EVALUATION OF HGF AND ENDOSTATIN IN THE BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA AND THE EFFECT OF PERIPHERAL BLOOD ADMIXTURE

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ABSTRACT

The aim of our study was to evaluate the concentration changes in the proangiogenic hepatocyte growth factor (HGF) and antiangiogenic endostatin during the sampling of large bone marrow volumes in patients with multiple myeloma. HGF and endostatin concentrations were measured in the 1st ml, the 5th ml, the 19th ml, the 30th ml, and the 45th ml of the sample. Concentrations of HGF, which is produced by bone marrow and myeloma cells, decreased significantly in the course of BM sampling due to the admixture of peripheral blood. Endostatin concentrations did not change significantly during the procedure of sampling, as endostatin is mainly secreted by the parenchymatous organs such as liver, lungs, and others. In conclusion, the admixture of the peripheral blood could be partly responsible for conflicting results of studies of HGF in multiple myeloma. Standardisation of HGF sampling is required for valid comparisons between different studies.

INTRODUCTION

Continually, new prognostic and predictive factors are being discovered that contribute to our understanding of the pathogenesis of multiple myeloma (MM) and to the development of evidence-based approaches in MM treatment. Presently, there are tests that are used routinely and/or for research purposes in MM. Various parameters are determined from the bone marrow (BM), and sometimes they require sampling of large volumes of BM. Angiogenesis plays a key role in myeloma pathogenesis [1]. The evaluation of angiogenesis is usually done from BM. However, there is concern that concentrations of angiogenic parameters may vary during the sampling of large volumes of BM due to increasing admixture of peripheral blood, as has been described for other types of studies, e.g. morphology assessment [2] and S-phase DNA content measurement [3]. The aim of our study

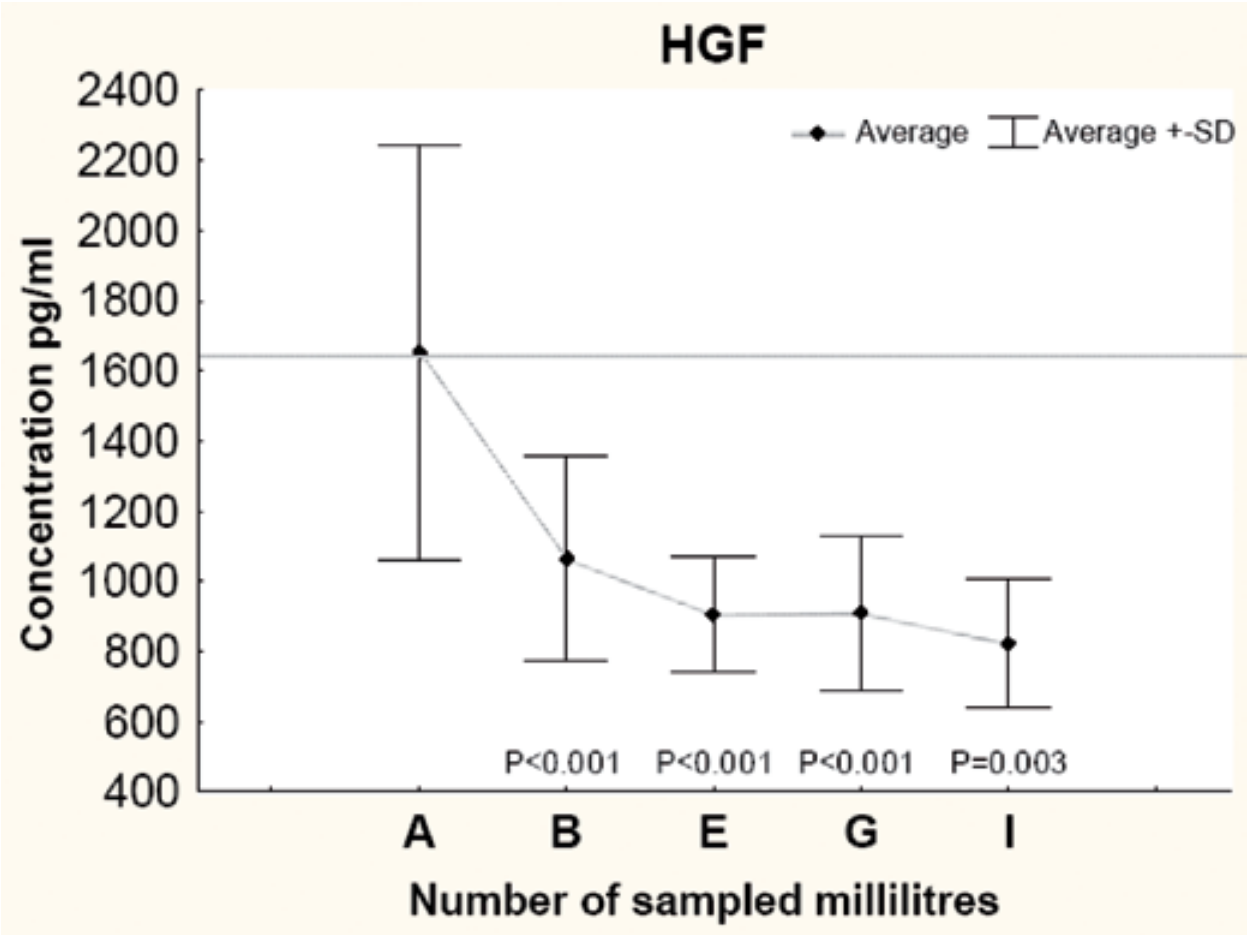

Figure 1A
Concentrations of HGF in pg/ml; p levels mean statistical difference between concentrations in 1st (A), 5th (B), 19th (E), 30th (G), and 45th (I) millilitre

was to evaluate the fluctuations in the concentrations of two molecules related to the formation of new vasculature – the proangiogenic hepatocyte growth factor (HGF) and the anti-angiogenic endostatin during the sampling of large BM volumes in patients with MM.

MATERIALS AND METHODS

BM was sampled from a standard sternal puncture after obtaining an informed consent. The maximum volume of BM sampled for diagnostic and research purposes was 45 ml. We tested samples from 9 men and 6 women. The type of the monoclonal protein produced was IgG in 11 patients and IgA in 4 patients. The average age of the patients was 64.8 years. HGF and endostatin concentrations were measured in the 1st ml, the 5th ml, the 19th ml, the 30th ml, and the 45th ml of the sample. ELISA kits HGF (Biosource, USA) and Endostatin (Chemicon, USA) were used according to the manufacturers' instructions. Statistical evaluation was carried out using Statistica software (StatSoft, USA).

RESULTS AND DISCUSSION

We sampled 45 ml of BM in eleven patients, 30 ml of bone marrow in two patients, and 5 ml of BM in two patients. Sampling of large BM volumes was well tolerated in all the patients. The concentration of HGF decreased progressively during the BM sampling (Figure 1A). Endostatin concentrations did not change significantly during the procedure of sampling (Figure 1B). HGF and endostatin are important factors thought to be involved in the angiogenesis in MM. Serum and BM levels of HGF have been reported to be higher in

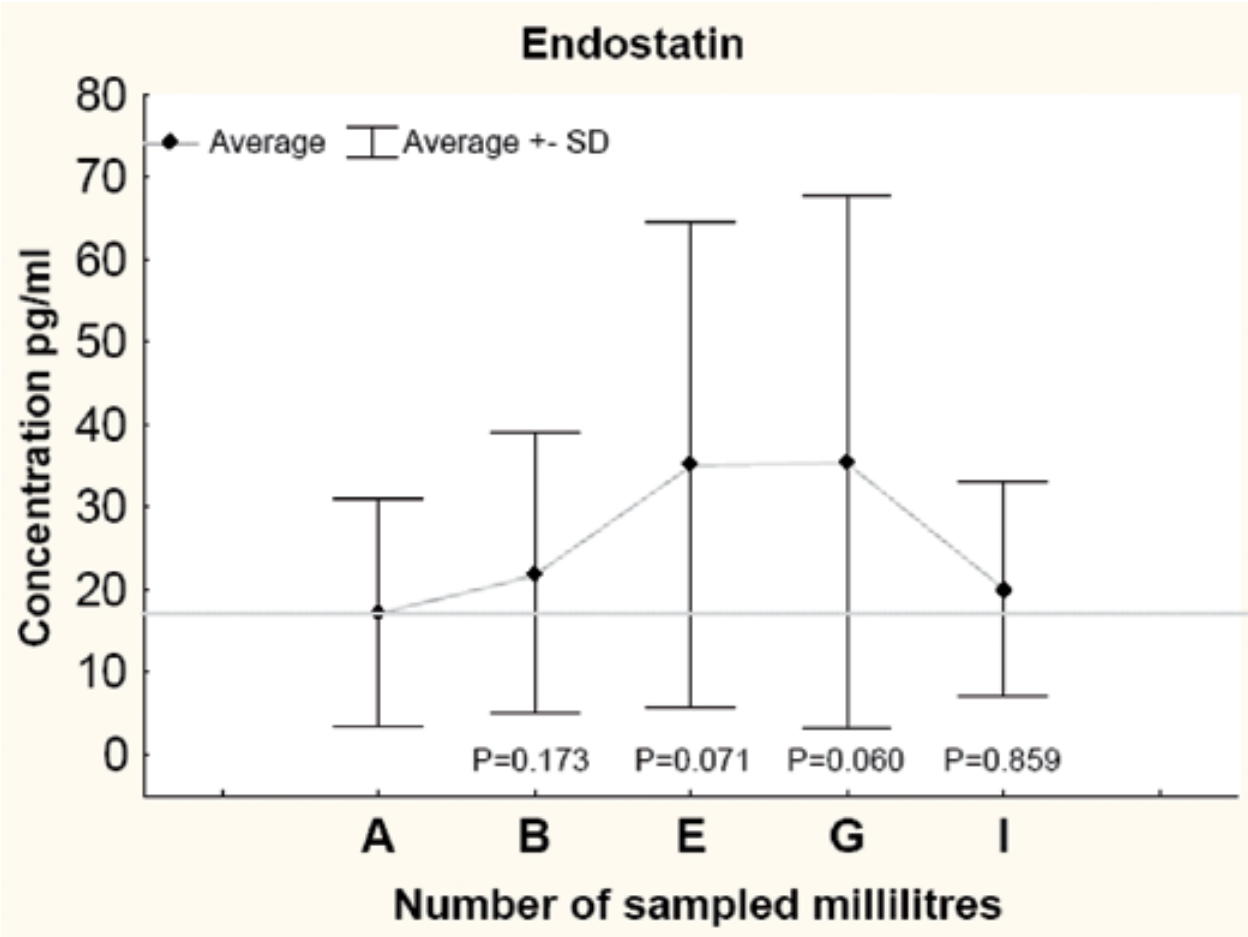

Figure 1B
Concentrations of endostatin in pg/ml; p levels mean statistical difference between concentrations in 1st (A), 5th (B), 19th (E), 30th (G), and 45th (I) millilitre

myeloma patients than in healthy controls [4]. However, the role of HGF in MM has yet to be fully elucidated. Its concentration was decreased after the treatment according to some reports [5], while according to others its level was not changed after the treatment [5]. Our study showing that HGF levels decrease significantly in the course of BM aspiration could at least partly explain the discrepancies between the various reports. The reason for the drop is probably the increased content of peripheral blood in the BM aspirate, because HGF is mainly produced by myeloma and BM stromal cells [6]. Endostatin is a terminal fragment of type XVIII collagen that arrests cell proliferation and is a potent inhibitor of tumour growth [7]. There are reports that the levels of endostatin are higher in myeloma patients compared to a healthy control [8]. Our results did not show statistically significant variations in BM endostatin levels in different phases of sampling. An explanation of this fact could be that endostatin is not produced in the BM but mainly in parenchymatous organs such as the liver, brain and lungs [7], and thus the admixture of peripheral blood does not alter BM endostatin concentrations. In conclusion, HGF levels decrease progressively during the sampling of BM. Therefore, it is best to evaluate HGF levels always in the same phase of sampling, preferably from the first portion. Standardisation of HGF sampling is required if different studies are to be compared.

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