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

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***Aim*: Evaluation of serum levels of 17 cytokines and 5 adhesion molecules in patients with acute lymphoblastic leukemia (ALL) and in healthy subjects using biochip array technology. This approach allows multi-analytical determination from a single sample.** ***Methods*: A total of 15 ALL patients and 15 healthy subjects (blood donors) were studied. Serum samples were analyzed by biochip based immunoassays on the Evidence Investigator analyzer. T-tests were used for statistical analysis.** ***Results*: Comparing cytokine and adhesion molecule levels in ALL patients and in healthy subject, we found significant increase in serum VCAM-1 ($p < 0.000001$), ICAM-1 ($p < 0.0001$), L-selectin ($p < 0.0001$), IL-8 ($p < 0.001$), MCP-1 ($p < 0.01$), and significant decrease ($p < 0.01$) in serum IL-3 and IL-4.** ***Conclusion*: Our results indicate that serum levels of specific cytokines and adhesion molecules (VCAM-1, ICAM-1, L-selectin, IL-8, IL-3, IL-4, MCP-1) are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. Further investigation is needed to establish if these alterations could be used as a prognostic indicator for ALL.**

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

Cytokines and adhesion molecules have been studied in many pathological states including hematological malignancies [1–3] and acute leukemias, both myeloid (AML) and lymphoblastic (ALL) [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 multi-analytical determination of cytokines and adhesion molecules could allow better diagnosis and management of hematological malignancies, 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 leukemias and myelodysplastic syndromes [8–11].

The aim of our pilot study was to evaluate serum levels of multiple cytokines and adhesion molecules in patients with newly diagnosed ALL and in healthy subjects using the innovative biochip array technology. This generates a patient profile, which is relevant when investigating interacting functional networks.

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Abbreviations used: ALL – acute lymphoblastic leukemia; 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.

Subjects. A total of 15 newly diagnosed ALL patients (median age 46, range 24–63 years, 11 males) and 15 healthy subjects (median age 41, range 25–58 years, 11 males) were studied. The study was approved by the local Ethics Committee and all patients gave a written consent.

Multi-analytical evaluation. We evaluated circulating 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). We analyzed serum samples at the diagnosis of ALL (active leukemia) and in healthy subjects (blood donors).

Statistical analysis. Statistical analysis was performed with the "Statistica" program. T-tests were used. The values were expressed as mean ± SD. Probability values (p) < 0.01 were considered statistically significant.

In newly diagnosed ALL patients, we found significant increase in serum VCAM-1 (1078.54 ± 456.96 mcg/L vs. 328.31 ± 88.66 mcg/L; $p < 0.000001$), ICAM-1 (499.57 ± 237.53 mcg/L vs. 196.69 ± 36.06 mcg/L; $p < 0.0001$), L-selectin (2366.33 ± 1035.37 mcg/L vs. 1104.54 ±

243.45mcg/L;p<0.0001),IL-8(34.07±28.52ng/Lvs.4.87± 3.09 ng/L; p < 0.001), MCP-1 (433.99 ± 328.59 ng/L vs. 153.25 ± 53.60 ng/L; p < 0.01). On the other hand, we found significant decrease in serum IL-3 (7.34 ± 3.41 ng/L vs. 11.53 ± 4.66 ng/L; p < 0.01), IL-4 (1.10 ± 1.08 ng/L vs. 3.27 ± 2.21 ng/L; p < 0.01). No significant differences were found in the levels of other evaluated cytokines and adhesion molecules.

To our knowledge, this is the first published study using the innovative biochip array technology to determine circulating levels of cytokines and adhesion molecules in ALL patients.

Altered levels of cytokines and adhesion molecules have been found in many pathological states and have been linked to many diseases such as autoimmune diseases, allergies, cardiovascular diseases and cancer [12–17]. 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 cytokines and adhesion 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 [18, 19].

Our results indicate that serum levels of specific cytokines and adhesion molecules (VCAM-1, ICAM-1, L-selectin, IL-8, IL-3, IL-4, MCP-1) are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. Further investigation is needed to establish if the alterations observed in the levels of these molecules could be used as a prognostic indicator for ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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