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“New molecular biomarkers for assessment of bone disease in multiple myeloma”

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Multiple myeloma (MM) is malignant disease characterized by infiltration of bone marrow by malignantly transformed plasma cells with concomitant bone destruction. Over 80% of patients with MM have osteolytic lesions at diagnosis. Their effective treatment and improvement of quality of life needs additional knowledge for the pathogenic mechanisms of bone destruction and the discovery of new biomarkers and targets for treatment. At present, studies on bone involvement in MM are mainly focused on the detection of biomarkers related only to osteoclast function, without considering bone remodeling as an integrated process between three main cell types (osteoclasts, osteoblasts and osteocytes).

The research problem posed in the current project is relevant and innovative, not only for Bulgaria but also worldwide due to the growing morbidity and the need for new specific and reliable molecular biomarkers to identify and monitor the most common complication of MM – the bone disease.

Therefore **the aim** of the current study is to evaluate in dynamics the prognostic and predictive value of new bone biomarkers characterizing osteoclast, osteoblast and osteocyte function in patients with MM at different stages of the disease.

The main tasks include: to determine in dynamics of the levels of periostin, osteopontin, sRANKL, dickkopf-protein -1, sclerostin, vitamin D status and the B-cell receptors CD229 and CXCR4 in patients with MM at diagnosis, on the 4th and 8th month after chemotherapy and autologous transplantation; to study the relationships between new biomarkers, conventional laboratory parameters and imaging studies.

Patients and methods: The current prospective study will last 3 years. Sixty adult patients with proven MM according to IMWG criteria and selected according to the inclusion criteria defined by the research team will be enrolled in the study.

The current project is based on an innovative and original combination of research - clinical, biochemical, immunochemical, flow cytometric, chromatographic, and statistical. ELISA assays, flow cytometry analysis of CD229 and CXCR4 expression, and vitamin D analysis by a modern chromatographic method will be used for testing the reliability of proteins regulating not only osteoclast, but also osteoblast and osteocyte function. These proteins will be evaluated as potential biomarkers for monitoring the bone changes and therapy efficiency in MM.

Expected outcomes: The study will provide new data for the levels of bone biomarkers assessing not only osteoclast but also osteoblast and osteocyte function, for vitamin D status, and for the expression of CD229 and CXCR4, insufficiently studied in MM. The changes of these biomarkers according to disease stage and the conducted therapy will help to assess their prognostic and predictive value. The diagnostic reliability of the tested biomarkers and vitamin D status in patients with MM will be evaluated by statistical modeling. In long-term aspect, the discovery of new and reliable biomarkers for assessment the bone lesions in MM is a prerequisite for adequate treatment of the disease and for improvement quality of life.

Achieved results:

Multiple myeloma (MM) is a hematologic neoplastic disease characterized by the accumulation of clonal plasma cells in the bone marrow. An essential feature of clinically manifested MM is the presence of bone lesions caused by an imbalance between osteoclast/ osteoblast differentiation and activity.

In the present study, changes in sRANKL, periostin, and osteopontin, as osteoclast stimulating factors and inhibitors of osteoblast function, DKK-1, and sclerostin, as inhibitors of osteoblastogenesis, and vitamin D status were dynamically monitored in newly diagnosed patients with multiple myeloma (NDMM). Their correlations with clinical indicators of disease course and severity were examined as well.

1. NDMM patients at diagnosis revealed significantly higher levels of Dickopf-1 protein (DKK-1) and sclerostin ($p<0.0001$) compared to controls. These two parameters showed a clear correlation with disease stage (sclerostin: $p<0.0012$; DKK-1: $p<0.025$).
2. The severity of osteolytic lesions was accompanied by a significant increase in both serum levels of sclerostin ($p<0.0001$) and DKK-1 ($p<0.05$).
3. In the course of therapy, both parameters gradually decrease being more pronounced after autologous stem cell transplantation.
4. At each stage of treatment, there was a significant decrease in DKK-1 and sclerostin compared to their baseline levels ($p<0.0001$).
5. Significantly lower serum sclerostin ($p<0.01$) and DKK-1 ($p<0.05$) levels were found in patients with complete and very good partial response to treatment compared to those with partial response, stable or progressive disease.

   Conclusion: Serum sclerostin and DKK-1 in NDMM reflect the severity of bone disease and the effect of therapy.

6. At diagnosis, serum levels of sRANKL, periostin, and osteopontin were significantly higher in patients with NDMM compared with controls ($p<0.0001$), correlating with disease stage, bone disease severity, and bone marrow infiltration with clonal plasma cells.
7. In the course of therapy, serum levels of sRANKL, periostin and osteopontin decreased, most markedly after autologous stem cell transplantation ($p<0.0001$).

8. Significant decrease in serum levels of sRANKL, periostin and osteopontin was found in patients who achieved a complete and very good partial response, compared to all other patient groups ($p<0.05$).

   Conclusion: Serum levels of sRANKL, periostin and osteopontin reflect the severity of bone disease in multiple myeloma and could be promising markers to monitor the course of bone disease in multiple myeloma and the effect of treatment.

9. We established reduced serum 25-hydroxyvitamin D levels in patients with NDMM compared to controls (43.55 nmol/L versus 57.08 nmol/L, $p<0.01$) and to patients with monoclonal gammopathy of undetermined significance (MGUS) (43.55 nmol/L versus 53.03 nmol/L, $p=0.4266$).
10. The frequency distribution of patients with NIDDM according to their vitamin D status was worse than that of controls ($\chi 2=10.28$, $p=0.0163$).
11. Patients in ISS-3 had significantly lower 25-hydroxyvitamin D levels compared to patients in ISS-2 (24.03nmol/L vs. 53.13nmol/L, $p=0.0003$) and ISS-1 (63.21nmol/L, $p<0.0001$).
12. Patients with more than 3 osteolytic lesions and/or pathological fractures had lower 25-hydroxyvitamin D levels compared to patients with lack or no more than 3 osteolytic lesions (33.14nmol/L vs. 60.05nmol/L, $p=0.0288$) and controls (57.08nmol/L, $p=0.0010$).

    Conclusion: Patients with worse clinical characteristics had significantly lower serum 25-hydroxyvitamin D levels. Vitamin D insufficiency/deficiency may cause secondary hyperparathyroidism, thereby increasing the number of osteoclasts. This could further influence the impaired bone remodeling process observed in the pathogenesis of MM. Vitamin D supplementation could have a beneficial effect at least on symptoms such as fatigue, muscle weakness and bone pain.