Analysis of thymidine kinase serum levels by novel method DiviTum™ in multiple myeloma and monoclonal gammopathy of undetermined significance – comparison with imaging methods 99mTc-MIBI scintigraphy and 18F-FDG PET/CT

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Aims. The study aimed at comparing two methods for evaluating thymidinekinase TK in serum – an older RIA method and novel DiviTum™ – in patients with MM and MGUS, and also comparing them with biochemical markers and degree of activity evaluated by imaging methods 99mTc-MIBI scintigraphy and 18F-FDG PET/CT.

Methods. Serum thymidinekinase TK levels were evaluated by DiviTum™ and an RIA method (TK REA kit by Immunotech); The study analyzed correlation of TK activity in serum with biochemical markers reflecting activity of MM: β 2-m, LDH, the ratio of kappa to lambda (κ/λ) free light chains and percentage of bone marrow plasma cells (BMPC). 99mTc-MIBI scintigraphy and 18F-FDG PET/CT were performed at the time of diagnosis. The degree of activity was expressed semiquantitatively. Scans were classified as 0 (normal activity), 1 (diffuse positivity) or 2 (focal positivity).

Results. We found a strong positive correlation between TK in serum evaluated by DiviTum™ and by TK REA.. The DiviTum™ analytic method extended the detection range and was able to detect higher levels of TK than the RIA method. DiviTum™ technique found positive correlation with β 2-m ($r = 0.497$) and LDH ($r = 0.502$) and moderate positive correlation with BMPC ($r = 0.368$). Significantly higher TK values measured by TK REA and DiviTum™ in the group of patients with MM (stages I, II or III) than in those with MGUS. Increased TK levels were observed in MIBI- or PET/CT-positive patients. Analysis of repeated measurements of TK in serum during treatment of MM patients found a correlation between change in TK measured by DiviTum™ and LDH during treatment.

Conclusions. Analysis revealed a significant correlation between TK in serum and LDH, β 2-m and BMPC. Increased levels of TK in serum were observed in MIBI- or PET/CT-positive patients. Combination of positivity of imaging methods which can localize active tumor lesions and increased levels of TK in serum can have an impact on decision-making and optimization of the therapeutic approach.

Key words: multiple myeloma, MGUS, thymidine kinase, beta-2 microglobulin, LDH, 99mTc-MIBI scintigraphy, 18F-FDG PET/CT

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INTRODUCTION

Cell proliferation activity is a central independent prognostic factor and one of the targets for personalized and risk-adapted treatment in multiple myeloma (MM) (ref.¹). Increased activity of thymidine kinase (TK) in serum is associated with higher proliferation rates in hematological malignancies. Imaging methods such as 99mTc-MIBI scintigraphy or 18F-FDG PET/CT can visualize active myeloma lesions and their positivity is a negative prognostic factor²⁻⁴. Accurate determination of

high TK levels at the time of diagnosis may identify patients with poorer treatment outcome^{5,6}. Serum TK levels may be correlated with other markers reflecting disease activity. These are serologic parameters lactate dehydrogenase (LDH), beta-2 microglobulin (β 2-m), plasma cell labeling index, uptake of FDG in FDG PET/CT examination, and activity of MIBI scintigraphy⁷⁻⁹. Serum TK levels may predict the risk of progression of monoclonal gammopathy of undetermined significance (MGUS) to MM (ref.⁹⁻¹¹). The presence of increased TK activity in serum and its correlation to malignant diseases was first

shown in 1982. Since then, radioactive assays for evaluation of TK have been used, e.g. Prolifigen® TK-REA (DiaSorin) or an Immunotech TK REA kit. These methods have demonstrated that levels of serum TK in patients with malignancies correspond to the number of dividing tumor cells. Thus, TK has indirectly been used for measuring cell division in cell culture by addition of radioactive thymidine followed by measurement of radioactivity incorporated in the DNA. Serum TK activity assays have found major clinical application in the prognosis and monitoring of therapy in some hematological malignancies^{6,12,13}. Recently, a novel non-radioactive method for evaluation of TK has been introduced. TK DiviTum™ (Biovica) is a non-radioactive high-sensitive *in vitro* diagnostic product intended for quantitative determination of TK activity in serum¹⁴. Its analytic method significantly extends the working range and is able to quantify higher levels than the conventional radioimmunoassay (RIA) method. A prospective study was conducted to analyze the predictive role of assessment of TK levels with DiviTum™ in patients with newly diagnosed MM and MGUS. In clinical practice, prognosis estimates are based on classical prognostic scores such as the International Staging System (ISS) and the presence of cytogenetic abnormalities. In high-risk patients who are candidates for intensive therapy, clinical decision-making should include as broad a spectrum of additional risk factors as possible. Analysis of TK levels at the time of diagnosis, during the treatment and at the time of restaging provides additional prognostic information even in samples treated with novel drugs.

AIMS

The study aimed at comparing two methods for evaluating TK in serum – an older RIA method and novel DiviTum™ – in patients with MM and MGUS, and also comparing them with biochemical markers and degree of activity evaluated by imaging methods 99mTc-MIBI scintigraphy and 18F-FDG PET/CT. Combination of biochemical and imaging methods may have higher prognostic significance for monitoring of the disease at the time of diagnosis and during its course.

METHODS

Serum TK levels were evaluated by DiviTum™ (normal range, 0-50 Du/L) and an RIA method (TK REA kit by Immunotech; normal range, 0-9 U/L). In DiviTum™, TK activity was measured with a refined ELISA-based method. A brief summary of the technique is as follows: on Day 1, 10 µL of serum sample are added to a total of 500 µL of a dilution buffer in a well on a microtiter plate. Then, 10 µL of the diluted sample are transferred to a well with 100 µL of a reaction mixture on an assay plate. Bromodeoxyuridine, a thymidine analogue, is used as a substrate to TK in the serum sample. The assay plate including two control samples is sealed and incubated at 32°C for 18 h. The product of the reaction binds to the

bottom of the well. On Day 2, the plates are washed and 100 µL of antibody conjugate solution is added to each well. The conjugate consists of a monoclonal anti-BrdU antibody linked to alkaline phosphatase. The plate is incubated at 32 °C for 1 h. The wells are then washed to remove unbound antibody and 120 µL of substrate solution (alkaline phosphatase; 4-nitrophenyl phosphate) is added to each well. The product of the alkaline phosphatase reaction is yellow and hence the absorbance is measured at 405 nm with a reference wavelength of 630 nm. Absorbance is measured after 30 and 180 min of incubation at 32 °C. The measured signal is proportional to TK activity of the tested sample. The DiviTumEval software was used for data evaluation. The coefficient of variation of the DiviTum™ assay measuring at 100 Du/L is better than 20%. The study analyzed correlation of TK activity in serum with the following biochemical markers reflecting activity of MM: β2-m, LDH, the ratio of kappa to lambda (κ/λ) free light chains and percentage of bone marrow plasma cells (BMPC). The β2-m serum levels were assessed with the SPA_{PLUS}® analyzer (Binding Site; normal range, 0.80-2.34 mg/L). The κ/λ ratio was determined with the Freelite® system (Binding Site). Infiltration of bone marrow with plasma cells was obtained from bone marrow smears. 99mTc-MIBI scintigraphy and 18F-FDG PET/CT were performed at the time of diagnosis. The degree of activity was expressed semiquantitatively. Scans were classified as 0 (normal activity), 1 (diffuse positivity) or 2 (focal positivity).

PATIENTS

The analyzed group of 83 patients consisted of 64 (77.1%) patients with MM and 19 (22.9%) individuals with MGUS. The median age was 64 years (range, 32–86 years; the male-to-female ratio was 1.1. There were 67% of IgG cases, 21% of IgA cases and 12% with Bence Jones protein production only. Patients were diagnosed according to the International Myeloma Working Group criteria and staged according to the Durie-Salmon (D-S) staging system and ISS as follows: D-S I (n=6), II (n=20) and III (n=38); MM ISS 1 (n=26), 2 (n=19) and 3 (n=19). Patients were assessed at the time of diagnosis prior to the treatment and during their therapy.

DATA ANALYSIS

Data were analyzed with the SPSS version 15 (SPSS Inc., USA) software. Statistical evaluation was carried out using Fisher's exact test, Spearman's correlation analysis and the Mann-Whitney U test at a significance level of 0.05.

RESULTS

We found a strong positive correlation between TK in serum evaluated by DiviTum™ and by TK REA

Fig. 1. ^{18}F -FDG PET/CT revealed glucose hypermetabolism in the right mandible and adjacent soft tissues, mediastinum, bone marrow in the iliac crest and both femurs.

(Spearman's correlation coefficient $r = 0.895$). After extreme values were excluded, the correlation coefficient was $r = 0.868$. The DiviTumTM analytic method extended the detection range and was able to detect higher levels of TK than the RIA method. There was a clear correlation of serum TK with biochemical markers reflecting activity of MM such as $\beta_2\text{-m}$, LDH or BMPC. TK REA found a positive correlation of moderate strength with $\beta_2\text{-m}$ ($r = 0.549$) and LDH ($r = 0.468$) and a weak correlation with BMPC ($r = 0.346$). The novel DiviTumTM technique found a moderate positive correlation with $\beta_2\text{-m}$ ($r = 0.497$) and LDH ($r = 0.502$) and a weak to moderate positive correlation with BMPC ($r = 0.368$). The Mann-Whitney U test showed significantly higher TK values measured by TK REA and DiviTumTM in the group of patients with MM (stages I, II or III) than in those with MGUS. The median values found by TK REA were 6.6 and 11.5 in MGUS and MM, respectively ($P = 0.0004$); the median values obtained by DiviTumTM were 47.0 and 152.5 in MGUS and MM, respectively ($P = 0.001$). No correlation was found between TK in serum and either the κ/λ ratio or age of patients. Imaging methods MIBI or PET/CT were positive in 21 patients and negative in 15 patients out of 36

Fig. 2. $^{99\text{m}}\text{Tc}$ -MIBI scintigraphy. Whole-body planar scintigraphy (anterior and posterior views) showed focal pathological accumulation of the radiopharmaceutical in the frontal and, less significantly, occipital skull. $^{99\text{m}}\text{Tc}$ -MIBI is accumulated in an area of osteolysis in the frontal region, suggesting myeloma relapse.

Fig. 3. Significantly higher TK values were in the group of patients with MM (stages I, II or III) than in those with MGUS. The median values obtained by DiviTumTM were 47.0 and 152.5 in MGUS and MM, respectively ($P = 0.001$).

investigated patients. Increased TK levels were observed in MIBI- or PET/CT-positive patients. The Mann-Whitney U test showed significantly higher TK in serum measured by DiviTumTM in the group of PET/CT- or MIBI-positive patients (0 = negative, 1 or 2 = positive); positive median 182 vs. negative 44, $P = 0.045$; AUC = 0.791. It means that DiviTumTM could be used as a predictor for MM. The Mann-Whitney U test did not show a significant difference in TK measured by TK REA in the group of PET/CT- or MIBI-positive or -negative patients ($P = 0.127$). Analysis of repeated measurements of TK in serum during treatment of MM patients found a correlation between change in TK measured by DiviTumTM and LDH during treatment after 2 months ($r = 0.525$) and after 5 months ($r = 0.515$).

DISCUSSION

Increased activity of TK in serum is associated with higher proliferation rates in hematological malignancies including MM⁷. Both DiviTumTM and TK REA found significantly increased levels in MM compared with MGUS patients, as reported previously⁷. The DiviTumTM method significantly extends the detection range and is able to accurately measure high levels of TK in serum. Accurate determination of high TK levels at the time of diagnosis may identify patients with poorer predicted treatment outcome. DiviTumTM found a stronger correlation with 99mTc-MIBI scintigraphy and 18F-FDG PET/CT than the older RIA method. Both 99mTc-MIBI scintigraphy and 18F-FDG PET/CT are useful for detecting active tumor lesions and the degree of positivity has a prognostic significance^{2,4}. During the treatment, TK levels change, and so do LDH levels, reflecting biological response to therapy. Monitoring of TK and LDH levels can be used for monitoring the effect of therapy¹³.

CONCLUSIONS

Statistical analysis revealed a significant correlation between TK in serum and LDH, β 2-m and BMPC. Increased levels of TK in serum were observed in MIBI- or PET/CT-positive patients. Combination of positivity of imaging methods which can localize active tumor lesions and increased levels of TK in serum can have an impact on decision-making and optimization of therapeutic approach.

ABBREVIATIONS

β 2-m, Beta-2 microglobulin; BMPC, Bone marrow plasma cell; D-S, Durie-Salmon staging system; ELISA, Enzyme-linked immunosorbent assay; ISS, International Staging System; LDH, Lactate dehydrogenase; MGUS, Monoclonal gammopathy of undetermined significance; MM, Multiple myeloma; REA, Radio-enzyme assay; RIA, Radioimmunoassay; TK, Thymidine kinase.

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Authorship contributions: JB: manuscript writing, study design, data analysis and interpretation; MM: figures, data interpretation; JM,VS,TP: literature search; JZ: statistical analysis; PV, MB, TA: data analysis; SG: study design.

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