ANTICANCER RESEARCH *37*: 1501-1506 (2017)
doi:10.21873/anticanres.11477

MIC1/GDF15 as a Bone Metastatic Disease Biomarker

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Abstract. *Aim: The aim of the study was to evaluate MIC1/GDF15 as a biomarker in the monitoring of bone metastases occurrence. Patients and Methods: The assessed group included patients diagnosed with: prostate cancer, breast cancer, lung cancer and colorectal cancer. Patients were divided into two groups based on the scintigraphy of the occurrence of bone metastases. Group 0 contained 55 patients without bone metastases, that served as the control group. Group 1 contained 75 patients with bone metastases. Results: Higher levels ($p<0.0001$) of MIC1/GDF15 were found in group 1 (with bone metastases) compared to the group 0. Receiver operating characteristic (ROC) analysis showed an area under the curve (AUC) 0.87. At the point of 90% specificity we found a 65% sensitivity and cut-off value of 1.48 ng/ml. Conclusion: Circulating MIC1/GDF15 is a powerful biomarker for bone metastatic disease but insufficient sensitivity calls for further studies incorporating combinations with other novel or routine markers.*

Current diagnostics of bone-metastatic disease is based on imaging methods which are not satisfactory for early detection or regular treatment monitoring. During our previous testing of circulating markers for the detection of bone-metastatic disease within the novel cancer metastatic multiplex panel, macrophage inhibitory cytokine-1/growth differentiation factor 15 (MIC1/GDF15) was identified as the best candidate (1). Today there exists accessible and effective biological treatment for patients but only given the early-detected bone-metastasis disease. New biomarkers could help improve the strategy for managing bone-metastatic disease.

The transforming growth factor-β (TGF-b) superfamily cytokine MIC-1/GDF15 circulates in all humans and is linked to the physiological regulation of appetite, energy storage, inflammation, cardiovascular diseases (CVD), and cancer pathophysiology. MIC1/GDF15 may play an anti-tumoral role by inhibiting tumor growth and inducing apoptosis during the early stages of cancer but, conversely, it can promote invasiveness and metastatic behavior at advanced stages and is involved in the epithelial-mesenchymal transition in tumors (2-4). The cleaved mature secreted peptide that rapidly diffuses into the circulation is a 112-amino acid protein with a variety of used names: MIC1, GDF15, NSAID-activated gene 1 protein (NAG1), NSAID-regulated gene 1 Protein (NRG1), placental TGF-beta, placental bone morphogenetic protein (PLAB), *etc*. In the presented study MIC1/GDF15, a promising cancer biomarker, was tested for its usefulness in monitoring bone metastases occurrence in 130 oncology patients.

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Key Words: MIC1/GDF15, biomarkers, cancer, bone metastasis, scintigraphy.

Patients and Methods

Group of patients. MIC1/GDF15 was studied in a cohort of 130 oncological patients with solid tumors who underwent whole-body skeletal scintigraphy using technetium (^{99m}Tc). The assessed group included patients with the following diagnosis: prostate cancer, breast cancer, lung cancer and colorectal cancer (CRC). The cohort was divided into two groups based on the scintigraphy of the occurrence of bone metastases. The first group 0, comprised of 55 patients with no signs of bone metastases and served as a control group for our study. The second group (group 1) contained 75 patients with bone metastases with no bone-related therapy. Age characteristics of both groups are presented in Table I. The Wilcoxon test showed that the groups of patients did not differ significantly in age. The study was approved by the local Ethical Committee on 13th July 2011 and all patients signed an informed consent form before participating in this study.

Table I. *Age distribution of patient groups.*

Group	N	Age (years)				*p*-Value Wilcoxon test
		Mean	Median	Minimum	Maximum	
Group 0						
With no bone metastases	75	67.6	67.0	35.0	87.0	0.4520
Group 1						
With bone metastases	55	65.4	65.0	39.0	85.0	

Table II. *Comparison of MIC1/GDF15 levels (ng/ml) in oncology patients with bone metastases compared to oncology patients with no bone metastases.*

Group	N	Mean	Median	25th percentile	75th percentile	*p*-Value Wilcoxon test
Group 0						
With no bone metastases	75	0.73	0.50	0.34	0.81	<0.0001
Group 1						
With bone metastases	55	4.28	2.09	0.93	6.87	

Serum samples. Peripheral blood was drawn before the scintigraphy using VACUETTE® tubes (Greiner Bio-One, Kremsmünster, Austria) and allowed to clot. Sera were separated by centrifugation at 1,700 × *g* for 10 min and all specimens were immediately aliquoted and frozen. Samples were stored at −80˚C. No more than one freeze-thaw cycle was allowed before analysis.

Analytical methods. Serum levels of MIC1/GDF15 were assayed by the xMAP technology – sandwich multiplex immunoassay on magnetic microspheres labeled by fluorescent dyes – using a Human Cancer Metastasis Biomarker Magnetic Bead Panel (Merck Millipore, Darmstadt, Germany) and measured by CCD imager MagPix instrument (Bio-Rad Laboratories, Hercules, CA, USA). For the assay, 10 μL of serum diluted 1:10 is used. Mean coefficient of variation was below 4.5% as verified in previous pilot study (1), which incorporated analytical verification of used method.

Statistical methods. Descriptive statistics, *i.e.* the median, the 25th and the 75th percentiles, were calculated for all markers. The Mann-Whitney test for independent samples was used to compare marker levels between groups. Significance was set for *p*-values lower than 0.05. Furthermore, a receiver operating characteristic (ROC) curve was drawn and the area under the curve (AUC) was calculated.

Results

Higher levels ($p<0.0001$) of MIC1/GDF15 were found in group 1 with bone metastases compared to the control group 0. Descriptive statistics of levels in groups are presented in Table II and comparisons of levels between groups in box-plots are shown in Figure 1. A ROC curve was plotted between group 0 and group 1 and is presented in Figure 2. ROC analysis showed AUC 0.87 with 95% confidence interval (CI)=0.80-0.92. At the point of 90% specificity we found 65% sensitivity and a cut off value of 1.48 ng/ml.

Discussion

In the presented study we analyzed MIC1/GDF15 cytokine as a promising cancer biomarker for the purpose of detecting bone metastases occurrence in 130 oncology patients. MIC1/GDF15 is linked with inflammation, cardiovascular diseases, and cancer pathophysiology. Furthermore, it has been suggested that it is involved in the physiological regulation of appetite, energy storage and thus body weight regulation by direct action on brain feeding centers (4). MIC1/GDF15 is predicted to be one of the major molecules mediating the relation between cancer, obesity and chronic inflammation. Serum levels are strong predictors of all-cause mortality pointing to a possible role in biological processes involved in ageing (2). Wicklund *et al*. studied cohorts of 876 male subjects and 324 twins who were monitored for overall mortality. Their study shows serum MIC1/GDF15 levels to be a predictor of mortality with an odds ratio of death of 3.38 independent of telomere length, IL-6 and C-reactive protein (CRP) and MIC1/GDF15 serum levels correlating with survival time (5). Wallentin *et al*. described coherent

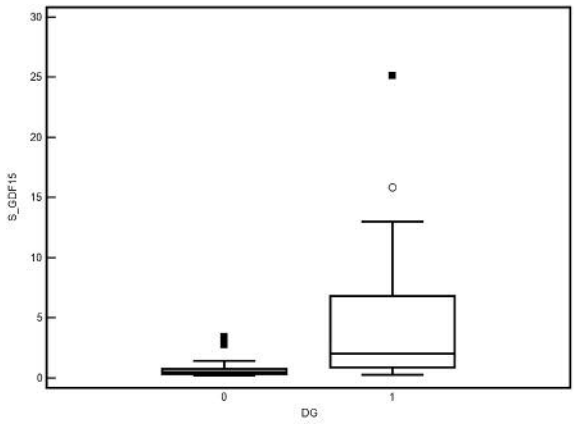

Figure 1. *Comparison of MIC1/GDF15 levels (ng/ml) in oncology patients with bone metastases compared to oncology patients with no bone metastases.*

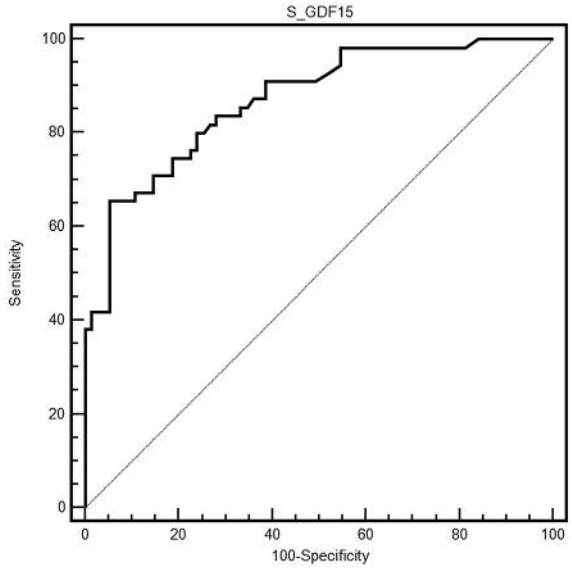

Figure 2. *ROC curve of MIC1/GDF15 between oncology patients with bone metastases compared to oncology patients without bone metastases.*

improvement of prognostication of both cardiovascular and cancer mortality and morbidity beyond established risk factors and biomarkers of cardiac, renal dysfunction and inflammation by MIC-1/GDF15 levels in a cohort of elderly men. The association of one SD increase of plasma log concentration and corresponding incremental increase of mortality and morbidity was studied. The risk of total mortality and risk of cardiovascular mortality was reported to be increased by 48%, in a total studied population, cancer mortality was increased by 46% and coronary heart disease morbidity and mortality was increased by 36% (6).

The use of MIC1/GDF15 as a biomarker could be affected by its biochemistry. It is synthesized as a 308-amino acid propeptide, which is attached after production to a local extracellular matrix (ECM) and subsequently cleaved by proteases. The association of propeptide with ECM components modulate its local bioavailability, cellular functions, and serum concentration with the possibility of latent storage in stroma. The cleaved mature secreted peptide, rapidly diffusing into the circulation, is a 112-amino acid protein (7).

MIC-1/GDF15 is the only known secreted p53-regulated cytokine and therefore can serve as a biomarker for p53 activation both *in vitro* and *in vivo*. In cancer, it can act as an anti-tumorigenic protein by inhibiting tumor growth and inducing apoptosis in the early stages of cancer while, on the contrary, in advanced stages of disease this secreted cytokine can instead promote the proliferation, migration and enhanced invasive and metastatic abilities of cancer cells (8). In this regard, the genetic and/or epigenetic alterations in the signaling elements influence the final cellular response induced by this cytokine in a given cell type involved in the mediation of MIC1/GDF15 effects on cancer cells or modulation of these pathways may occur during cancer progression. Cancer promoting effects of MIC1/GDF15 in advanced stages could be caused, not only by the promotion of more malignant behavior of cancer cells, but may also be linked to effects on anti-tumor immunity. In animal models MIC1/GDF15 was shown to have anti-inflammatory and immunosuppressive properties. Furthermore, in late-stage cancer, high levels of circulating MIC1/GDF15 suppress appetite and mediate cancer anorexia/cachexia (9, 10).

When considering MIC1/GDF15 as a novel cancer biomarker or tumor marker, the broad range of various types of tumors potentially suitable for application must be considered. An increase in MIC1/GDF15 levels has been linked to melanoma, oral squamous cell carcinomas, colorectal, pancreatic, prostate, breast, and cervical epithelial cancers (11).

MIC1/GDF15 could be considered as a novel prognostic marker for CRC and could be implemented in clinical practice. In the literature, high levels of MIC1/GDF15 in plasma or in tissue are referred to as a negative prognostic marker at the time of diagnosis. CRC patients with a higher intensity of MIC1/GDF15 immunostaining had an increased recurrence rate intensity at stages I-III. High plasma levels predict a shorter time to recurrence, reduced overall survival, and they are associated with greater CRC-specific mortality (12).

Serum MIC1/GDF15 levels are significantly elevated in most cases of pancreatic ductal adenocarcinoma (PDAC), including those negative for cancer antigen 19-9 (CA19-9) and with early-stage disease, and thus it may serve as a novel diagnostic marker for early diagnosis and postoperative monitoring of PDAC (13). Mohamed *et al*. not only

demonstrated that circulating levels of ADH, MIC1/GDF15 and CA19-9 were significantly higher in pancreatic cancer patients than in healthy controls even in early stages but also propose the addition of ADH and MIC-1/GDF15 to CA19-9 as an improvement to the efficacy of diagnosis (14).

Liu *et al*. confirmed increased MIC/GDF15 levels in hepatocellular carcinoma and cirrhosis versus healthy controls. Comparably, just as with pancreatic cancer, use of MIC1/GDF15 was proposed, in this case for hepatocellular diagnosis where a combination with AFP could improve the sensitivity and specificity of diagnosis (15).

MIC1/GDF15 has been put forward as a biomarker for gynecological cancers as well. Multivariate analysis demonstrated that MIC1/GDF15 expression was an independent predictor of poorer progression-free survival in epithelial ovarian cancer. Furthermore, MIC1/GDF15 expression was proposed to be a novel biomarker for early detection of epithelial ovarian cancer and a predictor of responses to chemotherapy, facilitating disease recurrence screening (16). MIC1/GDF15 concentration was elevated in ovarian cancer as compared to healthy controls and women with benign ovarian tumors or border ovarian tumors. MIC1/GDF15 plasma concentration correlated inversely with survival time and was an independent predictor of survival (17).

Association between MIC1/GDF15 levels and metastatic behavior has been proved in several studies. Xue *et al*. performed a complex study with an applied quantitative proteomics approach to compare the secretome of a primary colorectal cancer cell line and its lymph node metastatic cell line from the same patient; a total of 145 differentially expressed proteins were identified. An expression pattern of 6 candidate proteins was further validated by Western blot analysis including MIC1/GDF15 which was found to be up-regulated in the lymph node metastatic cell line. In subsequent studies of a large cohort of clinical tissue and serum samples, serum levels of MIC1/GDF15 were proved to be a discriminatory diagnostic test for predicting colorectal cancer metastasis (18). Data suggest that MIC1/GDF15 can be used as a serum marker for the diagnosis of metastases in uveal melanoma patients. Patients with clinically detectable metastases had significantly higher MIC1/GDF15 serum levels compared to those without clinically detectable metastases as well as healthy individuals (19).

In our study, we focused on the role of MIC/GDF15 as a biomarker of bone metastasis. We found higher levels in patients with bone metastases compared to the oncological patients with no bone metastasis. Our observation is supported by available data in literature. Roles for MIC1/GDF15 have recently been identified in the modulation of osteoclast differentiation and for therapy for bone metastases from prostate cancer (20). Westhrin *et al*. described the role of GDF15 in osteoclast differentiation and showed an association between high serum GDF15 levels and bone disease in multiple myeloma. (21). MIC1/GDF15 control tumors induced mixed sclerotic/lytic bone lesions but increased the osteolytic component of tumors. Osteoclast formation at the tumor-bone interface was significantly higher in the MIC1/GDF15 tumors in mice. MIC1 up-regulates TLR9 expression in various cells. MIC1/GDF15 stimulates both osteoblast and osteoclast differentiation *in vitro*, independently of TLR9. MIC1/GDF15 over-expression by prostate cancer cells that grow in bone induce osteoclast formation and cachexia (22). Lea *et al*. studied bone metastasis secretome in prostate cancer. Within the tumor microenvironment they identified 26 important proteins, both novel and previously published, including the MIC1/GDF15 molecule with both autocrine and paracrine influence on tumor cells and stromal cells – endothelial cells and osteoblasts. Furthermore MIC1/GDF15 treatment led to a small increase in SMAD1/5 phosphorylation in primary mouse osteoblasts and so MIC1/GDF15 signals stimulate osteoblast differentiation suggesting that MIC1/GDF15 may have effects on the metastatic and osteogenic potential of prostate cancer cells (23). In a comparable study to our presented work, Selander *et al*. from a group of 159 patients with prostate cancer, showed MIC1/GDF15 to be a predictor for the presence of baseline bone metastasis and to be significantly more accurate than carboxy-terminal telopeptide for type I collagen, prostate specific antigen, and PINP. Patients who experienced bone relapse had significantly higher levels of baseline MIC1/GDF15 compared with patients who did not (24).

Our observations as well as published data regarding bone, reviewed above, indicate the possible clinical use of MIC1/GDF15 as a biomarker for bone metastasis in oncology. This observation could potentially improve early detection and diagnosis of bone metastatic disease and help in optimal and sufficient treatment.

Literature data could engender discussion on the specificity of MIC1/GDF15 selectivity for bone localisation of metastatic process. In our study, no significant differences in MIC1/GDF15 levels between patients with or without non skeletal metastases were found.

Our study, in which patients were enrolled with several oncology diagnoses and a broad range of published oncology diagnoses in which MIC1/GDF15 levels were found to be increased, all illustrate the universality of the marker.

Our results show the insufficient sensitivity of MIC1/GDF15 for clinical use as a single marker of bone metastatic disease. We found 65% sensitivity at the point of 90% specificity and a related cut off value of 1.48 ng/ml. We propose studying this marker in combination with other potential candidates such as those described in our previous pilot study (1). This multi-marker approach was used for MIC1/GDF15 by Liu *et al*. (15) and Mohamed *et al*. but for different diagnostic purposes (14).

In conclusion, circulating MIC1/GDF15 is a powerful biomarker for bone metastatic disease, but its insufficient

sensitivity requires further studies incorporating its combination with other novel or routine tumor markers.

Acknowledgements

This study was supported by the research project of Ministry of Health of The Czech Republic IGA NT 13655-4.

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Received January 31, 2017
Revised February 27, 2017
Accepted February 27, 2017