# RESEARCH ARTICLE

# Hemostatic Abnormalities in Multiple Myeloma Patients

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## Abstract

**Background:** Multiple myeloma (MM) is a neoplastic plasma cell disorder characterized by clonal proliferation of plasma cells in the bone marrow. Diverse hemostatic abnormalities have been reported in patients with myeloma which predispose to bleeding and also thrombosis. **Methods:** Complete blood count, biochemical parameters and parameters of hemostasis i.e. platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), factor VIII assay results, plasma fibrinogen, D-dimer and lupus anticoagulant, were assessed in 29 MM patients and 30 age matched controls. **Results:** The most frequent abnormal screening parameter was APTT. Of the six indicative of a bleeding tendency i.e. thrombocytopenia, prolonged PT, APTT, TT, reduced plasma fibrinogen and factor VIII, at least one was abnormal in 8 (27.6%) patients. Of the four prothrombotic markers, lupus anticoagulant, D-dimer, elevated factor VIII and plasma fibrinogen, one or more marker was present in 24 (82.7%). D-dimer was the most common prothrombotic marker, being elevated in 22 (75.9%) patients. One or more laboratory parameter of hemostasis was abnormal in all 29 (100%) patients. Though thrombotic complications are reported to be less frequent as compared to hemorrhagic manifestations, one or more marker of thrombosis was present in 24 (82.7%) patients. **Conclusion:** This study provided laboratory evidence of hemostatic dysfunction which may be associated with thrombotic or bleeding complications at diagnosis in all MM patients. Hence, screening for these abnormalities at the time of diagnosis should help improved prognosis in such cases.

**Keywords:** Multiple myeloma- hemostatic abnormalities- thrombosis- bleeding

## Introduction

Multiple myeloma (MM) is a neoplastic plasma cell disorder characterized by the clonal proliferation of plasma cells in the bone marrow and presence of monoclonal protein in the blood or urine (Dispenzieri et al., 2009). Worldwide, it accounts for 1% of all malignancies, 10-13% of all haematological malignancies (Kumar et al., 2006) and 1% of all cancer deaths every year (Saraf et al., 2012). In India, the reported incidence is 0.3 to 1.9 and 0.4 to 1.3 per 1,00,000 in females and males respectively(Kumar et al., 2006).

Diverse hemostatic abnormalities have been reported in patients with myeloma which predispose the patient to bleeding (Saif et al.,2001) and also thrombosis (Leebeek, 2016). Inhibition of vWF, FVIII, dysfibrinogenemia and abnormalities in fibrinolysis have been described which are associated with bleeding (Saif et al., 2001; Zangari et al., 2007; Kotlín et al., 2008). In a study on 157 patients with MM and 34 patients of monoclonal gammopathy of unknown significance (MGUS), an isolated prolonged PT was the most common abnormal coagulation test, observed in 25% patients (Pandey et al., 2013). Similar results were reported by other authors (Saif et al., 2001). Besides these, development of autoantibodies such as antiprothrombin antibodies, lupus anticoagulant (LA) and activation of coagulation have been reported predisposing the patient to thrombosis (Zangari et al., 2007).

Hemostatic abnormalities are associated with a poor prognosis. As most patients with these abnormalities are asymptomatic; their early identification and treatment will help in improving the prognosis.

## Materials and Methods

*Subjects*

Twenty-nine patients of MM diagnosed as per standard criteria (Dispenzieri et al., 2009) and thirty matched controls were enrolled in the study. Written informed consent was obtained from all patients before their inclusion in the study. The study received clearance from the Institutional Ethics Committee for human research.

*Investigations*

The following tests were done on all patients and controls: Complete blood counts (Automated hematology analyser LH500), biochemical parameters including serum calcium, creatinine and total proteins, β-2 microglobulin, hemostatic parameters including prothrombin time (PT; Dade Behring Thromborel S), activated partial

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thromboplastin time (APTT; Dade Behring Actin FS), thrombin time (TT; Sigma- Aldrich), plasma fibrinogen (Quantia, Tulip), factor VIII assay (PZ Cormay S.A.), D-dimer (Zymutest, Hyphen Biomed) and LA (dRVVT screening and confirmatory test, Tulip) .

*Statistical analysis*

The data was subjected to statistical analysis using IBM SPSS software statistics 20 package. Mean value of continuous variables was compared using unpaired t-test. Level of significance and level of confidence were 5% and 95% respectively.

# Results

*Patient characteristics*

There was no significant difference in the age of patients (Range 29-75y, Mean ±SD 57±11.8 y) and controls (Range 29-75y, Mean ±SD 58±10.5 y). Both groups comprised of 17 males and 13 females with a M: F ratio of 1.4:1. The most common clinical manifestation was weakness and fatigue (23/29, 79.3%). Serum electrophoresis (agarose gel, pH 8.6) revealed a monoclonal band in all (100%) patients.

*Haematological parameters*

The haematological parameters of patients and controls are shown in Table 1. Hemoglobin, RBC count and MCH were significantly lower (p <0.01) in patients as compared to controls. There was no difference in MCV, TLC and platelet count of patients and controls. ESR was significantly (p <0.01) higher in patients as compared to controls and was elevated in all (100%) patients, being normal in controls. Anaemia and leucocytosis were identified in 29 (100%) and 5 (17.2%) patients respectively.

*Biochemical parameters*

Hypercalcemia was observed in 3 (10.3%) patients. Raised creatinine, total protein and β-2 microglobulin levels (reference range 1.1-2.4 mg/L) were observed in 10 (34.5%), 6 (20.7%) and 19 (65.5%) patients respectively.

Table 1. Hematological Parameters

| Parameters | Patients | Controls | p-value |
|---|---|---|---|
| | Mean ±SD | Mean ±SD | |
| Hemoglobin (g/dl) | 8.7±1.8 | 13.8±1.2 | p <0 .01;  significant |
| RBC count (x10^{12}/L) | 3.21±0.75 | 4.62±0.44 | p <0.01;  significant |
| MCV (fl) | 87.1±9.5 | 89.4±4.4 | p =0.240; not significant |
| MCH (pg) | 27.4±2.9 | 29.9±2.1 | p <0.01; significant |
| TLC (x10^9/L) | 8.9±2.6 | 8.0±1.4 | p =0.105; not significant |
| Platelet count (x10^9/L) | 211±127 | 246±75 | p =0.207; not significant |
| ESR(mm 1st hr) | 78±37 | 11±5 | p<0.01; significant |

SD, Standard Deviation; RBC, Red Blodd Cell; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; TLC, Total Leucocyte count; ESR, Erythrocytic Sedimentation

Table 2. Screening Tests of Hemostasis

| Parameter | Patients | Controls | Abnormality in patients | |
|---|---|---|---|---|
| | Mean±SD | Mean±SD | No. | % |
| PT (secs) | 14.1±3.3* | 12.4±0.9* | 14 | 48.3 |
| APTT (secs) | 39.6±10.1* | 29.6±1.3* | 20 | 68.9 |
| TT (secs) | 11.3±2.7* | 9.8±0.5* | 10 | 34.5 |

* p<0.01, significant; SD, Standard Deviation; PT, Prothrombin Time; APTT, Activated Partial Thromboplatin Time; TT, Thrombin Time.

Table 3. Plasma Fibrinogen and Factor VIII

| Parameter | Mean± SD | | p-value |
|---|---|---|---|
| | Patients | Controls | |
| Fibrinogen (mg/dl) | 272.9±185.6 | 225.0±58.7 | p=0.194; not significant |
| Factor VIII (%) | 63.5±45.8 | 102.8±28.4 | p<0.01; significant |

SD, Standard Deviation

*Screening tests of hemostasis*

PT, APTT and TT were significantly (p<0.01) higher in patients as compared to controls. PT and APTT were prolonged in 48.3% and 68.9% patients respectively. A prolonged TT was observed in 34.5% patients. The tests were within the reference range in controls (Table 2)

*Plasma fibrinogen and FVIII*

There was no significant difference in the level of plasma fibrinogen of patients and controls. FVIII was significantly (p <0.01) lower in patients (63.5±45.8%) as compared to controls (102.8±28.4%) (Table 3).

*D-dimer and Lupus Anticoagulant*

D-dimer was significantly (p <0.01) higher in patients (Mean ±SD; 2444.8±2566 ng/ml) as compared to controls (165.3±101.4 ng/ml). Lupus anticoagulant was detected in 2 (6.9%) patients being negative in all controls.

*Abnormal test of hemostasis*

Plasma fibrinogen and FVIII were reduced in 11 (37.9%) patients each. An elevated plasma fibrinogen was observed in 10 (34.5%) patients while FVIII was elevated in 1 (3.4%) patient. D-dimer was elevated in 22 (75.9%) patients (Table4).

*Markers of a Bleeding Tendency*

Of the six parameters indicative of a bleeding tendency i.e. thrombocytopenia, prolonged PT, APTT, TT, reduced plasma fibrinogen and factor VIII, one parameter was abnormal in 8 (27.6%) patients. Of these, an isolated prolonged APTT was the most frequent

Table 4. Abnormal Tests of Hemostasis

| Parameter | Cut off values | Patients | |
|---|---|---|---|
| | | Number | Percentage % |
| Fibrinogen(mg/dl) | <150 | 11 | 37.9 |
| | >400 | 10 | 34.5 |
| Factor VIII(%) | <50 | 11 | 37.9 |
| | >170 | 1 | 3.4 |
| D-dimer(ng/ml) | >400 | 22 | 75.9 |

abnormality seen in 4(50%) patients. One patient had only thrombocytopenia, 2 patients had reduced plasma fibrinogen and one patient had an isolated prolonged PT. Two parameters were abnormal in 7 (24.1%) patients while 3 parameters were abnormal in 8 (27.6%) patients. Five (17.2%) patients showed an abnormality in four tests with 1 (3.4%) patient having abnormality in all five test results.

*Prothrombotic markers*

Of the four prothrombotic markers including lupus anticoagulant, D-dimer, elevated factor VIII and plasma fibrinogen, one or more marker was present in 24(82.7%) patients.

One, two and three markers were present in 14 (48.3%), 9 (31.0%) and1 (3.4%) patients respectively. D-dimer was the most common prothrombotic marker, being elevated in 22 (75.9%) patients.

One or more laboratory parameter of hemostasis was abnormal in all 29 (100%) patients with myeloma.

## Discussion

This study evaluated haematological and hemostatic parameters in 29 patients of multiple myeloma and 30 matched controls. Hemoglobin was significantly ($p < 0.01$) lower in patients as compared to controls. Anemia was identified in all patients. Anemia is a common clinical feature in patients with MM and contributes to weakness and fatigue in 82% patients (Dispenzieri et al., 2009). Weakness and fatigue were the most common clinical features (80%) in this study.

Thrombocytopenia was observed in 5(8.5%) patients and thrombocytosis in 1(3.4%) patient. Kyle et al observed thrombocytopenia in 5% and thrombocytosis in 2% MM patients (Kyle et al, 2003). Perkin et al reported an abnormal platelet count more frequently in myeloma (20-30%) than in other macroglobulinemias (0%) (Perkin et al., 1970). Thrombocytopenia was the initial presentation in three patients who were subsequently diagnosed with MM (Gupta et al., 2000). Thrombocytopenia which is either chemotherapy induced or due to marrow replacement by plasma cells contributes to bleeding in these patients.

Prolonged PT, APTT and TT were observed in 14 (48.3%), 20 (69%) and 10 (34.5%) patients respectively. Both PT and APTT were prolonged in 10 (34.5%) patients. One or more screening coagulation test was abnormal in all (100%) patients. Bleeding was not observed in any of the patients. Abnormal screening coagulation tests have been commonly reported in patients with MM majority of whom were asymptomatic (Pandey et al., 2013; Eby, 2007). In a previous study on 22 patients of IgG myeloma, asymptomatic prolongation of PT, APTT and TT was seen in 59%, 18% and 71% patients respectively (Perkin et al, 1970).

In this study, the most frequent abnormal screening test was APTT. In contrast, other authors have reported an isolated prolonged PT as the most common abnormality (Pandey et al., 2013; Teng et al, 2007; Elice et al., 2006).

In a study on 252 patients of MM, an isolated prolonged PT was seen in 25% patients (Pandey et al., 2013). An isolated prolonged APTT was seen in 2 (<1%) patients while both PT and APTT were prolonged in 10 (4%) patients (Pandey et al., 2013). Elice et al., (2006) reported prolonged PT in 24% of newly diagnosed MM patients.

A correlation was then done between prolonged PT, APTT and TT and clinical and laboratory parameters of MM. No correlation was observed between any of the screening coagulation tests and other prognostic factors. In a study on 101 newly diagnosed patients of MM, there was no correlation between APTT and stage of MM or type of M protein and serum light chain concentration (Huang et al., 2015). In a study on 252 patients of MM, there was no significant difference with respect to age, gender and immunoglobulin type in patients with and without prolonged PT (Pandey et al., 2013). Huang et al., (2015) observed a significant ($p < 0.01$) increase in M protein in patients with prolonged PT. A significant ($p < 0.01$) increase in PT was observed as the stage of MM increased. Teng et al., (2007) found prolonged APTT to be an independent prognostic factor in IgA myeloma.

There was no difference in the level of plasma fibrinogen of patients and controls. Plasma fibrinogen and FVIII were reduced in 11 (37.9%) patients each. An elevated plasma fibrinogen was observed in 10 (34.5%) patients while FVIII was elevated in 1 (3.4%) patient. Both parameters did not show any correlation with other prognostic factors which may possibly be due to the small number of cases included in the study.

In a study on 252 patients of MM, plasma fibrinogen was significantly ($p < 0.001$) lower in patients with prolonged PT as compared to patients with normal PT. However, there was no correlation between fibrinogen activity and PT (Pandey et al., 2013). Huang et al observed a negative correlation between fibrinogen and M protein level (Huang et al., 2015).

As seen in this study, a reduced level of FVIII in patients with MM has been reported by other authors also (Perkin et al., 1970; Ouaaliti et al., 2016). The reduced levels increase the risk of bleeding in these patients. Komiya et al reported a case of bi clonal MM with reduced FVIII who subsequently developed intracerebral haemorrhage (Komiya et al., 1990).

D-dimer, a marker of fibrinolysis, was elevated in 22 (75.9%) patients. Excessive fibrinolysis has been reported in several cases of MM that manifest as bleeding and abnormal coagulation tests such as elevated D-dimer. In a study on 78 newly diagnosed cases of multiple myeloma, elevated D–dimer was observed in 63% patients (Elice et al., 2006). In a study on 101 newly diagnosed patients of MM, D-dimer mean was significantly higher than normal ($>0.5 \mu g/ml$) ($P \leq 0.01$) (Huang et al., 2015). However, in a study on 134 newly diagnosed patients of MM and 124 controls, no difference was observed in the level of D-dimer (Auwerda et al., 2017).

In this study, LA was positive in 2 (6.9%) patients. LA is infrequently reported in association with myeloma. Auwerda et al reported lupus anticoagulant in 12% myeloma patients (Auwerda et al., 2017). LA is a risk

factor for arterial and venous thrombosis, rarely these patients may also present with bleeding (Hara et al., 2013). However in this study, LA positive patients did not have clinical bleeding/features of thrombosis.

In the present study FVIII was increased in only 1 (3.4%) patient. An elevated FVIII has been reported in upto 50% of MM patients (Elice et al., 2006; Auwerda et al., 2017). Elevated FVIII increases the risk of thrombosis. In a study on 20 myeloma patients, 7 (35%) had experienced an episode of venous thromboembolism. Extremely high level of factor VIIIc (mean 352%) and vWF-Ag (mean 374%) were seen in all the cases. FVIIIc levels were significantly higher in patients who developed VTE as compared to patients without VTE (Minnema et al., 2003). Similar results were reported by Robak et al., (2012).

In this study, plasma fibrinogen was increased (>400 mg/dl) in 10 (34.5%) patients. An elevated plasma fibrinogen is a risk factor for thrombosis. Elice et al observed an elevated plasma fibrinogen in 68% newly diagnosed patients of MM (Elice et al., 2006). The elevated IL-6 levels in MM patients, increase plasma levels of fibrinogen and FVIII (Zangari et al., 2007).

This study observed laboratory evidence of hemostatic dysfunction which may be associated with thrombotic or bleeding complications at diagnosis in all patients. Though thrombotic complications are reported to be less frequent as compared to hemorrhagic manifestations, one or more marker of thrombosis was present in 24 (82.7%) patients. The coagulation abnormalities that are observed more frequently in patients with active disease can normalize with effective treatment. The risk of venous thromboembolism is highest at diagnosis, hence screening for these abnormalities at this time assumes importance and will help in improved prognosis.

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