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BIOCHIP ARRAY TECHNOLOGY AND EVALUATION OF SERUM LEVELS OF MULTIPLE CYTOKINES AND ADHESION MOLECULES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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***Aim*: Evaluation of serum levels of 17 cytokines and 5 adhesion molecules in patients with newly diagnosed acute myeloid leukemia (AML) and in healthy subjects using biochip array technology. *Methods*: A total of 15 AML patients and 15 healthy subjects (blood donors) were studied. Serum samples were analyzed by biochip based immunoassays on the Evidence Investigator analyzer. This approach allows multi-analytical determination from a single sample. T-tests were used for statistical analysis. *Results*: In newly diagnosed AML patients, we found significant increase ($p < 0.01$) in serum VCAM-1, ICAM-1, E-selectin, L-selectin, and significant increase ($p < 0.05$) in serum IL-6, IL-8. No significant differences were found in the levels of other evaluated cytokines and adhesion molecules. *Conclusion*: Our results indicate that serum levels of specific cytokines and adhesion molecules (VCAM-1, ICAM-1, E-selectin, L-selectin, IL-6, IL-8) are significantly altered in patients with newly diagnosed AML, showing activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. Further studies will be needed to define the potential role of these and additional markers in the risk stratification of AML.**

***Key Words*: cytokines, adhesion molecules, biochip array, acute myeloid leukemia.**

Cytokines and adhesion molecules have been studied in many pathological states including cancer [1–3] and acute leukemias [4, 5]. Alterations in this interacting functional network may have direct effect on the malignant cells or have indirect effect on leukemogenesis through altered functions of bone marrow stromal elements [6, 7]. The knowledge gained from multiple cytokine and adhesion molecule analysis could allow better diagnosis and disease management, since cytokines or their receptors may also represent a target for specific anticancer therapy at the molecular level. Recently, some studies reported the possible diagnostic and prognostic use of cytokine levels in newly diagnosed acute myeloid leukemia (AML) and myelodysplastic syndromes [8–10].

The aim of our pilot study was to evaluate serum levels of multiple cytokines and adhesion molecules in patients with newly diagnosed AML and in healthy subjects using the innovative biochip array technology.

Serum samples of 15 newly diagnosed *de novo* AML patients (median age 51, range 24–61 years, 8 males) and 15 healthy subjects (median age 41, range 25–58 years, 11 males) were analyzed. The study was approved by the local Ethics Committee and all patients gave a written consent.

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Abbreviations used: AML – acute myeloid leukemia; CR – complete remission; EGF – epidermal growth factor; ICAM-1 – intercellular adhesion molecule-1; IFN-gamma – interferon-gamma; IL – interleukin; MCP-1 – monocyte chemotactic protein-1; TNF-alpha – tumor necrosis factor-alpha; VCAM-1 – vascular cell adhesion molecule-1; VEGF – vascular endothelial growth factor.

We evaluated serum levels of the following 17 cytokines and 5 adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All analytes were measured by biochip array technology using chemiluminescent sandwich immunoassays applied to the Evidence Investigator analyzer (Randox Laboratories Ltd., Crumlin, UK).

Statistical analysis was performed with the "Statistica" program. T-tests were used. The values were expressed as mean ± SD. Probability values $p < 0.05$ were considered statistically significant.

Comparing serum cytokine and adhesion molecule levels in AML patients and in healthy subjects, we found significant increase in AML patients in serum VCAM-1 (716.22±364.38 mcg/L *vs* 328.31±88.66 mcg/L; $p < 0.01$), ICAM-1 (659.61 ± 259.50 mcg/L *vs* 196.69 ± 36.06 mcg/L; $p < 0.01$), E-selectin (30.19 ± 20.46 mcg/L *vs* 13.89 ± 4.80 mcg/L; $p < 0.01$), L-selectin (2179.35 ± 1169.39 mcg/L *vs* 1104.54 ± 243.45 mcg/L; $p < 0.01$), IL-6 (46.24 ± 83.14 ng/L *vs* 0.52 ± 0.44 ng/L; $p < 0.05$), IL-8 (104.99 ± 167.30 ng/L *vs* 4.87 ± 3.09 ng/L; $p < 0.05$). Serum levels of other evaluated cytokines and adhesion molecules were without significant differences.

Altered levels of cytokines and adhesion molecules have been found in many pathological states and have been linked to many diseases including cardiovascular

diseases and cancer [1–3, 11–13]. The cytokine system constitutes an interacting functional network where the contribution from single cytokines is modulated by the levels of other cytokines. It may therefore be more relevant to look at the total serum profile of these molecules.

Biochip array technology enables simultaneous detection of multiple cytokines and adhesion molecules in a single sample and provides valuable information relating to each tested analyte and possible associations between analytes in each sample [14, 15]. We recently published our experience with biochip arrays for cytokines and adhesion molecules in acute lymphoblastic leukemia patients [16]. The presented paper is among the first published studies using the innovative biochip array technology to determine circulating levels of cytokines and adhesion molecules in AML patients.

Our results indicate that serum levels of specific cytokines and adhesion molecules (VCAM-1, ICAM-1, E-selectin, L-selectin, IL-6, IL-8) are significantly altered in patients with newly diagnosed AML, showing activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. Further studies in a larger number of patients and comparing cytokine and adhesion molecule levels with established prognostic markers (cytogenetics, molecular genetics) will be needed to define the potential role of these and additional markers in the risk stratification of AML patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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