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Case Report

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Angiogenesis and osteopontin expression in paraskeletal myeloma with plasmablastic morphology and aggressive clinical course: a report of two cases

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Abstract

Extramedullary disease (EMD) of multiple myeloma (MM) can present as paraskeletal (paraosseous) plasmocytoma (PP) that arise from skeletal focal lesions or extramedullary plasmacytomas (EMP) that derive from hematogenous spread. The pathogenetic mechanisms that distinguish classical MM, PP, and EMP are still insufficiently known, as are the therapies that would be effective in EMD. The aim of this study was to evaluate immunohistochemically the angiogenesis, determined as microvessel density (MVD) and osteopontin expression in PP, of two patients with MM of plasmablastic morphology and an aggressive course of disease. We found high levels of MVD and osteopontin expression in both cases of PP. The role of angiogenesis and osteopontin in EMD should be clarified in future investigations, especially since there are no satisfactory therapeutic protocols for this form of multiple myeloma, and both of these biological factors can be the potential targets of new therapies.

Keywords: Extramedullary myeloma, paraskeletal plasmacytoma, osteopontin, angiogenesis, plasmablastic morphology

INTRODUCTION

Multiple myeloma (MM) is a plasma cell neoplasia characterized by diffuse tumor infiltration of the bone marrow. Occasionally, neoplastic plasma cells acquire a different growth pattern generating tumor masses that are referred to as extramedullary disease (EMD). EMD can arise from skeletal focal lesions, which disrupt the cortical bone and spread to adjacent tissue forming smaller or larger masses outside the bone, even though they are in continuity with it. They are called paraskeletal or paraosseous plasmocytoma (PP) or bone-associated EMD with MM. Furthermore, EMD can appear as extramedullary plasmacytoma (EMP) that derives from hematogenous spread forming tumor masses in soft tissues and different organs[1]. Although PP has a better prognosis than true EMP[2,3], in principles EMD has a poorer prognosis and different biological behavior than classical MM. The pathogenetic mechanisms that distinguish classical MM, PP, and EMP are still insufficiently known, as are the therapies that would be effective in EMD. Therefore, additional efforts and research are needed to better understand the pathogenesis of PP and EMP as the most important prerequisite for better treatment.

Angiogenesis is an important component of the pathogenesis of MM, and there is much evidence that higher angiogenesis is associated with poorer treatment outcomes and prognosis[4]. Thus far, the influences of several proangiogenic factors on neovascularization itself, as well as their association with the response to therapy, survival, and prognosis, have been studied in MM[5].

Osteopontin (OPN) is a chemokine-like protein involved in different aspects of tumor biology, including enhanced mobility and adhesion of tumor cells, improvement of tumor growth, survival, and promotion of neoangiogenesis in various hematological malignancies[6,7]. Some earlier studies showed an association among OPN production/expression, tumor burden, bone disease, and angiogenesis in MM[8,9].

We report two cases of PP with plasmablastic morphology in myeloma patients and an aggressive course of disease. Our aim was to evaluate the extent of angiogenesis (microvessel density) in paraskeletal masses and the expression of OPN in myeloma cells, since both factors may be potential therapeutic targets in EMD.

CASE REPORTS

Immunohistochemical staining

To estimate the angiogenesis and OPN expression in our patients, immunohistochemical staining with anti-CD34 class II (m7165 clone QBEnd10, DAKO, Glostrup, Denmark, dilution 1:500, incubation 30 min at room temperature) and anti-OPN (OP3 N, Novocastra, Newcastele upon Tyne, UK, dilution 1:100, incubation 60 min at room temperature) was performed on sections from formalin-fixed and paraffin-embedded tissue blocks of paraosseous plasmacytoma by using EnVision FLEX, High pH (Link) Code K8000 (Glostrup, Denmark). For negative control, the slides were stained by substituting the primary antibody with buffer solution (DAKO).

To determine the angiogenesis, slides were scanned under low power to identify the three areas with the greatest number of microvessels. These areas were then evaluated at 400 × magnification. The number of vessels in the entire field was determined for each, and the average was expressed as microvessel density (MVD). The immunoreactivity of OPN was evaluated on the basis of the percentage of positive plasma cells.

Case 1

Our first patient, a 67-year-old female with a previous history of ischemic heart disease, heart attack and percutaneous coronary intervention, arterial hypertension, and type II diabetes, was diagnosed as MM, IgG lambda, ISS II, with symptomatic anemia and extensive osteolytic lesions at presentation. The serum

paraprotein concentration was 73 g/L, the free lambda light chain value was 2190 mg/L, and the uninvolved/involved light chain ratio was 0. The bone marrow biopsy demonstrated infiltration with 90% of CD138+, lambda +, kappa- plasma cells. Unfortunately, cytogenetic analysis was not performed for technical reasons at the time of diagnosis. Initially, two cycles of the VCD (bortezomib, cyclophosphamide, and dexamethasone) regimen with bisphosphonates were administered. Due to symptoms and signs of pneumonia, a computed tomography (CT) of the thorax was performed, determining a large tumor of the posterior mediastinum infiltrating Th6-Th12 vertebrae and prolapsing into the spinal canal [Figure 1]. Surgical treatment and biopsy confirmed a plasmacytoma consisting of lambda-positive plasma cells [Figure 2A]. Radiotherapy of the mediastinal plasmacytoma and infiltrated vertebrae Th7-Th9 was performed and a second line of treatment with the DVd (daratumumab, bortezomib, and dexamethasone) regimen was administered. After only four cycles, further progression was observed (increase in serum paraprotein). Thus, a third line of treatment started with the KRd (carfilzomib, lenalidomide, and dexamethasone) regimen. After four cycles of therapy, radiological imaging showed significant regression of the residual thoracic extramedullary tumor mass without protrusion into the spinal canal. A humoral reevaluation of the disease showed a very good partial remission. Since the general condition of the patient was not good enough to perform an autologous hematopoietic stem cell transplantation, the KRd regimen was continued until the ninth cycle, when a new progression of disease occurred (pathological bone fractures of the ribs and progression of the mediastinal plasmacytoma with large pleural effusions, but without an increase of paraprotein concentration or involved free light chain in serum or urine). Further palliative radiotherapy was done, and chemotherapy with the VAD (vincristine, adriamycin, and dexamethasone) regimen in combination with pomalidomide was started. After five cycles of this therapy, severe infectious complications and sepsis occurred without significant tumor regression. The patient was discharged from the hospital with the best supportive care recommendations after approximately two years from diagnosis. Interestingly, while extramedullary tumor has progressed, bone marrow biopsy showed no plasma cell infiltration.

MVD in mediastinal paraosseous plasmacytoma was 32, while the percentage of OPN-positive cells was around 70% [Figures 2B and C].

Case 2

Our second patient, a 72-year-old female with a previous history of arterial hypertension, ischemic heart disease, and percutaneous coronary intervention, has been diagnosed as MM, light chain disease, lambda, ISS III, with symptomatic anemia and extensive bone marrow infiltration with 80% of CD138+, lambda+, kappa- plasma cells. The serum free lambda light chain value was 38 mg/L and the uninvolved/involved light chain ratio was 0.085. Unfortunately, cytogenetic analysis was not performed for technical reasons at that time. Initially, the patient refused the proposed treatment. A year after the diagnosis was made, the patient developed intermittent headaches and vision impairment in the right eye. Brain magnetic resonance imaging showed a 62 mm × 37 mm large tumor that infiltrated the right orbit, eroded the skull base, protruded into the middle cranial fossa suppressing the brain, and infiltrating the sphenoid and maxillary sinus [Figure 3]. PET-CT confirmed a high metabolic activity of the malignant process infiltrating the viscerocranium, as well as osteolytic lesions in the right iliac bone.

Pathohistological diagnosis established by endoscopic biopsy of the right maxillary sinus showed infiltration of MM [Figure 2D]. There were no malignant cells found in the cerebrospinal fluid. Considering possible side effects of radiotherapy (loss of vision on the right eye), the patient was not inclined to it. She was initially treated with eight cycles of the VCD protocol with bisphosphonates and showed partial remission. Brain CT also showed a significant reduction of the tumor. Maintenance therapy with bortezomib and dexamethasone was continued. Unfortunately, four months after maintenance therapy was started, the

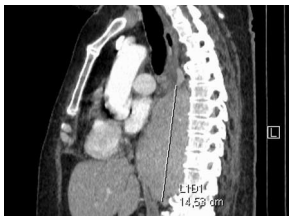

Figure 1. CT of the thorax presents a large tumor (14.5 cm × 9.4 cm) of the posterior mediastinum infiltrating Th6-Th12 vertebrae and prolapsing into the spinal canal. CT: Computed tomography.

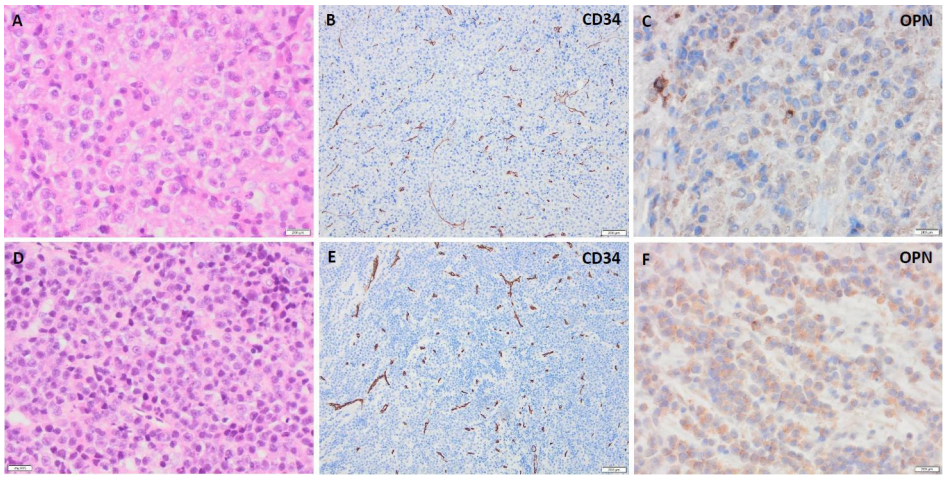

Figure 2. Mediastinal (A-C) and maxillary sinus (D-F) plasmacytomas stained immunohistochemically to visualize microvessel density (MVD) and expression of osteopontin (OPN). On hematoxylin-eosin staining, the poorly differentiated plasma cells can be seen in both extramedullary plasmacytomas (A,D), while immunostaining confirmed high grade of MVD (B,E) and high expression of OPN in plasma cells.

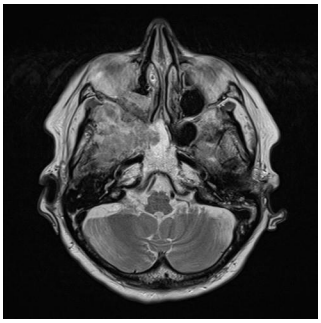

Figure 3. Brain MRI showed a 62 mm × 37 mm large tumor that infiltrated the right orbit, eroded the skull base, protruded into the middle cranial fossa suppressing the brain, and infiltrating the sphenoid and maxillary sinus. MRI: Magnetic resonance imaging.

disease progressed. The patient developed anemia, kidney damage, enlargement of the viscerocranial tumor mass with progression into the right orbit, and bone disease progression. The second line of treatment with the KRd regimen was started and initially had a good clinical response (regression of anemia, renal failure, and orbital tumor mass), but after the sixth cycle of therapy, tumor mass of the right orbit showed clinical and radiological progression causing protrusion of the right eye. This time, radiotherapy was clearly warranted, so the patient received a total dose of 30 Gy/10 fractions with a significant reduction of the tumor mass. After approximately 30 months from diagnosis, a third line of therapy with PVd

(pomalidomide, bortezomib and dexamethasone) is in progress.

In this case, maxillar sinus paraosseous plasmacytoma was analyzed, and MVD was 23, while expression of OPN was assessed as 100% [Figure 2E and F].

DISCUSSION

One of the largest European myeloma registries showed that 14.5% of myeloma patients had PP and 3.7% had EMP at diagnosis[10]. At relapse, the incidence of PP rises up to 34% and EMP up to 10%[11,12]. In the final stage of the disease, an extraskeletal involvement is observed in approximately 70% of cases studied with autopsy, with a peculiar involvement of visceral sites[13]. For now, there is no firm evidence that could establish an association between EMD and any of the drugs for the treatment of MM, nor can it be concluded that the use of novel drugs is associated with a more frequent occurrence of EMD. The biological mechanisms behind the acquisition of the EMD-forming phenotype have not yet been fully elucidated. It seems that TP53 deletions/mutations and RAS mutations are more frequent in EMD than in standard myeloma[14,15]. However, these cytogenetic changes are also characteristic of the later stages of relapsing and refractory disease without EMD. Diversely, the cyclin D1 pathway seems to favor the bone marrow homing, protecting from extramedullary localizations, as t(11;14) is not observed in MM patients with EMD[16]. All this suggests that different microenvironmental factors are the main drivers in pathogenesis of EMD. Therefore, an increased expression of CXCR4 and CXCL12 plays a major role in promoting a bone marrow-independent behavior, favoring dissemination and homing to distant and unusual sites[17]. Other mechanisms are represented by increased expression of CD44 and reduced expression of several adhesion molecules, in particular VLA-4 and CD56, and chemokine receptors, such as CCR1 and CCR2[18,19].

The role of angiogenesis in the pathogenesis of EMD in contrast to MM has not been very clearly elucidated. Hedvat *et al.*[20] demonstrated that different genes involved in angiogenesis and adhesion, such as angiopoietin-1, Notch3, and fibronectin-1, were upregulated in EMP compared with MM. The same authors reported that plasma cells in EMD are positive by immunohistochemical staining for CD31 and endoglin, both angiogenic factors[20]. These data suggest that angiogenesis may play a role in the pathogenesis of EMD. In general, EMD is associated with aggressive disease and shorter progression-free survival and overall survival (OS), albeit treated with new drugs[21]. Pour *et al.*[12] showed a significantly longer OS for myeloma patients without EMD in comparison with those who had EMD (109 months *vs.* 38 months). Similarly, Mangiacavalli *et al.*[22] found that patients without EMD showed a significantly longer OS compared to EMD patients (median OS 11 years *vs.* 2 years). The outcome was significantly worse for EMP patients compared to PP patients (median OS 1.6 years *vs.* 2.4 years)[22].

Our previous results show an association between serum OPN concentration and bone disease intensity in patients with MM[8]. Therefore, we believe that OPN is an important factor in the progression of MM that also affects bone metabolism and promotes angiogenesis, which is indicated by the works of some other researchers[23]. We did not find in the literature a description of OPN expression in EMD. In this short description of two patients, we decided to determine angiogenesis (MVD) and expression of OPN in PP with extensive extramedullary mass showing plasmablastic morphology that is otherwise a characteristic of aggressive EMP[24]. In both cases, the MVD was more than 20; thus, the angiogenesis could be established as high grade[25]. Therefore, high MVD in the tumor micro-ecosystem and OPN expression in plasma cells were found to be supporting our hypothesis that angiogenesis and OPN could play a biological role in EMD, both in EMP and in PP with plasmablastic morphology and an aggressive course of disease. Unfortunately, at the time of taking tumor tissue samples for immunohistochemical analysis, no serum samples were taken to determine the serum concentration of OPN. Additional interesting information

would have been gained if the values of MVD and OPN expression were taken at different time points, regarding therapy response, so this remains a goal for our next investigation.

It is obvious that nothing can be concluded on the bases of two cases, but this review of our two patients encourages us, and we hope other research groups to continue analyzing cases of extramedullary MM (especially in large clinical trials) and investigating various factors of the microenvironment in this particular type of MM, including angiogenesis and OPN. If angiogenesis were proven as an important pathogenetic factor in EMD, it would provide a wide range of possibilities for the treatment of the most aggressive forms of MM, such as EMD. The same could be true for OPN, whose biological function in MM and EMD should also be clarified in future research with the possibility of pharmacological inhibition of its biological effect.

DECLARATIONS

Authors' contributions

Designed the paper and wrote the manuscript: Valković T, Stanić Damić M, Valković F
Designed the paper, wrote the manuscript and reviewed and approved the manuscript: Jonjić N

Availability of data and materials

Authors declare that data and supplementary figures supporting their findings can be found in Clinical Hospital Center Rijeka.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Written informed consent was obtained from the patients.

Consent for publication

Written informed consent was obtained from the patients for publication of this case reports and any companying images.

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