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Prospective evaluation of coagulopathy in multiple myeloma patients before, during and after various chemotherapeutic regimens

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Abstract

Background: Venous thromboembolism (VTE) occurs frequently in multiple myeloma patients, especially during induction treatment with thalidomide in combination with anthracyclines and/or dexamethasone. Several coagulation abnormalities have been described in untreated myeloma patients, but these have not been prospectively evaluated during and after treatment.
Patients and methods: We performed a prospective study in 138 multiple myeloma patients in whom coagulation factor levels were evaluated longitudinally before, during induction and after intensification. Patients were randomized to induction treatment consisting of adriamycin and dexamethason, in combination with either vincristin (VAD), thalidomide (TAD), or bortezomib (PAD) followed by high-dose melphalan (HDM) and autologous stem cell transplant (ASCT).
Results: Factor VIII:C (FVIII:C) and von Willebrand factor (VWF) were significantly elevated before treatment (median FVIII:C 2.26 U/ml, VWF:Ag 1.95 U/ml). Irrespective of the type of induction regimen, these variables increased strongly during induction therapy (FVIII:C 2.55 U/ml and VWF:Ag 2.96 U/ml). Fibrinogen also showed a significant increase after induction therapy (3.5 g/l pre-treatment and 4.0 g/l after treatment, respectively, $P < 0.001$). This was significantly higher in TAD than VAD treated patients. Three to six month after ASCT levels of VWF and FVIII:C had decreased to values lower than observed before treatment (1.71 and 1.67 U/ml respectively). There was no correlation between the increased levels at start and the response of multiple myeloma to treatment. High levels of VWF, fibrinogen and FVIII:C before start of treatment were significantly associated with mortality. Fourteen patients (10%) developed a venous thrombotic event (VTE). The coagulation factor abnormalities before and during treatment were not associated with the development of VTE.
Conclusion: During induction treatment several changes in coagulation factor levels are observed, which may result in a prothrombotic state. Larger studies are required to establish whether the changes in these coagulation factors during induction treatment contribute to the increased risk of venous thromboembolism in multiple myeloma patients.

Keywords: Coagulation; Von Willebrand factor; Factor VIII; Multiple myeloma; Venous thrombosis; Thalidomide; Bortezomib

1. Introduction

Multiple myeloma is associated with an increased risk for venous thromboembolism (VTE). This risk is even higher when multi-agent chemotherapy and/or prednisone are combined with anti-angiogenic drugs, such as thalidomide [1–3]. The combination of thalidomide and doxorubicin is associated with the occurrence of venous thrombosis in 10–30% of the patients, whereas venous thrombosis occurs in only

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1–5% of the patients receiving more conventional medication [4–7]. Low molecular weight heparin has been shown to reduce effectively the risk of VTE during thalidomide treatment [8,9]. Treatment with the recently introduced proteasome inhibitor bortezomib has not been associated with an increased incidence of venous thrombosis [10]. However, bortezomib has so far only been used as rescue or second line treatment, whereas an increased incidence of VTE is especially seen during induction treatment of newly diagnosed multiple myeloma [11].

Multiple alterations in the hemostatic system promoting coagulation have been found in untreated myeloma patients, including elevated levels of factor VIII:C (FVIII:C) and von Willebrand factor (VWF), activated protein C (APC) resistance, and a hypofibrinolytic state [3,11–15]. Elice et al. studied APC resistance before and after treatment. Nine percent of the patients had a transient APC resistance, which was associated with an increased risk of VTE [16].

Because VTE occurs mainly during the induction treatment of multiple myeloma, it is of utmost interest to study the coagulation disorders during treatment. Therefore we evaluated coagulation factor levels longitudinally before, during and after different intensive chemotherapeutic regimens. The aim was to find coagulation factor abnormalities which might correlate with an increased VTE risk and outcome of therapy. A second aim was to investigate whether the various chemotherapeutic regimens are associated with different coagulation profiles.

2. Materials and methods

2.1. Patients

Consecutive patients under the age of 65 years with newly diagnosed multiple myeloma according to the Mayo clinic criteria who were admitted to the department of Haematology of the Erasmus MC Rotterdam or the University Medical Center Utrecht, both academic tertiary referral hospitals in the Netherlands, were included in the study. Only patients who were eligible for intensive chemotherapy followed by high-dose melphalan (HDM) and autologous stem cell support were included. Informed consent was obtained from all patients. According to the Declaration of Helsinki, the protocol was approved by the Research Ethics Board of both participating hospitals. Patients were randomized to receive 3 courses of adriamycin, dexamethasone in combination with either thalidomide (TAD) or vincristine (VAD) (HOVON 50-study) (17) or in combination with bortezomib (PAD, 1.3 mg/m^2, day 1, 4, 8, and 11) or vincristine (VAD) (HOVON 65-study, ongoing) as induction treatment in all patients followed by stem cell mobilization with cyclophosphamin, adriamycin and dexamethason (CAD) and intensive treatment with high-dose melphalan (200 mg/m^2) and autologous stem cell transplantation as previously described [17]. The starting dose of thalidomide was 100 mg and could be escalated to 200 mg. Thalidomide was stopped before stem cell mobilization and re-administered after the autologous stem cell transplantation in a lower dose (50 mg). Patients receiving thalidomide also received LMWH during the induction phase of treatment. Patients receiving VAD in the HOVON 50 trial also received IFN (3×10^6 IU, 3 times weekly) as maintenance therapy after stem cell transplantation. Patients treated with bortezomib received bortezomib (1.3 mg/m^2, twice monthly) as consolidation.

2.2. Methods

Blood samples were collected at time of diagnosis before start of treatment (time point 1), directly after therapy with VAD, TAD or PAD (time point 2), and 3–6 months after high-dose melphalan treatment (time point 3). Venous blood was collected using a vacutainer system in citrate (0.105 M, Beckton-Dickinson, Plymouth, UK) and centrifuged at 4 °C at 2000 × g for 10 min. The collected plasma was additionally centrifuged for 10 min at 2000 × g and stored in small aliquots at −70 °C until use. Genomic DNA was isolated from the white cell fraction of citrated blood, using a standard salting out procedure.

FVIII:C was measured by a one stage clotting assay using Platelin (Organon Teknika, Durham, USA) and factor VIII deficient plasma (Biopool, Ventura, USA) or a commercial coagulation method (Boehringer Mannheim, Mannheim, Germany). VWF:Antigen (VWF:Ag) was measured by using the LIA test (Boehringer Mannhein or an in-house sandwich enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human vWF and horseradish peroxidase conjugated anti-human vWF (DakoCytomation, Glostrop, Denmark). VWF collagen binding activity (vWF:CB) was measured by an in-house EIA using type I collagen (Sigma, St. Louis, USA) and horse radish peroxidase conjugated anti-human vWF. VWF Ristocetin Cofactor activity (vWF:RCo) was measured with an aggregometric method using formaline-fixed platelets and ristocetin (Diagnostiga Stago, Asnieres, France). All assays were calibrated with pooled normal plasma (factor assay control plasma, George King Bio-Medical, Kansas, USA). Fibrinogen was measured as described by Clauss [18]. Screening for lupus anticoagulant (LAC) was performed essentially as recommended by the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies (APA) and was described earlier [19,20]. Anticardiolipin antibodies (ACA) were tested by enzyme-linked immunosorbent sandwich assay, using cardiolipin (Sigma, Zwijndrecht, the Netherlands) and horseradish peroxidase conjugated-rabbit anti-human IgG and IgM (Dakopatts). Anticardiolipin antibodies were tested by enzyme-linked immunosorbent sandwich assay, using cardiolipin (Sigma, Zwijndrecht, the Netherlands) and horseradish peroxidase conjugated-rabbit anti-human IgG and IgM (Dakopatts). Anticardiolipin titers were calculated and considered positive if titers were above