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ORIGINAL RESEARCH

Evaluation of thrombosis-related biomarkers before and after therapy in patients with multiple myeloma

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Background: Thrombosis is one of the complications in the clinical course of multiple myeloma (MM). Vascular endothelial cells and/or the hemostatic-coagulatory system are thought to play an important role in thrombosis of MM. In addition to melphalan-prednisone (Mel-P) therapy, several new therapeutic drugs such as lenalidomide or bortezomib have been developed and show effectiveness against MM. However, these new drugs also have risk of therapy-related thrombosis.

Methods: We assessed 103 MM patients and 30 healthy controls, using enzyme-linked immunosorbent assays to evaluate five biomarkers: platelet-derived microparticles (PDMP), plasminogen activator inhibitor-1 (PAI-1), high mobility group box protein-1 (HMGB1), endothelial protein C receptor (EPCR), and soluble vascular cell adhesion molecule-1 (sVCAM-1). The effects of Mel-P, bortezomib, and lenalidomide on the plasma concentrations of these biomarkers were investigated.

Results: The plasma concentrations of PDMP, PAI-1, HMGB1, EPCR, and sVCAM-1 were higher in MM patients than in healthy controls. Mel-P, bortezomib, and lenalidomide therapies all reduced biomarker levels after treatment. However, when only patients with higher levels of EPCR were compared, differences were seen between the three therapies in the elevation of PDMP, HMGB1, and PAI-1.

Conclusion: These results suggest that both MM and therapies for MM can induce a hypercoagulable state. The elevated risk of thrombosis conferred by hypercoagulability increases patient morbidity and mortality. Attention should be paid to therapy-related thrombosis when new therapeutic regimens are selected for MM patients.

Keywords: multiple myeloma, bortezomib, lenaridomide, thrombosis, biomarker

Introduction

Multiple myeloma (MM) is an incurable malignancy of the plasma cells.[1] The majority of MM patients relapse, regardless of their initial treatment.[1] Melphalan-prednisone (Mel-P) has long been the treatment of choice for MM patients over 65 years of age.[2] Recently, several new therapeutic drugs, such as lenalidomide and bortezomib, have been developed and show effectiveness against MM.[3,4] However, the pathologic characteristics of individual MM patients differ, depending on the products secreted by MM cells. In addition, vascular endothelial cells and/or the hemostatic-coagulatory system are thought to play an important role in the clinical course of MM.[5,6]

Many individuals with cancer are in a hypercoagulable state, and the elevated risk of thrombosis conferred by hypercoagulability increases patients morbidity and mortality.[7–9] Cancer patients frequently develop venous thromboembolism (VTE),

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and various potentially predictive biomarkers have been evaluated for association with VTE in cancer progression.[10–12] For example, analysis of cell-derived microparticles (MP), high mobility group box protein 1 (HMGB1), plasminogen activator inhibitor-1(PAI-1), and soluble endothelial protein C receptor (sEPCR) can effectively predict the risk of VTE development.[13–16] Previous studies have further demonstrated a significant relationship between these markers and thrombosis with MM.[17–20] In addition, although new drugs such as bortezomib, lenalidomide, or monoclonal antibodies can improve the prognosis of MM, these drugs can also be accompanied by side effects such as VTE.[22–26] Therefore, here we measured various thrombosis-related biomarkers in patients with MM to study the clinical significance of these biomarkers in MM and their relationship with MM therapy.

Patients and methods

Patients

The study group consisted of 103 patients newly diagnosed with symptomatic, measurable MM,[21] defined in accordance with the Guidelines for Diagnosis and Treatment of MM in Adults,[27] presenting at our hospital between May 2010 and August 2016. As a control group, 30 healthy volunteers were recruited from the hospital staff and other sources. All study participants provided signed informed consent, and this study was approved by the Ethics Committee at Kansai Medical University, Hirakata, Osaka, Japan (no 110788).

Study design

A total of 91 patients were assigned to receive Mel-P (n=23), bortezomib (n=31), or lenalidomide (n=37) (Figure 1). The Mel-P regimen consisted of six 28-day cycles of melphalan (0.18 mg/kg body weight on days 1 through 4) and prednisone (2 mg/kg body weight on days 1 through 4). The bortezomib regimen consisted of subcutaneous bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11), oral cyclophosphamide (500 mg on days 1, 8, and 15), and oral dexamethasone (40 mg on days 1, 8, and 15). The cycle was repeated every 21 days.[28,29] The lenalidomide regimen consisted of either oral lenalidomide at 25 mg daily on days 1–21 plus oral dexamethasone at 40 mg daily on days 1–4, 9–12, and 17–20 of each 28-day cycle, or the same schedule of lenalidomide plus oral 40 mg daily on days 1, 8, 15, and 22 of the cycle.[30,31] Treatment was continued until disease progression stopped or unacceptable adverse effects developed. The primary study end point was an improvement in various biomarkers; secondary end points included response rate, response quality, and adverse events.

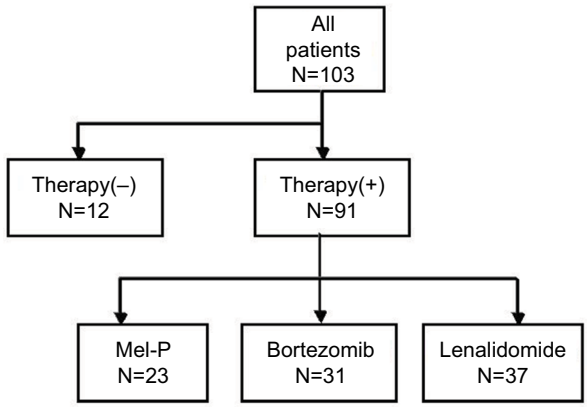

Figure 1 Randomization and follow-up of patients included in the trial.
Notes: A total of 91 patients underwent randomization: 23 were assigned to treatment with Mel-P, 31 with bortezomib, and 37 with lenalidomide.
Abbreviation: Mel-P, melphalan and prednisone.

Plasma levels of platelet-derived microparticle (PDMP), cytokines, soluble factors, and PAI-1

Fasting blood samples were obtained from the peripheral veins of patients and controls, using 21-gauge needles to minimize platelet activation, and were transferred into vacutainers containing ethylenediaminetetraacetic acid-citrate dextrose (NIPRO Co. Ltd., Osaka, Japan). The samples were gently mixed by inverting the tubes once or twice and kept at room temperature for a maximum of 2–3 hours. Samples were centrifuged at 8,000 × *g* for 5 minutes, and 200 μL at the top of each 2 mL upper layer was withdrawn to avoid contamination by platelets. These plasma samples were stored at –40°C until analysis. PDMP was measured by enzyme-linked immunosorbent assay (ELISA; JIMRO Co. Ltd., Tokyo, Japan).[32,33] Plasma concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1), tumor necrosis factor α (TNFα), and PAI-1 were measured using monoclonal antibody-based ELISA kits (Invitrogen International Inc., Camarillo, CA, USA). HMGB1 was measured using the HMGB1 ELISA kit (Shino-test Corp., Kanagawa, Japan). Plasma sEPCR levels were measured by ELISA (R&D Syetems Inc., Minneapolis, MN, USA). The recombinant products and standard solutions provided with the commercial kits were used as positive controls in each assay. All kits were used in accordance with the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± SD and analyzed using multiple regression (stepwise method), as appropriate. Between-group comparisons were made using the Newman–Keuls test and Scheffe's test. All statistical analyses were performed using Stat Flex (version 6; Artech Co., Ltd., Osaka, Japan) software, with *p*-values less than 0.05 considered statistically significant.

Results

The plasma concentrations of biomarkers in patients newly diagnosed with MM and in healthy controls are shown in Table 1. HMGB1, PDMP, sVCAM-1, PAI-1, and sEPCR concentrations were higher in patients than in controls.

The demographic and baseline characteristics of the treated patients were similar among the three groups (data nor shown). Treatment with Mel-P for 6 months significantly reduced the plasma concentrations of PDMP, relative to baseline, but did not significantly alter the plasma concentrations of TNFα, HMGB1, sVCAM-1, PAI-1, or sEPCR (Figures 2 and 3). In contrast, treatment with bortezomib or lenalidomide alone improved the levels of all tested biomarkers other than TNFα (Figures 2 and 3).

Table 1 Plasma levels of cytokines, PDMP, and soluble factors

Biomarker	Controls (n=30)	Patients (n=103)
TNFα (pg/mL)	13.2±11.5	23.4±16.8^{NS}
HMGB1 (ng/mL)	3.1±0.9	9.9±1.2$^{p<001}$
PDMP (U/mL)	8.2±1.5	27.9±4.6$^{p<0.001}$
sVCAM-1 (ng/mL)	627±219	1,866±1,182$^{p<0.001}$
PAI-1 (ng/mL)	9.3±2.5	36.8±7.9$^{p<0.001}$
sEPCR (ng/mL)	84±25	180±68$^{p<0.001}$

Notes: Data are shown as mean ± SD. *p*-value, patients versus controls.
Abbreviations: TNFα, tumor necrosis factor α; HMGB1, high mobility group box protein 1; PDMP, platelet-derived microparticles; sVCAM-1, soluble vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; sEPCR, soluble endothelial protein C receptor; NS, not significant.

Nineteen of 91 patients showed remarkable elevation of sEPCR (sEPCR >248 ng/mL) (including four patients treated with Mel-P, seven with bortezomib, and eight with lenalidomide). Four biomarkers (HMGB1, sVCAM-1, PAI-1, and PDMP) showed no significant concentration changes before and after treatment (Table 2). However, when only patients with higher levels of sEPCR were compared, differences were seen between the three therapies in the elevation of HMGB1, sVCAM-1, PAI-1, and PDMP (Figure 4). Specifically, only lenalidomide treatment was associated with the significant elevation of these four biomarkers after 6 months (Figure 4).

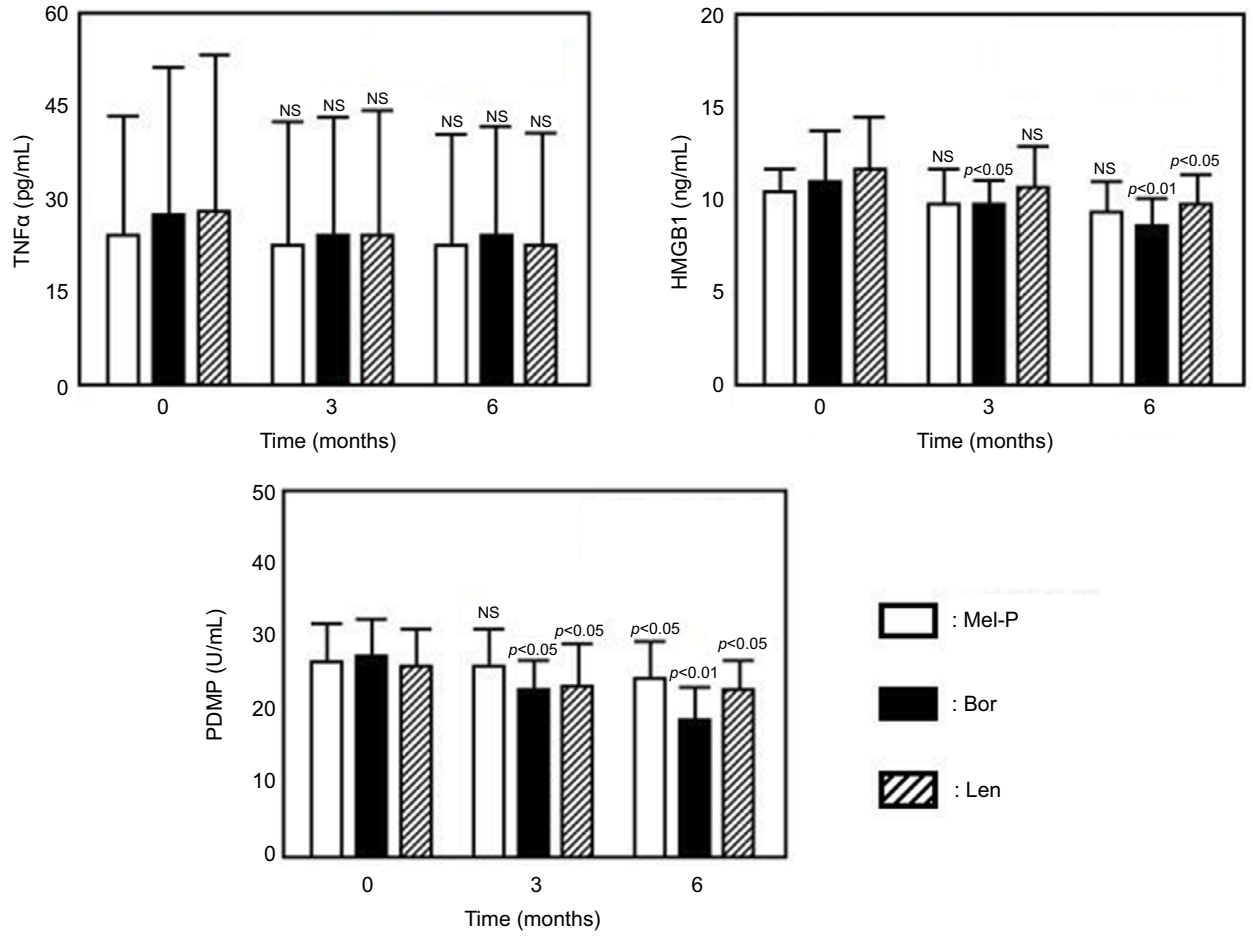

Figure 2 Changes in the plasma levels of TNFα, HMGB1, and PDMP before and after treatments.
Notes: Data are shown as mean ± SD. *p*-value, patients versus controls.
Abbreviations: Mel-P, melphalan and prednisone; Bor, bortezomib; Len, lenalidomide; TNFα, tumor necrosis factor α; HMGB1, high mobility group box protein 1; PDMP, platelet-derived microparticle; NS, not significant.

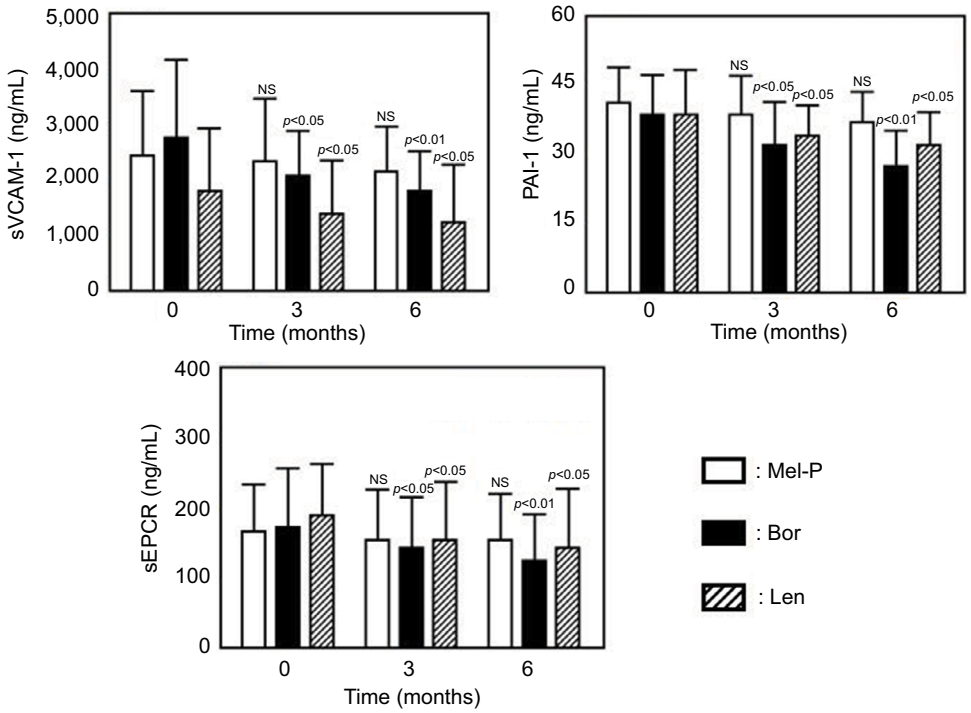

Figure 3 Changes in the plasma levels of sVCAM-1, PAI-1, and sEPCR before and after treatments.
Notes: Data are shown as mean ± SD. *p*-value, patients versus controls.
Abbreviations: Mel-P, melphalan and prednisone; Bor, bortezomib; Len, lenalidomide; sVCAM-1, soluble vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; sEPCR, soluble endothelial protein C receptor; NS, not significant.

Table 2 Changes in the plasma levels of PDMP, soluble factors, and cytokines/chemokines before and after all treatments of patients with elevated sEPCR

Biomarker	Before	3 months	6 months
HMGB1 (ng/mL)	15.1±4.9	15.8±5.2^{NS}	16.2±5.9^{NS}
sVCAM-1 (ng/mL)	2,230±1,060	2,295±1,102^{NS}	2,301±1,163^{NS}
PAI-1 (ng/mL)	31.5±19.6	32.3±21.2^{NS}	35.1±23.3^{NS}
PDMP (U/mL)	24.8±9.6	31.2±10.3^{NS}	32.1±11.4^{NS}

Notes: Data are shown as mean ± SD. *p*-value, before versus 3 or 6 months after treatment began.
Abbreviations: sEPCR, soluble endothelial protein C receptor; HMGB1, high mobility group box protein 1; sVCAM-1, soluble vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; PDMP, platelet-derived microparticle; NS, not significant.

However, these patients did not suffer from clinical thrombosis, because they received aspirin treatment.

Discussion

Bleeding and thrombosis are complications in patients with hematologic malignancies, with epidemiological, clinical, and pathophysiologic significance.[22,23,34] In patients with MM, several disease- and treatment-related factors have been found to affect the coagulation system, as well as increase the risks of bleeding and thrombotic complications.[34–36] Similar to findings in other cancers, malignant clones in patients with MM induce a cytokine environment responsible for a hypercoagulable state.[22] Circulating monoclonal proteins increase blood viscosity and impair platelet and coagulation function, which are considered key mechanisms in the hemostatic abnormalities frequently detected in patients with MM.[6] This study assessed the plasma concentrations of several biomarkers of hemostasis, coagulation, and endothelial dysfunction in patients newly diagnosed with MM. We found that the concentrations of HMGB1, PDMP, sVCAM-1, PAI-1, and sEPCR were higher in MM patients than in healthy controls. These results suggest that patients with MM likely have coagulation- and/or endothelial cell activation-related risk factors for coagulation abnormalities.[6,17–19,23–25]

Recently, the treatment strategy for MM has undergone a complete change.[37–39] In particular, Mel-P plus bortezomib has been reported to improve progression-free survival and overall survival when compared with Mel-P alone.[3,4,30,31,40] Furthermore, lenalidomide also provides good therapeutic effects.[30,31] Along with these treatments, new strategies using monoclonal antibodies can be anticipated.[41] However, an accurate understanding of therapeutic effects and/or side effects is necessary for the development of new MM treatments. This study therefore examined the response of various biomarkers to alternative treatments for MM. Treatment

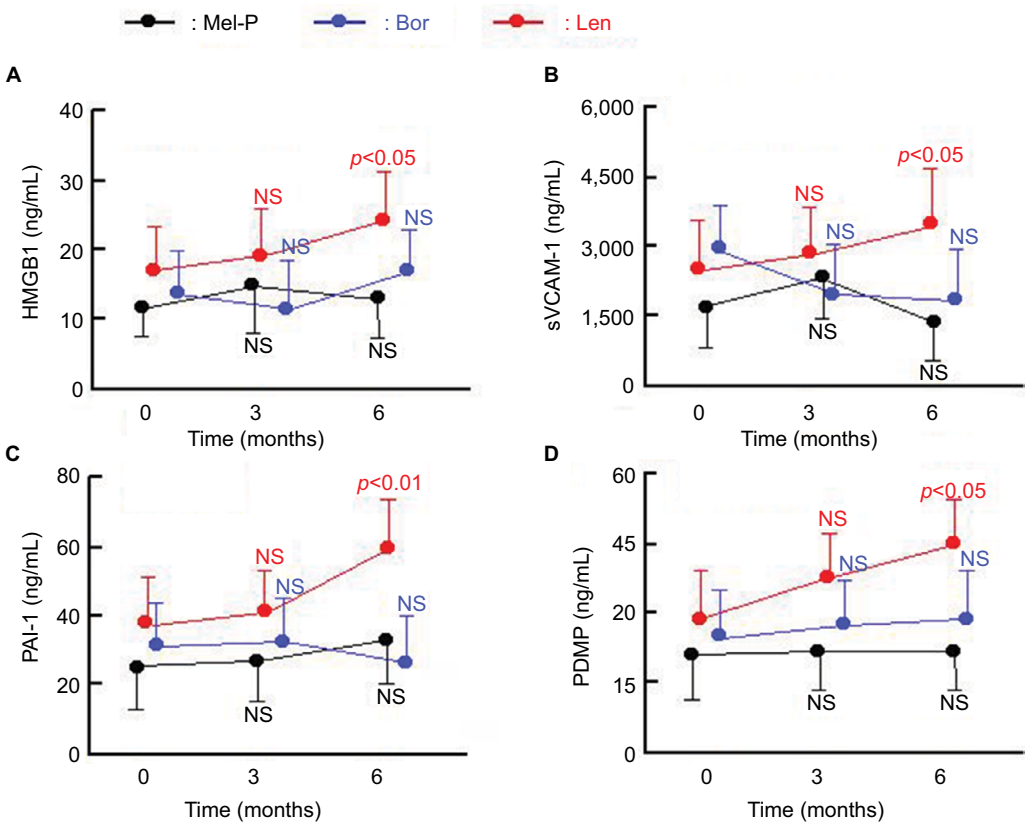

Figure 4 Changes in HMGB1 (**A**), sVCAM-1 (**B**), PAI-1 (**C**), and PDMP (**D**) before and after treatment of patients with higher levels of sEPCR.
Notes: Data are shown as mean ± SD. *p*-value, before versus 3 or 6 months after treatment began.
Abbreviations: HMGB1, high mobility group box protein 1; sVCAM-1, soluble vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; PDMP, platelet-derived microparticles; Mel-P, melphalan and prednisone; Bor, bortezomib; Len, lenalidomide; NS, not significant; M, months.

with Mel-P for 6 months significantly reduced the plasma concentrations of PDMP, relative to baseline, but did not significantly alter the plasma concentrations of TNFα, HMGB1, sVCAM-1, PAI-1, and sEPCR. In contrast, treatment with bortezomib or lenalidomide alone had a beneficial effect on all tested biomarkers other than TNFα. These results suggest the possibility that bortezomib and lenalidomide could improve the abnormality of thrombosis-related biomarkers that accompanies MM.

Activated protein C, combined with its cofactor protein S, acts as an anticoagulant, inactivating factor Va and factor VIIIa.[42] EPCR, which is a transmembrane glycoprotein found in the endothelium, enables activation of PC.[43] EPCR is also found in a soluble form, sEPCR, which binds activated protein C in competition with cell-surface EPCR.[44] Therefore, sEPCR can be considered to be a biomarker of cancer-related hypercoagulability in human malignancies.[16,17,45] In the present study, 19 of 91 patients showed remarkable elevation of sEPCR (sEPCR >248 ng/mL).

Four other biomarkers, HMGB1, sVCAM-1, PAI-1, and PDMP, showed no significant changes before and after treatment. However, when only patients with higher levels of sEPCR were compared, differences were seen in the elevation of these biomarkers between the three therapies described in this study. Only lenalidomide produced a significant elevation of the four biomarkers after 6 months of treatment. These results suggest the possibility that lenalidomide could cause the elevation of thrombosis-related biomarkers regardless of its effects on MM.[24,25]

The risk of venous thrombosis is increased not only in MM but also by chemotherapy, and is dependent on the combination of drugs administered.[46] In particular, VTE incidence greatly increases when thalidomide or lenalidomide is used in combination with dexamethasone or multiagent therapy.[47,48] The hazard ratio for a specific VTE risk should be taken into account when choosing an appropriate anticoagulant prophylaxis, because the relative risk of VTE is quite heterogeneous.[47] Our patients received aspirin treatment. Although these patients did not exhibit clinical thrombosis, their thrombosis-related biomarkers were elevated. Therefore, when a new multiagent therapy in addition to lenalidomide is chosen, it may mandate the delivery of more aggressive prophylaxis, such as low-molecular-weight heparin, full-dose warfarin, or the new oral factor Xa anticoagulants.

The exact mechanism by which lenalidomide treatment leads to an increase in sEPCR levels remains unclear. However, at least one possibility can be inferred: the participation of TNFα-converting enzyme (TACE or ADMM17).[49] TACE is a metalloproteinase that plays a pivotal role in the shedding of many cellular receptors.[50] During MM treatment, lenalidomide has critical immunomodulatory activity, and increases inflammatory cytokines such as TNFα and IL-8.[51,52] TNFα is closely linked to the production of TACE, and TACE ultimately causes the elevation of sEPCR.[49] A previous report demonstrated that the anti-inflammatory effect of lenalidomide may relate to its ability to block the production of TNFα in peripheral blood cells.[53] However, the effect of lenalidomide on TNFα is paradoxical, because an increasing level of TNFα after lenalidomide treatment has already been reported in chronic lymphoblastic leukemia patients.[51,52] Our results support these findings. However, we could not confirm a direct correlation between the TNFα and sEPCR. Therefore, further examination is required to elucidate the mechanism of sEPCR elevation caused by lenalidomide.

Conclusion

These results suggest that a hypercoagulable state can be induced by both MM and the therapies used to treat it. Hypercoagulability elevates the risk of thrombosis and hence increases patient morbidity and mortality. The risk of therapy-related thrombosis should be considered when new therapeutic regimens are selected for patients with MM.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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