Circulating activin-A is elevated in patients with advanced multiple myeloma and correlates with extensive bone involvement and inferior survival; no alterations post-lenalidomide and dexamethasone therapy


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Background: Activin-A is a transforming growth factor -β superfamily member, which seems to be implicated in the biology of osteolytic disease in multiple myeloma.

Design and methods: Circulating activin-A was evaluated in 98 newly diagnosed myeloma patients (85 with symptomatic disease), in 40 patients with relapsed myeloma before and after four cycles of lenalidomide and dexamethasone (RD), in 27 healthy controls and in 10 monoclonal gammopathy of undetermined significance disease, in 40 patients with relapsed myeloma before and after four cycles of lenalidomide and dexamethasone.

Results: Patients with newly diagnosed symptomatic myeloma had increased circulating activin-A compared with controls (P < 0.001), while patients with relapsed disease had elevated activin-A even compared with symptomatic patients at diagnosis (P < 0.001). High activin-A correlated with advanced International Staging System stage.
including lytic bone disease and survival. To explore possible correlations with clinical and laboratory data, we evaluated prospectively. The standard dose of lenalidomide, 25 mg per os (PO) daily on days 1–21 of a 28-day cycle, was given to patients with a baseline creatinine clearance (CrCl) >50 ml/min. For patients with lower CrCl, lenalidomide dose was adjusted according to standard recommendations. Dexamethasone was administered at a dose of 40 mg PO on days 1–4 and 15–18 for the first four cycles and only on days 1–4 thereafter. Serum was obtained from patients with relapsed myeloma on day 1 of the first RD cycle and then on day 28 of the fourth cycle and was stored at −80°C until the day of measurement of activin-A and bone markers. A grading of bone involvement in three groups according to the radiographical evaluation of the skeleton was made for all newly diagnosed patients and patients at the time of relapse. Group A included patients with no lytic lesions, group B included patients with osteolytic lesions in one to three areas of the skeleton and group C included patients with lytic lesions in more than three areas of the skeleton and/or a pathological fracture due to MM.

Forty myeloma patients, with relapsed myeloma, who received the combination of lenalidomide and high-dose dexamethasone were also evaluated prospectively. The standard dose of lenalidomide, 25 mg per os (PO) daily on days 1–21 of a 28-day cycle, was given to patients with a baseline creatinine clearance (CrCl) >50 ml/min. For patients with lower CrCl, lenalidomide dose was adjusted according to standard recommendations. Dexamethasone was administered at a dose of 40 mg PO on days 1–4 and 15–18 for the first four cycles and only on days 1–4 thereafter. Serum was obtained from patients with relapsed myeloma on day 1 of the first RD cycle and then on day 28 of the fourth cycle and was stored at −80°C, till the date of measurement of activin-A and bone remodeling markers. Ten patients with monoclonal gammopathy of undetermined significance (MGUS) were also included in the study. Finally, 27 healthy controls of similar age (median age: 67 years, range: 35–80 years) and gender (15 M/12 F) were also studied in order to be used as an internal control group. Each patient in the control group was examined to ensure that there was no evidence of osteopenia/osteoporosis (assessed by bone mineral density measurement using dual-emission X-ray absorptiometry scan), osteoarthritis or autoimmune disorder, no receipt of medication that could alter normal bone turnover during the last 6 months and no evidence of infection.

The study was conducted after approval from the institutional Ethical Committee and under the guidelines of the Declaration of Helsinki. All patients were given written consent for participating in this study.

**activin-A and biochemical markers of bone remodeling measurements**

In all patients and controls, serum activin-A was measured using an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN), according to manufacturer instructions. The following serum indices of bone metabolism were also measured in all studied population, using ELISA methodology: (i) bone resorption markers [C-terminal cross-linking telopeptide of collagen type-I (CTX), and tartrate-resistant acid phosphatase isofrom-5b (TRACP-5b)] and (ii) bone formation markers [bone-specific alkaline phosphatase (bALP) and osteocalcin (OC)], as previously described.

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**introduction**

Activin-A is a dimeric multifunctional glycoprotein, which belongs to the transforming growth factor-β (TGF-β) superfamily and regulates a broad spectrum of biological functions, including gonadal function, hormonal homeostasis, muscle growth, inflammation and immunity, but also bone remodeling [1, 2]. Activin-A has growth stimulatory effects on osteoclasts and possibly inhibitory effects on osteoblast function [3, 4]. Osteoblasts produce activin-A and follistatin (an activin-A antagonist) in a differentiation-dependent manner, leading to autocrine regulation of extracellular matrix formation and mineralization [5]. Furthermore, activin-A administration has strongly inhibited mineralization in osteoblast cultures, whereas follistatin increased mineralization in in vitro experiments [5].

Deregulation of the activin-A signaling pathway has been reported in patients with solid tumors and bone metastases [6]. Circulating activin was found elevated in patients with breast and prostate cancer with bone metastases compared with patients without bone metastases [7].

Multiple myeloma (MM) is characterized by the development of osteolytic disease, which is present in up to 80% of patients at diagnosis and up to 90% of patients at some stage of their disease, leading to devastating skeletal complications [8]. In a recent study, it has been reported that malignant plasma cells induce the secretion of activin-A by stromal cells, which leads to osteoblast inhibition [7]. Therefore, the aim of this study was to evaluate circulating activin-A in newly diagnosed patients with MM, as well as in relapsed MM patients who received the combination of lenalidomide plus high-dose dexamethasone (RD), and explore possible correlations with clinical and laboratory data, including lytic bone disease and survival.

**patients and methods**

**patients and healthy controls**

Ninety-eight, consecutive newly diagnosed MM patients (13 with asymptomatic and 85 with symptomatic MM) were studied. Patients with infection at the time of diagnosis were excluded for participation in this study. All symptomatic patients received frontline treatment with novel agent-based regimens (either bortezomib- or immunomodulatory drug-based therapies) and were followed up in the Department of Clinical Therapeutics of the University of Athens. All patients had serum samples collected before initiation of any kind of treatment, including bisphosphonate administration, and stored at −80°C till the day of measurement of activin-A and bone markers.

Conclusions: High circulating activin-A correlates with advanced features of myeloma, supporting the rationale for the use of activin-A antagonists, such as sotatercept in myeloma. The inability of RD to reduce activin-A reveals RD as a good candidate for combination therapies with activin-A antagonists in myeloma.

Key words: activin-A, bone disease, lenalidomide, multiple myeloma, survival
statistical analysis

Differences between patients and controls as well as between different patient subsets were evaluated using the Mann–Whitney U test. Differences between baseline and four-cycle values of the studied parameters post-RD were evaluated using the Wilcoxon signed rank test. The Spearman Rank correlation test was employed to examine relationships between various parameters and clinical patient characteristics. Survival probabilities were calculated by the Kaplan–Meier method and comparisons made using the log-rank test to identify potential prognostic factors. Variables found to be statistically significant at the $P<0.05$ level were entered into a multivariate model using Cox regression analysis to identify the most statistically significant model. All $P$ values were two-sided, the level of significance is $<0.05$ and confidence intervals (CIs) refer to 95% boundaries.

results

activin-A in newly diagnosed myeloma

The characteristics of all studied patients are shown in Table 1. Circulating levels of activin-A of newly diagnosed patients with symptomatic MM (median: 555 pg/ml, range: 129–2336 pg/ml) were increased compared with controls (381 pg/ml, 204–899 pg/ml; $P<0.001$; Figure 1A). There was no significant difference regarding activin-A levels between asymptomatic patients (462 pg/ml, 255–840 pg/ml), MGUS patients (457 pg/ml, 361–839 pg/ml) and healthy controls (Figure 1A). However, six (60%) patients with MGUS and six (46%) patients with asymptomatic myeloma had a value of circulating activin-A above the 95% CI of the normal controls (mean activin-A value of controls: 404 pg/ml, 95% CI 322–485 pg/ml). Regarding symptomatic myeloma patients at diagnosis, 63.5% of them (54/85) had an activin-A value above the 95% CI of the normal controls.

High circulating levels of activin-A correlated with advanced disease stage at diagnosis; the median values (range) for International Staging System (ISS)-1, ISS-2 and ISS-3 were 462 pg/ml (255–840 pg/ml), 536 pg/ml (210–1183 pg/ml) and 681 pg/ml (302–2336 pg/ml), respectively; $P$-ANOVA = 0.002 (Figure 1B). Activin-A levels showed also slight correlations with serum creatinine ($r=0.519$, $P<0.001$), β2-microglobulin ($r=0.450$, $P<0.001$; Figure 2A), serum lactate dehydrogenase ($r=0.247$, $P=0.034$) and plasma cell per cent infiltration of the bone marrow ($r=0.257$, $P=0.029$) at diagnosis. Regarding bone markers, serum activin-A positively correlated with both markers of bone resorption: CTX ($r=0.574$, $P<0.001$; Figure 2B) and TRACP-5b ($r=0.481$, $P<0.001$) but had no correlation with markers of bone formation. Patients with extensive bone disease (group C) had higher levels of circulating activin-A (618 pg/ml, 211–2043 pg/ml) compared with all others (477 pg/ml, 129–2336 pg/ml; $P=0.03$; Figure 3).

activin-A and survival in newly diagnosed symptomatic myeloma

All symptomatic myeloma patients were treated with novel agent-based therapies as first-line treatment: 52 received thalidomide-based regimens (cyclophosphamide, thalidomide and dexamethasone-CDT or prednisone and thalidomide-MPT) and 33 received bortezomib-based regimens (bortezomib and dexamethasone-VD, bortezomib, adriamycin and dexamethasone-PAD or melphalan, prednisone and bortezomib-MPV). The median survival of newly diagnosed symptomatic MM patients was 63 months. The univariate analysis showed that levels of activin-A treated as a continuous variable were inversely associated with survival ($P<0.001$). Thus, low levels of activin-A were associated with superior overall survival: the median survival of patients who had a serum activin-A of <422 pg/ml (lower quartile, $n=22$ patients) has not been reached yet, while the median survival of all other patients was 59 months ($P=0.04$) (Figure 4). Other factors associated with survival in the univariate analysis included: serum β2-microglobulin [either as a dichotomous variable (cut-off value of 3.5 mg/l, $P<0.01$) or as a continuous variable, $P=0.01$], serum creatinine [either as a dichotomous variable (cut-off value of 2 mg/dl, $P=0.018$) or as a continuous variable: $P=0.001$] and serum albumin [either as a

Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th></th>
<th>Newly diagnosed symptomatic MM</th>
<th>Newly diagnosed asymptomatic MM</th>
<th>Relapsed MM</th>
<th>MGUS</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>85</td>
<td>13</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>47/38</td>
<td>8/5</td>
<td>22/18</td>
<td>7/3</td>
</tr>
<tr>
<td>Age, years, median</td>
<td>69 (31–92)</td>
<td>69 (46–81)</td>
<td>68 (41–80)</td>
<td>56 (43–75)</td>
</tr>
<tr>
<td>Type of MM: IgG/IgA/IgD/NS</td>
<td>53/20/9/1/2</td>
<td>8/4/1/0/0</td>
<td>24/15/1/0</td>
<td>4/4/2/0/0</td>
</tr>
<tr>
<td>Stage at diagnosis (ISS)</td>
<td>1/1/1/II/III</td>
<td>12/1/0</td>
<td>0/12/28</td>
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<tr>
<td>Bone disease status: group A/B/C</td>
<td>27/27/31</td>
<td>0/12/28</td>
<td></td>
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<tr>
<td>Median number of lines of previous treatment (range)</td>
<td>2(1–4)</td>
<td></td>
<td></td>
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<tr>
<td>Parameters at baseline</td>
<td></td>
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<tr>
<td>Hb &lt;10 g/dl</td>
<td>32 (38%)</td>
<td>16 (40%)</td>
<td></td>
<td></td>
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<tr>
<td>Creatinine more than upper normal limit</td>
<td>27 (32%)</td>
<td>25 (62.5%)</td>
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<tr>
<td>Creatinine &gt;2 mg/dl</td>
<td>11 (13%)</td>
<td>17 (42.5%)</td>
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<tr>
<td>Albumin &lt;3.5 g/dl</td>
<td>46 (55%)</td>
<td>19 (47.5%)</td>
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<td>Serum β2-microglobulin &gt;3 mg/l</td>
<td>59 (69%)</td>
<td>26 (65%)</td>
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<tr>
<td>LDH &gt;240 U/l</td>
<td>22 (26%)</td>
<td>5 (12.5%)</td>
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</tr>
</tbody>
</table>

ISS, International Staging System; LDH, lactate dehydrogenase; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma.
dichotomous variable (cut-off value of 3.5 g/dl, $P = 0.018$) or as a continuous variable: $P < 0.01$. However, in the multivariate model, activin-A was not an independent factor for survival (neither as a dichotomous variable nor as a continuous variable).

circulating activin-A in relapsed myeloma
All patients with relapsed myeloma were receiving zoledronic acid every 4–8 weeks before relapse. Patients with relapsed myeloma had higher levels of circulating activin-A (725 pg/ml, 272–2088 pg/ml) compared with healthy controls ($P < 0.001$), MGUS ($P < 0.001$), asymptomatic myeloma at diagnosis ($P < 0.001$) and also compared with symptomatic newly diagnosed patients ($P = 0.008$). More specifically, 29/40 (72.5%)
activin-A levels were comparable to those of healthy controls. In contrast, in patients with newly diagnosed symptomatic MM and in patients with relapsed disease. In patients with relapsed disease, there was no correlation with markers of bone resorption or with lytic disease status at the time of relapse in these patients.

Treatment with RD did not reduce circulating activin-A levels, which remained elevated (790 pg/ml, 302–2384 pg/ml) after four cycles of therapy, compared with controls \((P < 0.001)\) or even compared with symptomatic myeloma patients at diagnosis \((P < 0.001)\). After four cycles of RD therapy, 16 (40%) patients had achieved at least a partial response to therapy. Even these patients continued to have elevated circulating activin-A (735 pg/ml, 302–2178 pg/ml) compared with controls and symptomatic myeloma at diagnosis \((P < 0.01)\) for both comparisons). However, responding patients had lower values of activin-A compared with all other patients (801 pg/ml, 485–2384 pg/ml), but this reduction did not reach statistical significance \((P = 0.217)\).

**discussion**

Osteolytic bone disease represents the most frequent complication of MM. Current therapeutic options include the inhibition of osteoclast function using bisphosphonates [8]. However, bisphosphonates have some important side-effects including osteonecrosis of the jaw and renal impairment, while they have no effect on osteoblast function [14]. Thus, the discovery of novel targets for the development of new drugs to enhance osteoblast function and thus to improve the management of myeloma-related bone disease is of great interest. Activin-A is a TGF-β superfamily member with regulatory effects on bone remodeling [1–3]. In our study, we showed for the first time that circulating activin-A is elevated in patients with newly diagnosed symptomatic MM and in patients with relapsed disease. In contrast, in patients with MGUS and with asymptomatic/smoldering myeloma, serum activin-A levels were comparable to those of healthy controls. This result may be due to the low number of patients with asymptomatic myeloma or MGUS in this study or due to the elevation of activin-A only in active myeloma. Indeed, we found higher values of activin-A in symptomatic newly diagnosed patients with ISS-3 compared with ISS-1 and 2 and in patients who relapsed after previous response to antmyeloma therapies compared with symptomatic patients at diagnosis. These results support the presence of high circulating activin-A in myeloma patients with advanced disease.

The elevation of activin-A in symptomatic MM patients at diagnosis is in accordance with a recent finding in 28 myeloma patients, in whom activin-A was elevated in the bone marrow plasma of ~50% of them, especially of those with more than one lytic lesion [9]. In our study, 63.5% and 72.5% of patients with symptomatic myeloma at diagnosis and at relapse, respectively, had higher circulating activin-A levels than the 95% CI of the activin-A levels of normal controls. Furthermore, we found that high serum levels of activin-A correlated with extensive bone disease (more than three lytic lesions or a pathological fracture) and increased bone resorption (assessed by elevated CTX and TRACP-5b levels). Thus, activin-A seems to be implicated in the biology of bone destruction in MM. *In vitro* studies have shown that myeloma cells enhance the production of activin-A in their microenvironment, which leads to inhibition of osteoblast differentiation [9]. Furthermore, in two myeloma mouse models, the administration of a soluble activin receptor type IIA fusion protein (ActRIIA.muFc) or an activin-A inhibitor (RAP-011) stimulated osteoblastogenesis, prevented myeloma-induced suppression of bone formation and blocked the development of osteolytic bone lesions [9, 14]. These data indicate that activin-A is a crucial cytokine for the development of myeloma bone disease and that the inhibition of activin-A may be a promising approach for the management of myeloma-related bone disease.

Of interest, in the above mouse models, the authors reported reduction of tumor load and increased survival in mice treated with activin-A antagonists [9, 14]. In our study, elevated activin-A correlated with inferior survival in the univariate analysis, although all patients had been treated upfront with novel agent-based therapies. However, serum levels of activin-A were not independently associated with survival in the multivariate analysis, possibly due to the correlation of activin-A with β2-microglobulin, one of the most important prognostic factors in MM.

In a very recent study, lenalidomide, an immunomodulatory drug with significant efficacy in myeloma patients, upregulated the expression of activin-A in myeloma bone marrow stromal cells *in vitro*, and especially in those that have not had high activin-A expression before their exposure to lenalidomide [11]. Our group has also shown that lenalidomide has no effect on osteoblast function, although it reduces osteoclast activity in responding patients [15]. Thus, we evaluated the effect of lenalidomide on activin-A circulating levels in 40 consecutive patients with relapsed disease who were treated with the RD combination. We found that RD did not reduce the levels of circulating activin-A, which remained stable in responding patients, while there was some increase of activin-A in...
nonresponders, which did not reach a statistical significant level. This result suggests that lenalidomide in combination with dexamethasone has either no effect or a modest effect on activin-A production by the marrow stromal cells or that the increased expression of activin-A in bone marrow stromal cells in the myeloma microenvironment is not reflected in the circulation. Whatever is true, it will be of great interest to see the effects of the combination of RD with activin-A antagonists, such as sotatercept, on both bone formation and response/survival of myeloma patients. Such studies are going to start very soon and their results are eagerly anticipated.

In conclusion, our study provides evidence that activin-A is elevated in myeloma patients with advanced disease and correlates with adverse disease features, including extensive bone disease and inferior survival. This study supports the rationale for the use of activin-A antagonists, such as sotatercept, in symptomatic myeloma patients with osteolytic disease. Furthermore, it shows that RD does not alter the circulating activin-A, suggesting that RD may be a good candidate for combination therapies with activin-A antagonists in MM patients.

acknowledgements

Part of the paper has been presented as a poster presentation in ASH 2010 Annual Meeting (Blood 2010; 116: Abstr 2967). ET and MAD designed the study, analyzed the data, followed up the patients and wrote the paper. EK, DC, MG, EEP and NK followed up the patients. ET and AP carried out all laboratory parameters of the study. DC and EK carried out the statistical analysis. All authors have provided comments on the drafts of the paper and approved the final draft for submission to Annals of Oncology.

disclosure

The authors have declared no conflicts of interest.

references