

ORIGINAL ARTICLE

Coagulation activation and changes during treatment of acute myeloid leukaemia: A prospective cohort study

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Abstract

Objective: Many patients with malignancies demonstrate coagulation abnormalities as activated coagulation and activation of fibrinolysis. Chemotherapy used for the treatment of leukemia may influence the coagulation changes. The purpose of this study was to examine hematological changes in patients with *de novo* acute myeloid leukemia (AML) before and after the start of induction treatment and during the following two weeks.

Methods: In 13 patients with *de novo* AML, markers of coagulation were determined before induction treatment and daily for 13 days.

Results: At start, Hb and platelets were low and levels diminished during induction therapy. International Normalized Ratio (INR) was elevated at days 3-5 and activated partial thromboplastin time (APTT) was prolonged at days 13-14. Von willebrand factor antigen (VWF: Ag), von willebrand factor ristocetin cofactor activity (VWF: RCo), and factor VIII (FVIII) were elevated during the observation period but the increase was lower during chemotherapy. The von willebrand factor multimeric sizing (VWF: MS) quotient rose. The activated protein C (APC)- protein c inhibitor (PCI) complex, thrombin-antithrombin complex (TAT), fragment 1+2 (F1+2) and D-dimer were all increased before start of induction therapy, peaked during chemotherapy, and afterwards returned to more normal values. Antithrombin and protein C were within normal limits at the onset and remained relatively stable.

Conclusions: Results suggest that coagulation is activated in patients with *de novo* AML, with a peak during induction treatment, and decline in activation subsequently. Increased thrombin generation occurs before consumption of clotting factors is apparent in global hemostatic tests (e.g. prolonged APTT).

Key words

Acute myeloid leukemia, Blood coagulation disorders, Global hemostatic tests, Induction treatment, Thrombin generation

Introduction

Malignant hematological disorders may be associated with coagulopathy causing bleeding and thromboembolic complications^[1]. Severe infections, as well as chemotherapy, may augment this risk by the action of inflammatory mediators and the release of proteases. Secondary activation of fibrinolysis may partly compensate the activated coagulation, but in metastatic prostate cancer and acute promyeloid leukemia (APL), the fibrinolysis may prevail^[2, 3]. Molecular markers for increased thrombin generation that can detect a pre-thrombotic state include a prothrombin activation fragment, Fragment 1+2 (F1+2), and a complex formed between thrombin and its cognate serpin antithrombin, the thrombin-antithrombin complex (TAT)^[4-7]. The complex formed between activated protein C (APC) and the serpin, protein c inhibitor (PCI), has been shown to be a sensitive indicator of thrombin formation and, thus, of the activation of blood coagulation^[8]. However, these methods are not widely used in clinical practice.

Hematological malignancies are often associated with hemostatic disturbances^[9, 10] which seem, at least partly, to be associated with von willebrand factor (VWF) defects. Both increased activity of the coagulation system as well as hyperfibrinolysis have been described. In addition, patients often experience low platelet counts ($<40 \times 10^9/L$) which may cause life-threatening hemorrhages^[11]. Other pathophysiological mechanisms that may add to an increased bleeding tendency include a massive proteolytic state, triggered by procoagulant substances, plasminogen activators and proteinases released into circulation from leukemic cells as well as proteolytical degradation of the VWF^[12]. A VWF multimeric pattern resembling type 2A von willebrand disease (VWD) mediated by an IgG antibody directed against the GPIb binding site has been described^[13]. Furthermore, an abnormal VWF multimeric pattern has been associated with elevated platelet as well as leukocyte counts^[14, 15].

In Sweden, the annual incidence of acute myeloid leukemia (AML) is approximately 3.5 per 100,000 population and increases progressively with age to a peak of 13 per 100,000 at 65 years of age or older. Adverse prognostic factors include age, secondary AML, and hyper leukocytosis. Hemorrhage is common and can be the cause of death.

The aim of this study was to examine hematological changes in patients with *de novo* AML prior to the start of induction treatment and during the following two weeks of therapy.

Patients and methods

Study design and subjects

We conducted a prospective study of patients admitted to the hematology units of Malmö and Lund University Hospitals between November, 2002 and May, 2004. The criterion for inclusion was hospitalization for induction treatment of *de novo* AML. Patients less than 18 years of age, and those for whom consent to participate could not be obtained were excluded. Intravenous chemotherapy consisted of receipt of idarubicin hydrochloride in bolus injections for three days and cytarabine in continuous infusion for seven days. Blood samples were collected in the morning prior to treatment on day one, and then once daily until day 14. Assessment of prior and ongoing bleeding and treatments, e.g. drugs and transfusions, were recorded.

The procedures were in accordance with the Helsinki Declaration of 1975 and the study was approved by the Ethics Committee, Lund University.

Laboratory assays

Hb and platelet count were analyzed by methods used in routine care at the Department of Clinical Chemistry at the respective hospitals. Both are accredited by the Swedish Board for Accreditation and Conformity Assessment (Swedac). All other blood coagulation analyses were performed from citrated plasma (Plasma for APC-PCI complex analysis - see

below) stored at -70°C . INR measures the activity of factor II, VII and X (Method according to Owren)^[16] and was determined using Stago Prothrombin complex Assay (SPA; Diagnostica Stago, Asnières, France). APTT was analyzed with Platelin LS reagent (Organon Teknika, Durham, NC, USA).

For measurement of VWF: Ag, an immunoassay, the STA Liatest VWF (Diagnostica Stago), was performed. The VWF: RCo assay was carried out using the BC von Willebrand reagent (Siemens, former Dade Behring, Marburg, Germany) containing lyophilized platelets and ristocetin. The method was slightly modified by Strandberg et al.^[17].

The multimeric pattern of VWF (VWF: MS) was analyzed by electrophoresis in 1.9% agarose gel in the presence of sodium dodecyl sulphate followed by immunoblotting with specific antibodies against VWF as previously described^[18, 19]. As a reference standard a normal plasma pool of 40 healthy volunteers was used. The VWF: MS quotient was calculated as the quotient between large and small multimer content (Compared to pooled normal plasma). The classification of small multimers (Number 1 to 5) and large multimers (Number 11 or larger) is according to the ISTH criteria^[20].

FVIII was measured with Coatest FVIII (Chromogenix, Milan, Italy). F1+2 and TAT were assayed using Enzygnost F1+2 micro and Enzygnost TAT micro (Siemens, former Dade Behring). D-dimer was analyzed with Auto D-dimer (Trinity Biotech, Bray, Ireland). Antithrombin activity was determined with Coamatic LR Antithrombin kit (Chromogenix). Protein C was analyzed by a chromogenic substrate method (Coamatic Protein C; Chromogenix).

Blood for measurement of the APC-PCI complex was collected in vacuum tubes with low pH citrate (Biopool® Stabilyte™ tubes, 5.0mL, containing 0.5mL 0.5M citrate buffer, pH4.3, Trinity Biotech)^[21] and the APC-PCI complex concentration was determined as described by Strandberg et al.^[8]. The assay kit can be purchased from BioPorto Diagnostics A/S, Gentofte, Denmark.

Statistical methods

Calculations were performed using PASW Statistics version 18.0 (IBM SPSS North American Headquarters, Chicago, IL, USA). *P*-values of less than 0.05 were considered to be statistically significant. As noted above, samples were taken on day 1 (Before treatment) and each day thereafter. Results of the coagulation activation markers APC-PCI complex, F1+2, and TAT are presented daily. To facilitate presentation of the other hematological analyses, these data were clustered into three intervals: Results during induction treatment (Days 3-5) were collected into one interval, shortly after chemotherapy (Days 8-10) into a second interval and at the end of the observation period (Days 13-14) into a third interval. For results shown in intervals that included multiple days, a median value for the variable of interest was first determined for each individual. Subsequently, these medians were then used in further calculations. The Wilcoxon signed ranks test was used to compare results at a given day or interval to those observed initially.

When results of laboratory assays exceeded the range of measurement, dilution of plasma was performed. This did not occur, however, in 21 of 148 morning samples analyzed for VWF: RCo (Values $>2.02\text{kIU/L}$ were set to 2.03kIU/L) and in 16 of 148 morning samples analyzed for D-dimer (Values $>32\text{mg/L}$ were set to 33mg/L).

Results

Thirteen patients met the requirements for study entry: Nine females and four males aged 32 - 73 years (Median 60 years). According to the French-American-British (FAB) classification of acute leukaemias^[22], one patient was categorized as M0, two as M1, four as M2, three as M4, and one as M5. One patient was assessed as having chronic myelomonocytic leukemia with transformation to acute leukemia, and the last patient was determined to have secondary AML.

Coagulation activation marker analyses are given in Figure 1. The median of the APC-PCI complex was greatly elevated at day 1, almost twice as high as the upper limit of the reference interval. At days 3-5, the median concentration was even higher - 2.5 times the upper limit of the reference interval. It subsequently declined to within the reference interval, and at days 7 and 11-14 the median was significantly lower compared to that of day 1. The levels of F1+2 and TAT varied in a similar way.

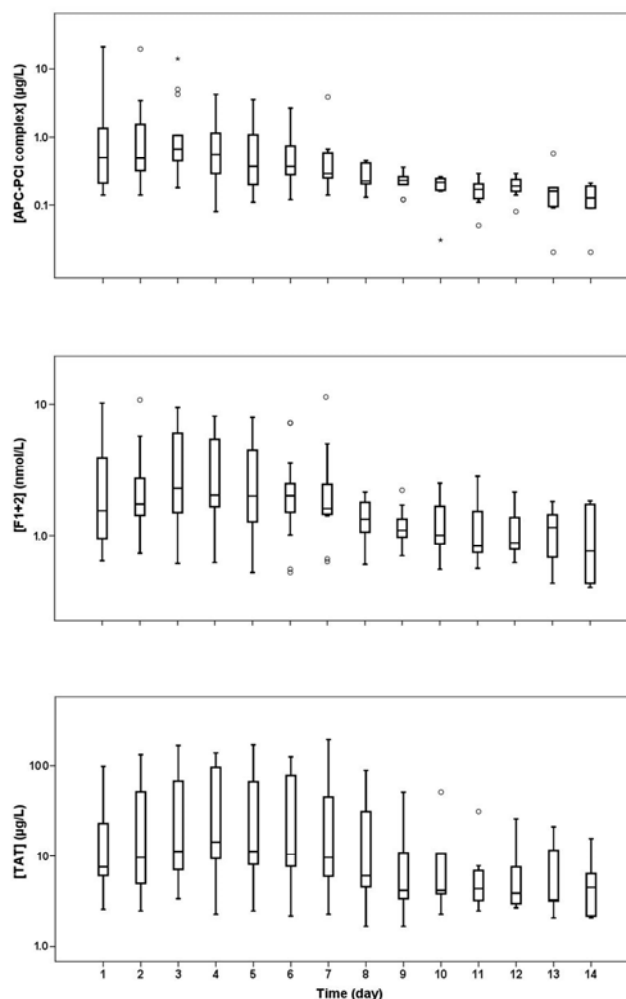


Figure 1. Results of coagulation activation marker analyses. Circles represent outliers and asterisks extreme-values. NB Scale of respective concentration is logarithmic. Reference interval (95% interval) APC-PCI complex 0.07-0.26µg/L, F1+2 0.4-1.1nmol/L, TAT 1.0-4.1µg/L. Number of patients (APC-PCI complex, F1+2, and TAT): n=12 at day 1, 6 (APC-PCI complex and TAT), and 8, n=13 at day 2-5, 6 (F1+2), and 7, n=10 at day 9, n=8 at day 10 and 11, n=7 at day 12 and 13, and n=6 at day 14. Significance levels (Wilcoxon signed ranks test) between day 1 and actual time. $P < 0.050$: APC-PCI complex at day 7, 11-14, F1+2 at day 3, 4, 13, and 14, TAT at day 3, 4, 9, 11, and 14.

Results of the remaining hematological analyses, shown as before chemotherapy (Day 1), during induction treatment (Days 3-5), shortly after chemotherapy (Days 8-10), and at the end of the observation period (Days 13-14), are presented in Table 1. Hb was decreased during the study period with the lowest values observed shortly after chemotherapy commenced. Platelets were low prior to the beginning of treatment and declined significantly thereafter. INR was significantly elevated at days 3-5 and APTT was significantly prolonged at days 13-14, at the other time points the values were well within their respective reference intervals.

VWF: Ag was slightly increased during the entire observation period. VWF: RCo was most elevated prior to induction of treatment and at days 13-14, and the increase in FVIII peaked during days 13-14. The VWF: MS quotient rose after treatment, although the differences were not significant.

D-dimer varied in a similar manner as the coagulation activation markers. The antithrombin and protein C levels were all within normal limits and relatively stable during the two weeks of observation.

During the study period, eight patients had hemorrhagic incidents. One patient had oozing from a central venous catheter. Two patients had epistaxis. Two had gastrointestinal bleeding only, one had gastrointestinal bleeding and oozing from a central venous catheter, one had gastrointestinal bleeding and eye bleeding, and one had gastrointestinal bleeding, oozing from a peripheral venous catheter, and a hematoma of a lower limb. Twelve patients were transfused with a median (Range) of 6 (3-9) units of erythrocytes and the same patients had 4 (1-6) units of platelets. One of these patients was transfused with fresh frozen plasma as well. Four patients received tranexamic acid and one had desmopressin (DDAVP). Two patients with epistaxis received absorbable hemostatic gelatin sponges.

One of the patients died during the study period (day 8) due to hemorrhagic pulmonary infarcts. One patient died on day 23 due to pulmonary bleeding. One patient had a distal deep vein thrombosis diagnosed on day 34.

Table 1. Results of hematological analyses

Hematological analysis	Time			
	Day 1 (Before)/n=13	Days 3-5/n=13	Days 8-10/n=12	Days 13-14/n=7
Hb, g/L	94 (88, 105)	85 (81, 90) 0.003	92 (83, 98) 0.41	87 (83, 89) 0.12
Platelet count($\times 10^9$ /L)	70 (50, 138)	39 (29, 83) 0.001	22 (17, 34) 0.002	18 (11, 26) 0.018
INR	1.1 (1.0, 1.2)	1.1 (1.1, 1.4) 0.020	1.1 (1.0, 1.3) 0.52	1.2 (1.0, 1.4) 0.068
APTT, s	32 (26, 36)/n=12	33 (29, 38) 0.16	34 (29, 36) 0.79	34 (30, 38) 0.046
VWF: Ag, kIU/L	1.75 (1.06, 3.40)/n=12	1.45 (0.99, 2.48) 0.055	1.47 (1.12, 2.17) 0.20	1.58 (1.02, 1.92) 0.18
VWF: RCo, kIU/L	1.99 (1.21, 2.81)/n=12	1.66 (1.17, 1.99) 0.026	1.69 (1.32, 2.03) 0.26	1.92 (1.35, 2.96) 0.80
VWF: MS quotient	1.13 (0.79, 1.40)/n=12	1.13 (0.86, 1.30) 0.48	1.34 (1.07, 1.56) 0.21	1.45 (1.24, 1.53) 0.24
FVIII, kIU/L	1.53 (1.26, 2.04)/n=12	1.42 (1.00, 1.74) 0.047	1.53 (1.46, 1.75) 0.42	2.05 (1.77, 2.28) 1.00
D-dimer, mg/L	0.6 (0.2, 11.2)	1.6 (0.6, 18.8) 0.004	0.5 (0.2, 1.3) 0.53	0.4 (0.2, 0.9) 0.075
Antithrombin, kU/L	1.06 (0.89, 1.08)/n=12	0.99 (0.93, 1.11) 0.81	1.09 (0.87, 1.13) 0.76	1.12 (1.01, 1.17) 0.18
Protein C (%)	79 (59, 85)/n=12	65 (52, 77) 0.099	76 (68, 95) 0.53	71 (67, 95) 0.13

Note: Numbers are given as median (First and third quartiles within parentheses). Significance levels (Wilcoxon signed ranks test) between day 1 and actual time interval. Reference interval (95% interval) Hb 117-153/134-170g/L (Females/males, respectively), platelet count $125-340 \times 10^9$ /L, INR <1.2, APTT 24-37s, VWF: Ag 0.60-2.73kIU/L, VWF: RCo 0.52-1.58kIU/L, VWF: MS quotient not applicable, FVIII 0.50-2.00kIU/L, D-dimer <0.2mg/L, antithrombin 0.82-1.11kIU/L, protein C 70%-149%.

Discussion

The APC-PCI complex, TAT, and F1+2 were all increased before start of induction therapy, peaked during chemotherapy, and declined afterwards to more normal values. APTT was normal at inclusion and slightly prolonged at the end of the observation period. INR behaved in a similar way. Our findings show an initial ongoing increased thrombin generation that occurs before consumption of clotting factors is apparent in global hemostatic tests (e.g. prolonged APTT). In our previous studies, we have found APTT to be the global test which best predicts outcome in the ICU [23, 24]. The indicator of fibrinolysis, D-dimer, showed the same pattern as the markers of thrombin generation. VWF (Measured as VWF: Ag and VWF: RCo) was at a stable elevated level, and then dipped somewhat during induction therapy. The VWF: MS quotient rose, possibly due to an increase in the proportion of large multimers. This may be caused by decreased proteolytic degradation of large multimers after start of induction treatment. Protein C was initially at the lower limit of the reference interval, in accordance with findings of other investigators [25, 26]. We found that concentrations of protein C remained at that level throughout the observation period, contrary to the findings of the same investigators who reported normalization after induction therapy. Of interest is that despite the low and stable protein C levels during the observation period, changes in the APC-PCI complex concentration could be observed. The APC-PCI complex concentration is about 10^4 -fold lower than the zymogen protein C concentration and the key feature of the method [8] is that the level of uncomplexed protein C does not interfere in the APC-PCI determination.

Although the size of the material in the current investigation limits the ability to investigate survival related to APC-PCI complex concentration or other coagulation parameters, the variation of levels was similar for the APC-PCI complex,

F1+2 and TAT, indicating thrombin generation and coagulation activation that were not detected with other hemostatic parameters (As e.g. global hemostatic tests). A sensitive, specific and early marker of activation of the coagulation system would potentially provide the clinician with a tool for making timely decisions in the treatment of coagulation disturbances, thereby perhaps improving the outcome for this patient group. Currently, replacement with factor concentrates is used as a second line or last resort treatment when the clotting system is severely exhausted and the patient is in a poor condition, likely decreasing the potential effectiveness of the treatment. Our data indicate a potentiated procoagulant state at days 3 to 5 after start of induction treatment, which at least partially may be caused by the release of intracellular proteolytic enzymes. This should be considered in patients with fulminant septicemia and in those at high risk of more severe general hemostatic disturbances.

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Conflict of interest

The authors declare that there is no conflicts of interest statement.

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