

Evaluation of D-dimer and lactate dehydrogenase plasma levels in patients with relapsed acute leukemia

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Abstract. Despite the outstanding advances made over the past decade regarding our knowledge of acute leukemia (AL), relapsed AL remains to be associated with a dismal prognosis. A better understanding of AL relapse and monitoring of the D-dimer and lactate dehydrogenase (LDH) plasma levels following chemotherapy may aid clinicians in determining whether relapse may occur in the subsequent phases of the disease. The present study evaluated D-dimer and LDH levels in 204 patients with relapsed AL. Data were collected at the initial onset of AL, at complete remission (CR) and in patients with relapsed AL. D-dimer plasma levels were significantly increased in patients with initial AL and in patients with relapsed AL ($P=0.005$ and $P=0.007$, respectively) but not in those with CR. LDH levels were significantly increased in AL patients at the initial onset of disease and at relapse compared with patients achieving CR, irrespective of cell type. Plasma prothrombin time, activated partial thromboplastin time and fibrinogen levels were not significantly different across patients (with the exception of acute promyelocytic leukemia patients) at the initial onset, relapsed AL or CR. Routine hematological parameters (white blood cell count, hemoglobin, platelet count) were significantly different at the initial onset of AL ($P=0.002$, $P<0.001$ and $P=0.001$, respectively) and during relapsed AL ($P=0.009$, $P=0.003$ and $P<0.001$, respectively) compared with patients achieving CR, suggesting an association between D-dimer, LDH and relapsed AL. These results also indicate that determination of D-dimer and LDH levels may be useful for predicting the probability of relapse during chemotherapy, but should also be combined with routine hematological parameters.

Introduction

Acute leukemia (AL) is a clonal disease that progressively produces novel sub-clones, which exhibit altered phenotypic and cytogenetic traits. AL is divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). AML is the most common type of leukemia in adults. Acute promyelocytic leukemia (APL) is a distinct subtype of AML characterized by coagulopathy and signs of disseminated intravascular coagulation (1,2). It is not known whether coagulation results are associated with AL except in APL. Therefore, the comparison of certain clinical parameters of AL patients, including activated partial thromboplastin time (aPTT), prothrombin time (PT), D-dimer and fibrinogen (FIB) should be eliminated due to interference from the APL cohort. Despite high sensitivity to standard chemotherapy, which leads to increased rate of complete remission (CR) in the two subtypes of AL, $\sim 1/3$ of AL patients subsequently relapse (3-6). AL relapse following standard chemotherapy remains a significant therapeutic challenge (7-10). AL continues to have a low survival rate compared to other cancers (11). Disease-free survival rates are 5-15% in older adults and 25-40% in younger patients (12).

Metabolic changes occur as part of oncogenesis and tumor progression. A better understanding of AL relapse and the careful monitoring of clinical parameters following chemotherapy may aid clinicians in determining the best treatment options on an individual patient basis. As a result of local activation of intravascular coagulation at the onset of some forms of malignancy (13,14), the fibrinolytic marker D-dimer increases and accumulates (14). LDH exists in numerous cell systems and, subsequent to tissue or cell damage, serum LDH levels may increase (15). Although it is generally understood that there is a poor outcome for adults with AL that develop bone marrow relapse (16) and various risk factors predicting outcome are continuously analyzed, there are few studies concerning LDH and D-dimer in these types of patients. The present study was performed to determine whether D-dimer and LDH levels during treatment are associated with relapse in patients with AL.

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Materials and methods

Patients. The present study evaluated data from 204 patients that were newly diagnosed with AL at the The First Affiliated

Hospital of Wenzhou Medical University (Wenzhou, China) between February 2010 and October 2014, several of whom relapsed. At the initial onset, 204 patients were treated uniformly for AML or ALL. AL was classified according to the FAB criteria (17). In total there were 40 ALL patients and 164 AML patients (M1, 44; M2, 26; M3, 32; M4, 36; M5, 20; M6, 6). AML patients only were treated with daunorubicin (45 mg/m² per day on days 1-3) and cytarabine (150 mg/m² per day for 7 days) according to the 3 plus 7 regimen. APL patients were treated based on an all-trans retinoic acid (20 mg/m² per day on days 1-15) plus anthracycline (idarubicin; 8 mg/m² per day on days 1-3) protocol. The induction chemotherapy in ALL patients included vincristine (2 mg per day on days 1, 8, 15 and 22) and daunorubicin (40 mg/m² per day on days 1-3), prednisone [1 mg/kg per day on days 1-14 and 15-28 (2/3 dose)] and L-asparaginase (6,000 IU/m² per day on days 11, 14, 17, 20, 23 and 26). The AL patients were free from any systemic, cardiovascular or inflammatory illnesses. The Ethics Committee at The First Affiliated Hospital of Wenzhou Medical University approved the present study. Written informed consent was obtained from the patients.

Blood collection. Blood samples were taken from the antecubital vein and placed in plastic tubes containing 3.8% trisodium citrate (9 volumes of blood and 1 volume of 0.1 M trisodium citrate) or ethylenediaminetetraacetic acid (EDTA)-K₂ anticoagulant. For plasma separation, blood was centrifuged at 2,500 × g for 15 min at 4°C; blood samples were obtained prior to the initiation of any treatment for AL. The present study adhered to the tenets of the Declaration of Helsinki.

Data collection. Blood counts were performed using an XE 2100 Sysmex™ automated hematology analyzer (Sysmex, Kobe, Japan). aPTT, PT, D-dimer and FIB were measured using a STAGO Coagulation analyzer (Diagnostica Stago, Asnières, France). The STA®-Liatest® D-Di kit (Diagnostica Stago) is intended for use with analyzers of the STA® line suitable with these reagents for the quantitative determination of D-dimer in plasma by immuno-turbidimetric method. The STA®-fibrinogen kit (Diagnostica Stago) is intended for use with STA-R® analyzers for the quantitative determination of FIB levels in plasma using the clotting method of Clauss. The STA®-néoplastine Cl Plus and STA®-APTT kits (Diagnostica Stago) are intended for use with STA-R® analyzers for the determination of PT and aPTT in plasma by the clotting method, respectively. Lactate dehydrogenase (LDH) plasma levels were measured using the LDH substrates method (Beckman Coulter Experiment System Co., Ltd., Suzhou, China). Markers were compared against normal ranges, which were as follows: White blood cell (WBC) count, 3.5-9.5×10⁹/l; platelet (PLT) count, 125.0-350.0×10⁹/l; hemoglobin (Hb), 115.0-175.0 g/l; PT, 11.6-14.9 sec; aPTT, 29.0-43.0 sec; FIB, 2.0-4.0 g/l; D-dimer, 0.0-0.5 mg/l; and LDH, 0.0-247.0 units (U)/l. The laboratory data were collected promptly at the initial onset of AL, CR and in patients with relapsed AL.

Response evaluation. The diagnostics were comprised of cytomorphology, cytochemistry, cytogenetics, molecular genetics and immunophenotyping of bone marrow or peripheral blood. Bone marrow aspirate smears were applied to assess the

therapeutic effect of AL. The morphological diagnosis and classification were performed according to the WHO 2008 diagnostic criteria (18).

Statistical analysis. The results were graphed using line charts depicting medians and range. The data were analyzed using the Wilcoxon matched pairs test. Spearman's rank correlation coefficient was used to analyze correlations between serum D-dimer or LDH level and selected blood routine parameters. P-values of <0.05 were considered to indicate a statistically significant difference. Data were analyzed using SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

Clinical parameters of AL patients at various stages of disease. Laboratory data in patients with initial onset, CR and relapsed AL are shown in Table I. The WBC count was significantly different at the initial onset of AL (P=0.002) and during relapsed AL, compared with patients in the CR group (P=0.009). Hb levels (P<0.001 and P=0.003) and PLT counts (P=0.001 and P<0.001) were significantly reduced in the initial onset and relapsed AL groups, compared with the CR group. D-dimer (P=0.005 and P=0.007) and LDH levels (P=0.007 and P=0.008) were also significantly increased in the two groups compared with the CR group (Figs. 1 and 2). In addition, these levels were significantly different across the two groups compared with AL in CR, regardless of whether APL patients were included. Plasma PT and FIB levels were significantly (P=0.020) increased in the initial onset and relapsed AL groups compared with patients achieving CR. Plasma FIB levels were significantly decreased in the initial onset (P=0.008) and relapsed AL groups (P=0.009) compared with patients achieving CR. Plasma aPTT levels were not significantly different between patients with initial onset or relapsed AL and AL in CR. When coagulation data results were eliminated in patients with APL, plasma levels of aPTT were elevated, but plasma PT, aPTT and FIB levels were not significantly different between patients with relapsed AL and AL in CR.

Plasma levels of D-dimer and LDH levels in all subtypes of AL at various stages of disease. In the AML-M1 group (Table II), LDH (P=0.030) and D-dimer (P=0.020) plasma levels were significantly increased at the initial onset of AL compared with during CR. The plasma levels of D-dimer and LDH were significantly increased in the relapsed group compared with the CR group (P=0.010 and P=0.020, respectively), which were higher during relapse compared with at initial onset of AL. In the AML-M2 group, LDH (P=0.008 and P=0.010) and D-dimer (P=0.008 and P=0.007) plasma levels were significantly increased in the initial onset and relapsed groups compared with CR; LDH plasma levels were increased in patients with initial onset of M2 compared with relapsed patients. The highest values of D-dimer were observed in AML-M3 patients. D-dimer plasma levels were significantly increased at initial onset of APL compared with during CR, and significantly increased during relapse compared with CR. Plasma LDH levels were significantly greater in patients at the initial onset and in patients with relapsed AL compared with patients achieving CR (P=0.009 and P=0.007, respectively).

Table I. Blood routine and coagulation measurements in patients at various stages of AL.

Characteristics	AL disease including APL, median (range)			AL disease except for APL, median (range)		
	Initial onset	CR	Relapse	Initial onset	CR	Relapse
WBC, $\times 10^9/l$	11.6 (0.1-658.4) ^a	4.8 (2.1-13.2)	5.8 (0.1-328.0) ^c	21.9 (1.1-200.0) ^a	4.2 (2.4-10.3)	10.6 (0.1-254.4) ^c
Hb, g/l	79.0 (34.0-149.0) ^a	105.0 (64.0-155.0)	92.0 (45.0-148.0) ^c	80.0 (34.0-148.0) ^a	101.0 (64.0-140.0)	90.0 (45.0-145.0) ^c
PLT, $\times 10^9/l$	30.0 (5.0-235.0) ^a	180.0 (6.0-460.0)	32.0 (4.0-167.0) ^c	41.0 (6.0-235.0) ^a	143.0 (21.0-462.0)	32.0 (6.0-167.0) ^c
PT, sec	15.6 (12.5-33.6) ^b	13.7 (11.7-18.1)	14.6 (11.9-22.8) ^d	14.2 (12.5-21.6)	13.3 (11.7-18.1)	14.1 (12.1-21.4)
aPTT, sec	38.8 (26.6-75.5)	38.8 (29.1-58.8)	39.8 (25.9-59.6)	38.1 (30.0-75.5)	38.4 (29.4-57.8)	39.3 (25.9-55.5)
FIB, g/l	3.5 (0.2-9.1) ^a	3.7 (0.6-8.5)	2.9 (0.8-8.4) ^c	3.77 (0.8-8.5)	3.4 (0.6-8.5)	3.3 (1.26-7.39)
D-dimer, mg/l	4.2 (0.5-47.0) ^a	1.2 (0.1-7.2)	2.7 (0.3-40.0) ^c	2.2 (0.5-10.4) ^a	0.6 (0.1-1.6)	1.6 (0.3-17.4) ^c
LDH, units/l	385.0 (140.0-2,894.0) ^a	198.0 (29.0-405.0)	335.0 (88.0-4,919.0) ^c	395.0 (156.0-2,405.0) ^a	173.0 (69.0-293.0)	376.0 (88.0-4,919.0) ^c

^aP<0.01, ^bP<0.05; significant difference between initial onset AL patients and CR. ^cP<0.01, ^dP<0.05; significant difference between relapse patients and CR. AL, acute leukemia; APL, acute promyelocytic leukemia; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; PT, prothrombin time; aPTT, activated partial thromboplastin time; FIB, fibrinogen; LDH, lactate dehydrogenase.

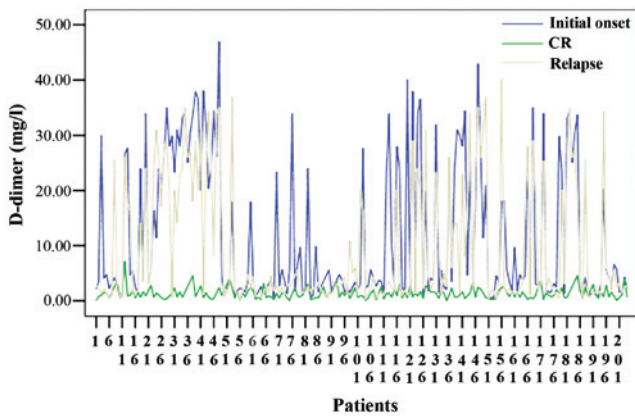


Figure 1. A paired analysis line chart demonstrating significantly elevated D-dimer levels in patients with relapsed acute leukemia compared with the same patients with CR. CR, complete remission.

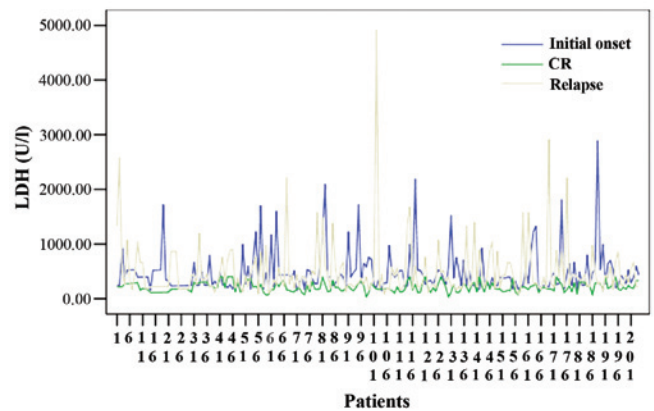


Figure 2. A paired analysis line chart demonstrating significantly elevated LDH levels in patients with relapsed acute leukemia compared with the same patients with CR. CR, complete remission.

In the AML-M4 patients group, D-dimer plasma levels were significantly increased in patients with initial onset and patients with relapsed AL ($P=0.010$ and $P=0.030$, respectively), but not with CR. LDH levels in AML-M4 patients were significantly increased at initial onset and in relapsed AL compared with at CR. In the AML-M5 patients group, D-dimer ($P=0.030$ and $P=0.040$) and LDH ($P=0.020$ and $P=0.040$) plasma levels significantly increased in the relapsed group and initial onset of disease compared with the CR group. In the AML-M6 patient group, D-dimer plasma levels increased during relapsed AL ($P=0.030$), but not at initial onset of AL compared with patients with CR. Plasma LDH levels were significantly greater in patients with initial onset and relapsed AL compared with

CR ($P=0.030$ and $P=0.020$, respectively). In the ALL patient group, D-dimer plasma levels were significantly increased in the initial onset and relapsed groups compared with the CR group, but were higher at the initial onset of ALL compared with during relapse. LDH plasma levels significantly increased at initial onset and during relapse compared with patients achieving CR ($P=0.005$ and $P=0.002$, respectively).

Serum LDH levels were elevated in the majority of AL patients following initial diagnosis and in relapsed patients; however, there does not appear to be a correlation between increased LDH levels and blood routine parameters in AL patients. Increased D-dimer concentrations demonstrated no correlation with any blood routine parameters in AL patients.

Table II. Clinical parameters of patients with various stages of AL subtypes.

Subtype	No. of cases	D-dimer (median, range)			LDH (median, range)		
		Initial onset	CR	Relapse	Initial onset	CR	Relapse
M1	44	1.8 (0.7-18.0) ^b	1.0 (0.2-3.0)	1.9 (0.9-40.0) ^d	359.0 (156.0-704.0) ^b	166.0 (77.0-400.0)	424.0 (100.0-588.0) ^d
M2	26	2.6 (0.9-4.2) ^a	1.8 (0.3-3.6)	3.2 (0.9-6.0) ^c	432.0 (217.0-760.0) ^a	176.0 (69.0-334.0)	260.0 (163.0-984.0) ^d
M3	32	29.0 (1.3-47.0) ^a	1.0 (0.3-7.2)	24 (0.5-37.0) ^c	253.0 (180.0-929.0) ^a	200.0 (140.0-405.0)	297.0 (110.0-1,200.0) ^c
M4	36	3.3 (1.2-34.0) ^b	1.4 (0.1-5.3)	2.4 (1.0-13.5) ^d	484.0 (140.0-2,405.0) ^b	192.0 (29.0-334.0)	363.0 (167.0-2,584.0) ^c
M5	20	2.3 (1.0-3.7) ^b	0.6 (0.1-1.9)	1.7 (0.3-3.4) ^d	419.0 (162.0-1,725.0) ^b	154.0 (76.0-287.0)	263.0 (88.0-569.0) ^d
M6	6	0.5 (0.5-1.0)	0.4 (0.2-1.1)	1.6 (1.5-1.8) ^d	379.0 (240.0-644.0) ^b	148.0 (128.0-187.0)	409.0 (376.0-424.0) ^d
ALL	40	4.1 (0.7-24.0) ^a	1.2 (0.1-3.5)	2.1 (0.5-25.6) ^d	496.0 (214.0-2,894.0) ^a	255.0 (114.0-400.0)	630.0 (119.0-4,919.0) ^c

^aP<0.01, ^bP<0.05; significant difference between initial onset AL patients and CR. ^cP<0.01, ^dP<0.05; significant difference between relapse patients and CR. AL, acute leukemia; LDH, lactate dehydrogenase; CR, complete remission; ALL, acute lymphocytic leukemia.

Discussion

Despite the outstanding advances made over the past decade regarding our knowledge of AL, AL relapse following routine conventional-dose chemotherapy remains to be associated with a dismal prognosis (3-6,8,10). D-dimers are a sensitive measure of endogenous fibrinolysis and are used to screen for deep vein thrombosis (19-24). Elevated LDH levels have frequently been observed in animal and human malignancies (15,25,26); in addition, there appears to be a strong correlation between disease activity and tumor mass (27). The association, pathogenesis and significance of D-dimers and LDH levels with relapse within the subtypes of AML and ALL are currently unknown. A clear understanding of the associations between these parameters and relapsed AL patients requires additional research.

Coagulation disorders are frequently observed in APL and can also occur in other AL subtypes (28-30). The pathogenic mechanism underlying the blood coagulation disorder in these patients is complex. The levels of D-dimer complex generally reflect the functional state of the clotting and fibrinolysis systems and support the existence of a hypercoagulable state (19,20,31,32).

In the present study, during the initiation of AL, PT was found to be significantly prolonged ($P=0.020$), plasma levels of FIB were significantly decreased ($P=0.009$), the levels of D-dimer were elevated in all subtypes of AML and ALL, and the highest levels of D-dimer were found in AML-M3 (Tables I and II); however, there was no significant difference in aPTT between patients with initial onset and those with CR, or between relapse and CR. When coagulation data results were eliminated in patients with APL, plasma PT, aPTT and FIB levels were not significantly increased at the initial onset of AL or during AL relapse compared to patients with CR.

The results of the present study indicate that the determination of D-dimer levels may be useful for predicting the probability of relapse during chemotherapy, as the PT, aPTT and FIB tests were not reliable markers of AL relapse.

Increased D-dimer levels at initial onset, which further increased during relapse, strongly suggest a hypercoagulable state, with secondary activation of fibrinolysis in the majority of patients (33,34). Activation of coagulation and secondary activation of fibrinolysis are likely to occur continuously and simultaneously throughout circulation (20,24,34). The activation of coagulation is most likely associated with the leukemic cells circulating in the blood, which may contain procoagulants, and the content of these substances depends on the leukemia subtype (35,36). These substances may be released into the blood from disintegrating cells during relapse.

Tanaka *et al* (35) confirmed that the development of disseminated intravascular coagulation in patients with AL prior to chemotherapy is associated with the presence of tissue factor (TF) on the surface of the leukemic cells. TF is a major procoagulant that initiates blood coagulation *in vivo* and is the membrane protein receptor for factor VII. The resulting factor VIIa activates factors IX and X, leading to thrombin generation and fibrin formation (35,37,38). The TF gene is expressed in cells from patients with AL.

In the present study, cells from AML patients expressed particularly high VII activity; these levels become essentially undetectable when patients are in CR. Other procoagulant mediators, including tumor necrosis factor α (39,40), cysteine proteinase (41), interferon- γ (40), asinterleukin-1 (42) and vascular permeability factor (43), are regarded as indirect procoagulants as they initiate coagulation by inducing TF in endothelial cells and monocytes (44). In addition, natural apoptosis may contribute to thrombogenesis in AL via the release of microparticles from the damaged leukemic

cells (35). Coagulation disorders may also occur due to leukemia-associated complications, including infection or organic impairment (45).

Notably, for patients that achieved CR following the induction of chemotherapy, D-dimer levels did not return to a normal value. The increased D-dimer levels during CR following chemotherapy treatment suggest a hypercoagulable state with secondary activation of fibrinolysis. Velasco *et al* (46). Observed an increase in the D-dimer level during treatment in patients with AML. In the present study, the results showed that the LDH levels were moderately elevated in the majority of AL patients with the exception of the CR phase, irrespective of cell type. Significantly elevated levels were recorded in the majority of patients with ALL but there was no significant difference in serum LDH levels between AML and ALL patients during relapse; in addition, no significant difference was found at initial onset of AL and during relapse.

In patients with increased levels of LDH at diagnosis, AL relapse was not found to lead to significant elevation. LDH activity reflects increased glycolysis in the cytoplasm of malignant cells accompanied by a high metabolic rate (15,25). The increase of serum LDH activity may be due to thrombotic microangiopathy, intravascular hemolysis or tumor lysis (25,26). Certain values from the ALL patients were remarkably high (Table II), and the majority of these patients had a high WBC counts during relapse. This phenomenon is likely due to the correlation between LDH levels and the number of circulating ALL blasts during relapse (47). Cumulative evidence indicates that serum LDH levels can be a good and reliable prognostic marker of ALL patients (48-50), suggesting an association between LDH levels and relapse.

Although D-dimer and LDH levels have been shown to be elevated in all subtypes of AML and ALL, none of these parameters provide diagnostic specificity. In the present study, a significant change in routine hematological parameters was indicated in patients with relapsed AL, which is consistent with previous findings (51,52). The most important and most common associated risk factors for the hematological relapse of AL are thrombocytopenia, leukocyte count and lower hemoglobin, which is associated with the proliferating leukemic clone (53,54). Ambulatory monitoring of D-dimer, LDH, and routine hematological parameters are recommended for the assessment of relapse in AL patients.

In conclusion, the present study demonstrated that D-dimer and LDH plasma levels were significantly increased at initial onset and during relapse in AL patients compared to those with CR. D-dimer and LDH levels may be useful for predicting AL relapse; therefore, the present study recommends that monitoring D-dimer and LDH for the assessment of AL relapse.

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