13 (vWF-cleaving protease), a metalloprotease of the ADAMTS family.[3] ADAMTS-13 is crucial for maintaining the normal size distribution of vWF multimers. Large vWF multimers accumulate in the absence of ADAMTS-13 and elevated large vWF multimer concentration result in excessive platelet aggregation.[4,5] As reported, low ADAMTS-13 activity (<5% that in normal plasma) is regarded as the cause of thrombotic thrombocytopenic purpura (TTP).[4–6]

Reduced ADAMTS-13 is not only happened in TTP, but also in metastasizing malignancies.[7–9] Oleksowicz et al[7] had reported ADAMTS-13 deficiency in patients with disseminated malignancies. In addition, patients with both localized and disseminated malignancies were reported to have reduced levels of ADAMTS-13.[9] Mild ADAMTS-13 deficiency has been reported in a variety of advanced malignancies and colon cancer patients.[10] As a major form of hematologic malignancy, acute lymphoblastic leukemia (ALL), including leukemia of both T and B lineages, was characterized by overproduction and accumulation of lymphoblasts.[11] However, the knowledge of expression and potential role of ADAMTS-13 in ALL is still limited and few research had been reported.

In this research, we aimed to explore the expression of ADAMTS-13 in plasma of ALL patients and inquire into the relationship between inflammation factors and ADAMTS-13. We would like to clarify the significance of ADAMTS-13 in the pathogenesis process of ALL.

2. Materials and methods

2.1. Patients

35 newly diagnosed ALL patients who were admitted to the hospital from September 2014 to September 2015 were enrolled in our research, as determined by the criteria for the classification of acute leukemia.[12] Blood samples for coagulation analysis were collected and the remaining plasma was used. ADAMTS-13, C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)α, and IL-1β levels were measured. All these patients were stratified for risk stratification with reference to the NCCN guideline.[13] Patients with infections were recorded according to

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the diagnosis by physicians, and infection types included soft tissue infection, perianal infection, upper respiratory tract infection, and pulmonary infection. 30 healthy adults who had taken physical examinations were enrolled in the control group, and their remaining plasma was collected in order to measure ADAMTS-13 levels. The research had been performed in accordance with the ethical standards of Declaration of Helsinki and all selected individuals had given written informed consent. All these patients were without irrelevant complications, chronic diseases, medication intake, or thromboembolic events. The procedures had been approved by the ethics committee of Peking University People's Hospital.

2.2. Blood sampling and analysis of ADAMTS-13 activity

A citrated plasma sample is assayed for measuring ADAMTS-13 level, using recombinant vWF86-ALEXA FRET substrate. Standard curve was constructed using normal plasma with a known concentration of ADAMTS-13. The ADAMTS-13 levels in plasma were determined by interpolating the change in fluorescence from the standard curve. The kit was purchased from SEKISUI Diagnostics, LLC (Stamford, CT).

2.3. ELISA

IL-6, IL-1β, and TNFα concentrations in plasma of the patients were measured using ELISA kits from Biolegend (San Diego, CA), according to the manufacturer's instruction. Each sample was assayed in triplicates. The concentrations were calculated using standard curves.

2.4. Clinical indicator analysis

Activated partial thromboplastin time, prothrombin time, and vWF in plasma were measured using ACL TOP700 (Instrumentation Laboratory, Bedford, MA) with regent from Instrumentation Laboratory (Lexington, MA). Platelet counts were measured using Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan) with blood collected in Vacutainer K3-EDTA tubes (Becton Dickinson, Franklin Lakes, NJ). CRP was measured using a fluorescence-based immunochromatographic method, namely an i-CHROMA hs-CRP assay (Boditech Med Inc, Gangwondo, Korea).

2.5. Statistics

All analyses were carried out with GraphPad Prime 5.5 software. All data are expressed as mean ± standard deviation unless otherwise stated. Student *t* test and χ^2 statistics (Fisher exact test) were used to evaluate differences between groups. Multivariate analyses were carried out using SPSS software with *F* test to evaluate the factors influencing ADAMTS-13 levels. The Pearson correlation coefficients (*r*) were estimated between ADAMTS-13 and inflammation factor levels. All of the statistical tests were 2-tailed. A *P* value <0.05 was considered to be statistically significant.

3. Results

3.1. Reduced ADAMTS-13 levels in the plasma of ALL patients

35 ALL patients were enrolled in this research and the control group included 30 healthy adults. The demographic and clinical characteristics of both groups are shown in Table 1. ADAMTS-13 level of each individual was measured and the comparison between ALL patients and healthy controls is shown in Fig. 1A. We found that ALL patients possessed lower level of ADAMTS-13 than healthy controls. As the substrate of ADAMTS-13, vWF in plasma was also analyzed, the activity of vWF in plasma of ALL patients was increased compared with controls (Table 1). Correlation analysis between ADAMTS-13 level and vWF activity was conducted and there was significant inverse correlation, as shown in Fig. 1B.

Table 1

Summary of the clinical characteristics of the patients and HCs.

	ALL	HC	
Number	35	30	ns
Gender			
M	21	18	ns
F	14	12	
Age	29.63 ± 12.80	29.97 ± 10.71	ns
ADAMTS-13	432.5 ± 97.3	577.4 ± 86.1	***
<481	24	1	***
≥481	11	29	
Lineage			
T	10/35		
B	25/35		
FAB classification			
L1	11/35		
L2	24/35		
APTT	33.14 ± 8.69	31.31 ± 2.91	ns
PT	13.08 ± 4.77	11.17 ± 0.94	ns
PLT	48.76 ± 47.21	183.80 ± 45.65	***
vWF	119.2 ± 21.2	104.2 ± 11.6	**

Values are given as means ± standard deviations.

ALL = acute lymphatic leukemia, APTT = activated partial thromboplastin time, F = female, FAB = French-American-British subtypes, HC = healthy control, M = male, ns = not significant, PLT = platelets, PT = prothrombin time, vWF = von Willebrand factor.

** $P<0.01$.

*** $P<0.001$.

We then studied the level of ADAMTS-13 in ALL patients of different lineages. ADAMTS-13 levels were compared between ALL patients of T or B lineage, which is shown in Fig. 1C. We found that ADAMTS-13 levels in both lineages were with no difference but both were significantly lower than healthy controls. ALL patients with different French-American-British subtypes (FAB) classifications were also analyzed and there was no difference between L1 and L2 ALL patients, but both L1 and L2 patients had lower ADAMTS-13 than healthy controls (Fig. 1D).

Multivariate analyses of variance were used to determine whether the differences in ADAMTS-13 levels among ALL patients are caused by the factors of gender, age, FAB classification, and/or lineage. As shown in Table 2, the *P* values of multivariate analyses for these factors were not <0.05, meaning that they do not significantly influence ADAMTS-13 levels.

3.2. ADAMTS-13 levels negatively correlate with CRP and IL-1β in plasma of ALL patients

ADAMTS-13 activity was found to be reduced in patients with acute systemic inflammation,[14] suggesting a possible relationship between inflammation and ADAMTS-13 deficiency. To clarify the relationship between ADAMTS-13 level and inflammation among ALL patients, we measured levels of inflammation factors in

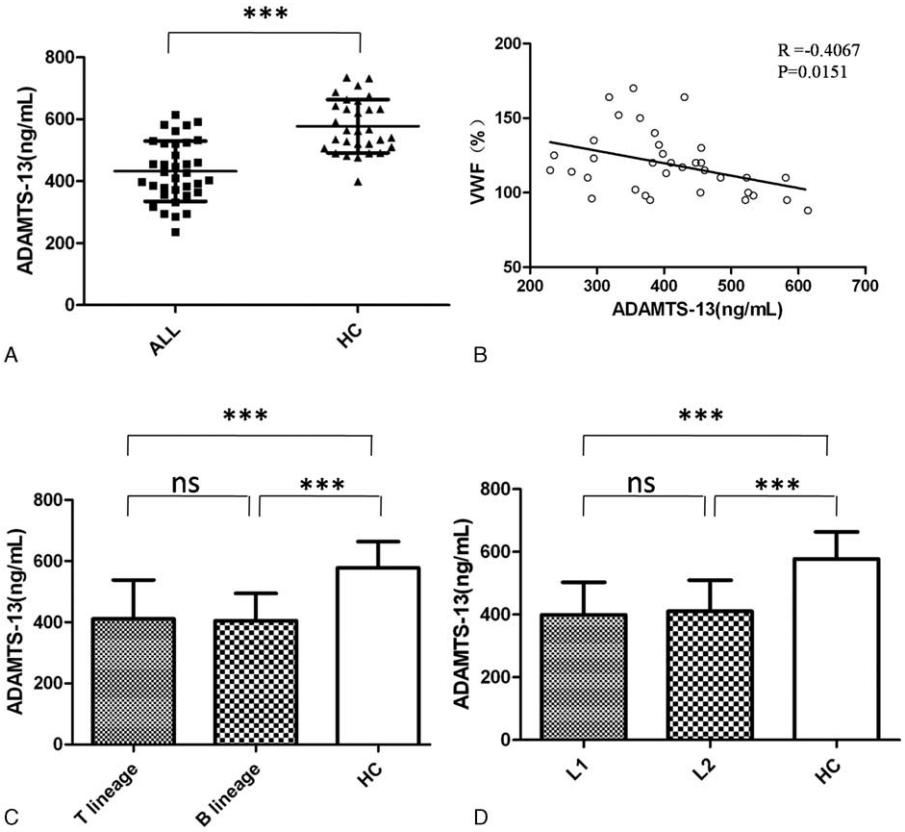

Figure 1. Level of ADAMTS-13 in ALL patients compared with HCs. (A) ADAMTS-13 levels in plasma of ALL patients and HCs were measured and compared. (B) Correlation analysis was conducted between ADAMTS-13 and vWF levels in ALL patients. Scatter plots with linear fit are shown and Pearson *r* and *P* values are listed. (C) The levels of ADAMTS-13 in ALL patients from T and B lineage were analyzed and compared with HCs. (D) The levels of ADAMTS-13 in different FAB classification of ALL patients were analyzed and compared with HCs. Data are shown as means ± standard deviations for A and C, D, and Student *t* test was conducted to compare 2 groups. ALL = acute lymphoblastic leukemia, FAB = French-American-British subtypes, HC = healthy control, ns = not significant, vWF = von Willebrand factor. ***$P < 0.001$.

plasma of ALL patients. CRP, IL-6, TNFα, and IL-1β were measured and correlation analysis was conducted with ADAMTS-13. The results are shown in Fig. 2. Interestingly, we found that ADAMTS-13 was inversely correlated with CRP and IL-1β ($r < 0$) and both the correlations were significant ($P < 0.05$). For IL-6 and TNFα, however, the correlations were not significant. These data suggested that reduced ADAMTS-13 level in ALL patients was related to inflammation factors in plasma.

Table 2

Multivariate analysis of the influence of age, gender, FAB classification, and lineage for ADAMTS-13 level in ALL patients.

ALL				
Source	**df**	**Mean square**	***F***	***P***
Age	23	11,217.736	1.185	0.425
FAB	1	2825.487	0.299	0.600
Lineage	1	18.937	0.002	0.965
Gender	1	15,999.453	1.691	0.230
Error	8	9462.580		
Total	34			

ALL = acute lymphatic leukemia, FAB = French-American-British subtypes.

3.3. ALL patients with infections had lower levels of ADAMTS-13 in plasma

ALL patients are always concurrent with infections, which is a major complication of acute leukemia. Considering the relationship between inflammation factors and ADAMTS-13, we further compared levels of ADAMTS-13 in ALL patients with or without infections. According to Fig. 3, we found that ADAMTS-13 levels were significantly lower in patients with infection than patients without infection, and both of them possessed significantly decreased ADAMTS-13 than healthy controls.

3.4. ADAMTS-13 levels are related to risk stratification of ALL patients

The ALL patients were stratified for risk stratification with reference to the NCCN guideline.[13] All these patients were divided into low-risk group, intermediate-risk group, and high-risk group by risk evaluation. ADAMTS-13 level was analyzed for each group and the results are shown in Fig. 4. We found that the level of ADAMTS-13 in low-risk group was significantly higher than other 2 groups. ADAMTS-13 levels in plasma of high- and intermediate-risk patients were not significantly different. These results suggested that ADAMTS-13 level is related to risk stratification of ALL patients.

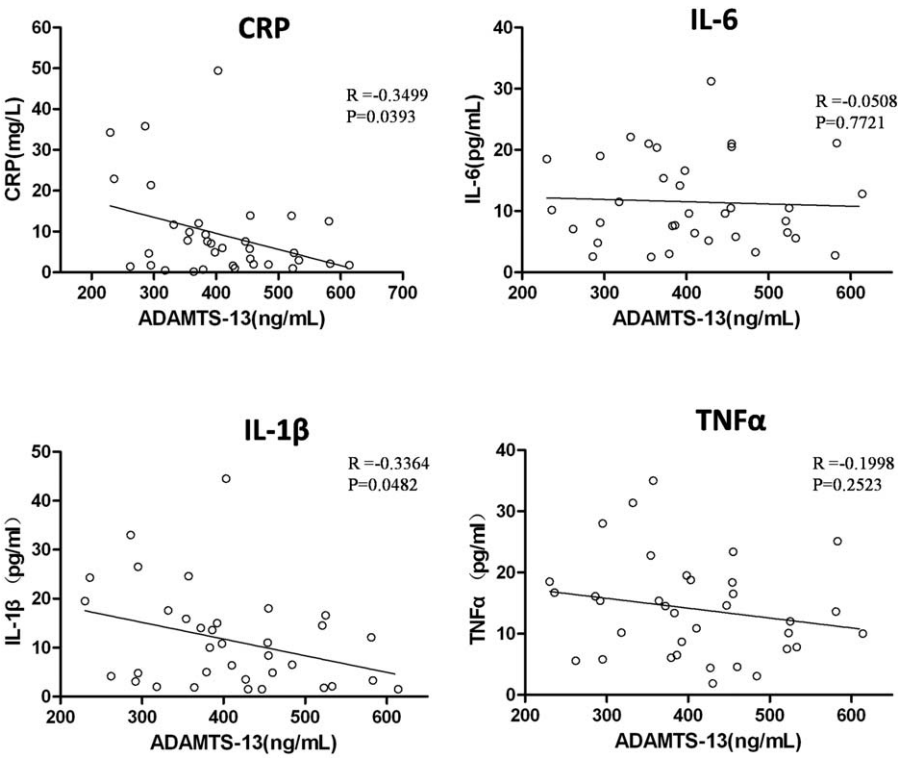

Figure 2. Correlation analysis between ADAMTS-13 and inflammation factor levels in ALL patients. CRP, IL-6, TNFα, and IL-1β levels of each ALL patients were measured. Correlation analysis was conducted between ADAMTS-13 levels and these 4 inflammation factors among all these ALL patients. Scatter plots with linear fit are shown and Pearson *r* and *P* values are listed. *P* values <0.05 are regarded as significant. ALL = acute lymphoblastic leukemia, CRP = C-reactive protein, IL = interleukin, TNF = tumor necrosis factor.

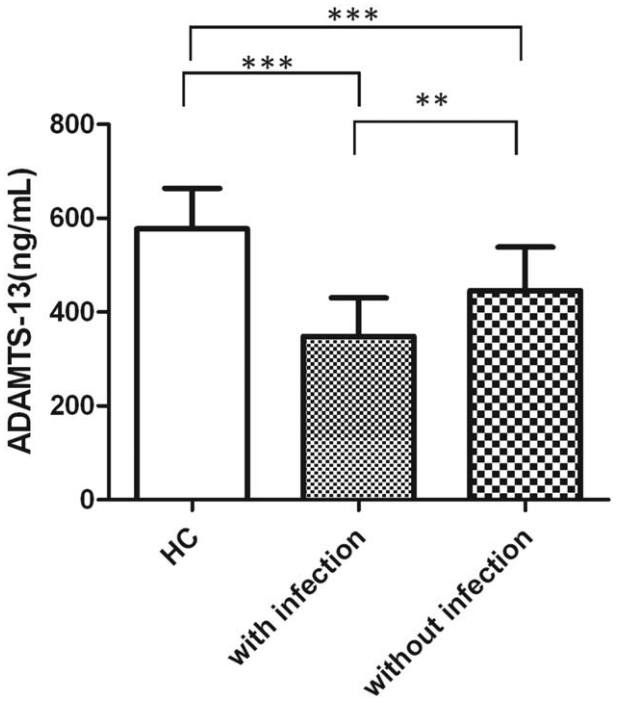

Figure 3. ADAMTS-13 levels in plasma of ALL patients with or without infection. ALL patients were divided into 2 groups, patients with infection and patients without infection according to the diagnosis by physicians. The level of ADAMTS-13 in 2 groups and healthy controls were analyzed and compared with each other. Data points are shown with means ± standard deviations and Student *t* test was conducted to compare 2 groups. $^{**}P < 0.01$, $^{***}P < 0.001$. ALL = acute lymphoblastic leukemia.

4. Discussion

Low level of ADAMTS-13 (<5% of normal) was always regarded as the etiology of TTP, which had been thoroughly studied and used for diagnosis of TTP.[7–9] In many pathological conditions unrelated to TTP, ADAMTS-13 levels are also low or undetectable.[10,15–17] Patients with both localized and disseminated malignancies had been reported to have reduced levels of ADAMTS-13.[16,17] In the case of leukemia, however, the change of ADAMTS-13 expression has not received sufficient attention. In this study, we have demonstrated that level of ADAMTS-13 was decreased in the plasma of ALL patients. Meanwhile, ADAMTS-13 levels were negatively correlated with CRP and IL-1β. Furthermore, the level of ADAMTS-13 was related to risk stratification of ALL patients. Low-risk patients had relatively higher level of ADAMTS-13 than patients at high or intermediate risk.

The effect of inflammation on ADAMTS-13 reduction was studied in pediatric patients with severe sepsis and ADAMTS-13 activity was reduced in patients with acute systemic inflammation.[14] In addition, serious deficiency of ADAMTS-13 had been shown to be associated with levels of interleukin-6 in plasma.[18] We found that ADAMTS-13 levels were negatively correlated with CRP and IL-1β in ALL patients. We hypothesized that inflammatory refection during ALL may play a role in the reduction of ADAMTS-13 levels. It has been found that ALL patients with infections have lower level of ADAMTS-13, which may be due to the inflammatory environment in these patients. Our results suggest that ADAMTS-13 levels are associated with risk stratification in ALL patients, suggesting a potential clinical

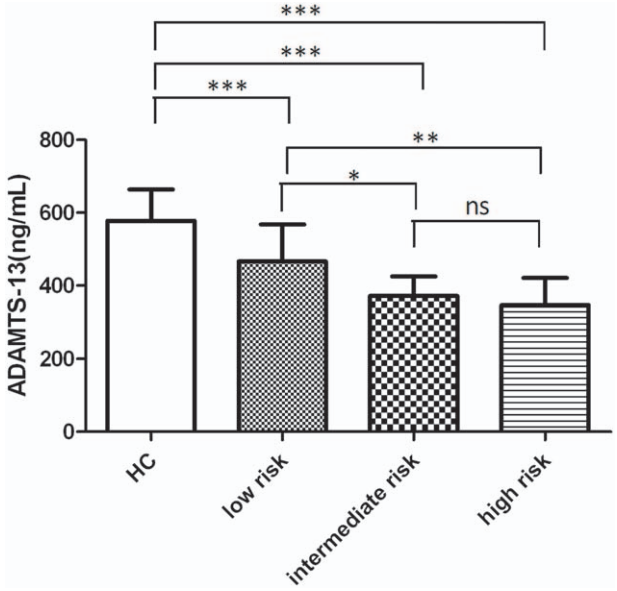

Figure 4. ADAMTS-13 levels in ALL patients of different risk stratification. ALL patients were stratified for risk stratification. These patients were divided into low-risk group, intermediate-risk group, and high-risk group by risk evaluation. ADAMTS-13 level of each group was analyzed and compared. The levels of each group were also compared with healthy controls (HC). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. ALL = acute lymphoblastic leukaemia, ns = not significant.

significance of ADAMTS-13. We hypothesized that ADAMTS-13 may partially reflect the condition of ALL patients.

We also studied the expression and clinical significance of ADAMTS-13 in acute myeloid leukemia (AML) patients and found that ADAMTS-13 levels were also decreased in plasma of AML patients and the level of ADAMTS-13 was also related to infections and risk stratification of AML patients.[19] It means that the decrease of ADAMTS-13 is not limited to ALL patients and the cause of reduced ADAMTS-13 may be the common characteristics of acute leukemias. Besides, ADAMTS-13 was found to be negatively correlated with International Society on Thrombosis and Haemostasis scores in AML patients and low ADAMTS-13 level was a potential risk factor for AML patients,[19] which had not been found in ALL patients, suggesting that the situations in AML and ALL patients were not completely the same.

There was still much more work to do about the significance of decreased ADAMTS-13 levels in acute leukemia. The sample size needs to be increased and multicentered research needs to be done as well. The detailed mechanism for the decrease of ADAMTS-13 also needs to be concerned in future. In this study, we inquired into the change of ADAMTS-13 levels in ALL patients and studied the relationship between ADAMTS-13 and inflammation factors, as well as infections. We also studied the change of ADAMTS-13 in ALL patients at different risk stratification, which could contribute to a comprehensive understanding of the clinical significance of ADAMTS-13.

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