Haemostatic parameters in acute promyelocytic leukemia patients treated with All Trans-Retinoic Acid (ATRA).

Dr. Mohammad Shnain Ali, Professor Saad Sh. Mansoor and Dr. Ali Muslim Al-Amiry.

Abstract

Background: Acute Pormyelocytic Leukemia (APL) is commonly associated with Disseminated Intravascular Coagulation (DIC) [1,2].

Objective: The work was conducted to identify patients with APL who show laboratory evidence of DIC (clinical or subclinical) with serial laboratory follow up.

Subjects and Methods: In this study 44 newly diagnosed APL patients were included. For each patient, full hemostatic investigations were done at time of diagnosis and repeated on day 3 and day 7 of therapy.

Results: Before starting therapy, all patients had elevated levels of D-dimer, indicating the presence of DIC. On day 7 of treatment, plasma D-dimer levels were normal in ATRA-treated group, but were high in the chemotherapy treated group, indicating that DIC was essentially resolved in ATRA treated patients.

Conclusion: DIC is commonly encountered at presentation in patients with APL, necessitating a rapid and full coagulation study in all patients. ATRA therapy in APL patients is associated with rapid improvement of coagulopathy, hence it will aid those patients to pass quickly the critical period of DIC. Therefore, ATRA therapy is justified to be used from day one of treatment in APL patients.

Keywords: All-trans retinoic acid, Acute promyelocytic leukemia & hemostatic parameters.

Introduction

About 54% of APL patients present initially with hemorrhagic manife-stations[1]. Fatal intracranial hemorrhage is the leading cause of death in 9-40% of patients[1]. APL-associated hemostatic disorders result from at least two distinct mechanisms due to the release of procoagulants and plasminogen activators from the leukaemic cells. Infections in all types of acute leukaemias play major roles in producing hypercoagulable states. The situation is rather complex in APL and an associated infection may greatly

affect haemostasis. Laboratory assessments show profound haemostatic imbalance compatible with clinical picture of DIC[3]. An important pathogenic role is attributed to the leukaemic cell properties for the activation of haemostatic mechanisms[4]. Both clinical DIC, (defined clinically by the presence of bleeding, oliguria, renal failure, and thrombocytopenia unresponssive to platelet transfusion), and subclinical DIC, (defined as a clinically compensated DIC in which slow cascade activation gives a time for the liver to compensate for coagulation factors as rapid as they are activated and removed), are common in APL with an incidence of (80%)[5]; however, DIC does occur in AML and ALL with an incidence of 18% & 9%, respectively[6].

Patients and methods

In this prospective study (from October 2002 to October 2003 excluding the period from 20/3/2003 to 2/5/2003 due to the war), a total of 44 newly diagnosed, untreated APL patients were admitted to Baghdad Teaching Hospitals [AL-Yarmook (30 case), Baghdad Medical City (10 cases), and Alkadhimiya (4 cases)] and they were included. Full clinical evaluation was done for each patient. Out of 44 patients, 24 patients were treated with ATRA (45mg/m^2/day, twice orally) plus the conventional chemotherapy [(16 of them were treated with Daunorubicin (DNR) in a dose of 45mg/m^2/day IV. for the days 1,3 and 5) and (8 patients were treated with cytosin arabenoside (Ara-C)in a dose of 100mg/m^2/day IV.for the first 7 days)], and 17 patients were treated with chemotherapy without ATRA [(14 of them were treated with DNR and Ara-C with the same above doses and durations) and (3 patients were treated with Etoposide in a dose of 100mg/m^2/day IV. for 1-5 days in addition to DNR and Ara-C)]. Before starting treatment, 3 patients were died. Blood samples were used for performing Complete blood count, PT, PTT, plasma fibrinogen level, and D-dimer test before starting treartment and repeated on day 3 and day 7 after starting treartment. Complete blood count was done using the manual methods. PT was determined using the commercially available kit (Biomerieux/Isimat 1-68 815/France) which consisted of thromboplastin with calcium. APTT was determined manually using the commercially available kit (Biomerieux / Cephalite-68 522/France). The calcium chloride

used in the procedure was provided at a concentration of 0.025mol/L (Biomerieux/Calcium Chloride-68 502/France).

Plasma fibrinogen level was determined by an automated coagulometer, using fibrinogen kit (Biomerieux/Fibrinomat-68 452/France). The test was done by making a 1:10 dilution of patients plasma (50 uL plasma and 450 uL Owren-Koller buffer, PH 7.35) in a glass tube at 37^0C. Then 0.2 ml of the diluted plasma was placed in a precise position in the instrument in which the temperature is exactly 37^0C. The position was already supplied manually with a small specially made ball. Exactly after 2 min. (incubation time), the beep emitted by the instrument to indicate the time of addition of 0.2 ml of the reagent (Human Calcium-Thrombin solution, 1.5 NIH Unit/ml). Results were automatically printed on a strip of paper and expressed in seconds. Assay results in g/L were taken directly from a table provided with the kit. Normal value was 1.5-4 g/L. D-dimer test was done using the commercially available kit (Biomerieux/FDP slidex direct-73 101/France). It is a rapid latex agglutination slide test for qualitative and semiquantitative determination of plasma D-dimer by agglutination of latex particle coated with anti-D-dimer monoclonal antibody.

The positive reaction is the detection of a visible agglutination within 2 min, by mixing equal volumes (20 uL was used) of the test plasma and the latex particle suspension against a black background (black test cards were supplied with the kit).The negative result is when no visible agglutination had occurred within 2 min. Normal value is ≤ 0.5 ug/ml.

Results

1. Before treatment:

Complete blood count, PT and PTT were variable. Low plasma fibrinogen levels (for the age) were found in 42 (95.5%) of patients with a median value of 0.9 g/L while normal levels (for the age) were found in 2 (4.5%) of patients with a median value of 2.2 g/L.

Plasma D-dimer test was positive in all patients with a median value of 6 ug/ml, ranging between 1-32 ug/ml. Plasma D-dimer concentration had a statistically significant relation (P value <0.05) with clinical and subclinical DIC. Initial haemostatic parameters were consistent with DIC in all patients.

2. Results after starting treatment:

Three patients were died before starting treatment so that the total number of patients became 41(100%). On day 3 of therapy, PT, PTT and fibrinogen levels show no statistically significant differences between the two groups of patients.

All chemotherapy-treated patients (without ATRA) who had high Plasma D-dimer concentrations before treatment showed significantly (P value <0.05) higher D-dimer concentrations (with a median value of 16 ug/ml, ranging from 8 to 32 ug/ml) than before treatment. Vise versa is true in ATRA treated patients. On day 7 of treatment, all ATRA-treated patients [24 (100%)] had normal plasma fibrinogen levels (with a median value of 1.8 g/L, ranging from 1.6 to 2.3 g/L) and normal Plasma D-dimer concentrations with a median value of ≤ 0.5 ug/ml while chemotherapy treated patients had low plasma fibrinogen levels, but with higher values than day 3 of therapy and high Plasma D-dimer concentrations but with lower values than day 3 of therapy (with a median value of 8 ug/ml, ranging from 4 to 16 ug/ml).

Unlike day 3 of treatment, day 7 of treatment had plasma D-dimer concentrations which had no statistically significant differences (P value > 0.06) between the two groups of patients.

Discussion

Hypofibrinogenaemia was present in 42 (95.5%) of patients at time of diagnosis. Many studies had showed that plasma fibrinogen level is variable in cases of subclinical DIC. Hypofibrinogenaemia was repoted to be present in 60, 80, and 100% of cases [7,8 &9].

Plasma D-dimer test was positive in 44 (100%) of patients at time of diagnosis with a median value of 6ug/ml. This finding is consistent with findings of previous studies which showed positive results in 100% of cases with a median of 13ug/ml.The differences in the median values were due to the different methods[5]. False positive tests can occur in the presence of high titer of rheumatoid factor[10]. In the studied patients, no patient had arthritis on clinical background and the positivity of D-dimer test was associated with clinical and laboratory features of DIC; however, rheumatoid factor assay is sometimes necessary[10].

The results demonstrate that ATRA therapy in patients with APL and DIC rapidly improves abnormal haemostatic parameters.

These data are consistent with previous reports which showed favourable outcome of coagulopathy in ATRA-treated APL patients[5]. The earliest indicators of response to treatment with ATRA was reported to be a normalization of coagulopathic abnormalities such as hypofibrinogenaemia and increased plasma D-dimer concentration[11] which were clearly found in this study.

Red cells and platelet transfusions during therapy can play a role in improving haemostatic parameters[12]; however, all patients included in this study had been transfused; however, no improvement was seen in the chemotherapy-treated group untill day 7 of treatment.

Recommendations

1. The availability of well-equipped laboratory supplied with all recent highly sensitive methods for diagnosis with greater certainty like plasma D-dimer assay, thrombin/anti-thrombin complex assay, and plasmin/antiplasmin complex assay.

2. Because of a great role in minimizing haemostatic derrangement, ATRA should be available for all APL patients.

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