Exp Oncol 2021
43, 3, 234–236

Experimental ONCOLOGY

EVALUATION OF SERUM LEVELS OF SELECTED CYTOKINE RECEPTORS IN ADULT B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR ASSOCIATION WITH PROGNOSTIC FACTORS AND SURVIVAL

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***Aim*: To evaluate serum levels of selected cytokine receptors in B-cell precursor acute lymphoblastic leukemia (B-ALL) and their association with acknowledged prognostic factors, relapse-free survival (RFS) and overall survival (OS). *Materials and Methods*: A total of 42 *de novo* adult B-ALL patients, 19 BCR/ABL positive, were included in this study. Soluble receptor α for IL-2 (sIL-2Rα), soluble receptor for IL-6 (sIL-6R), soluble receptor for TNF-α type I and II (sTNFR-1, sTNFR-2) and matrix metalloproteinase-9 (MMP-9) were measured by biochip array technology at diagnosis and in complete remission (CR). *Results*: At diagnosis of B-ALL, we found significantly higher levels of sIL-2Rα, sIL-6R, sTNFR-1, sTNFR-2 and significantly lower levels MMP-9 in comparison with CR ($p < 0.001$ in all cases). BCR/ABL positive patients had higher levels of sIL-2Rα at diagnosis ($r = 0.484$; $p = 0.014$). Serum levels of evaluated cytokines were not associated with achievement of CR after one cycle of induction therapy, RFS or OS. *Conclusion*: Serum levels of all evaluated cytokines are significantly altered in newly diagnosed B-ALL reflecting activity of the disease. No significant correlations with response to first induction therapy, RFS or OS were found. Further studies with a longer follow-up will be needed.**

***Key Words*: cytokine, soluble cytokine receptor, sIL-2Rα, prognosis, survival, acute lymphoblastic leukemia.**

DOI: 10.32471/exp-oncology.2312-8852.vol-43-no-3.16548

Dysregulated production of cytokines and adhesion molecules has been implicated in the onset and progression of various types of cancer including hematological malignancies [1, 2]. Better understanding of tumor microenvironment is essential for development of new treatment approaches [3–5]. Some studies reported diagnostic and prognostic value of cytokine and adhesion molecule levels in acute leukemias and other hematological malignancies [6–9].

The aim of this study was to evaluate serum levels of selected cytokines in adult B-cell precursor acute lymphoblastic leukemia (B-ALL) at diagnosis and in complete remission (CR) and their association with acknowledged prognostic factors, relapse-free survival (RFS) and overall survival (OS).

MATERIALS AND METHODS

A total of 42 *de novo* B-ALL patients (median age 49, range 19–75 years; 28 males, 14 females; all Caucasian) were included in this study. Nineteen patients were BCR/ABL positive. The study was approved by the local Ethics Committee and all patients gave a written consent.

Serum samples were taken at diagnosis and in CR. We used Cytokine IV Array (Randox Laboratories Ltd., UK) containing the following analytes: soluble receptor α for IL-2 (sIL-2Rα), soluble receptor for IL-6 (sIL-6R), soluble receptor for TNF-α type I (sTNFR-1), soluble receptor for TNF-α type II (sTNFR-2) and matrix metalloproteinase-9 (MMP-9). All analytes were measured by biochip array technology on Evidence Investigator analyzer (Randox Laboratories Ltd., UK).

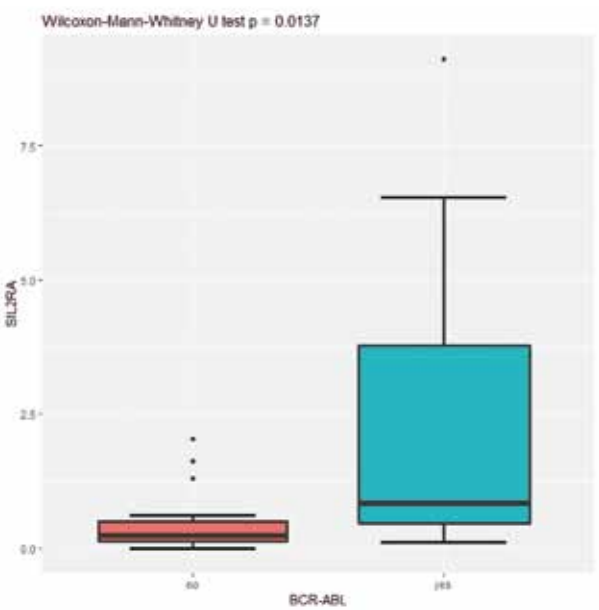

Figure. Correlation between BCR/ABL positivity and serum sIL-2Rα levels at diagnosis of B-ALL

Submitted: March 20, 2021.

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Abbreviations used: ALL – acute lymphoblastic leukemia; B-ALL – B-cell precursor acute lymphoblastic leukemia; CR – complete remission; MMP-9 – matrix metalloproteinase-9; OS – overall survival; RFS – relapse-free survival; sIL-2Rα – soluble receptor α for IL-2; sIL-6R – soluble receptor for IL-6; sTNFR-1 – soluble receptor for TNF-α type I; sTNFR-2 – soluble receptor for TNF-α type II.

Correlations between cytokines, acknowledged prognostic factors (age, white blood cell count, immunophenotype, BCR/ABL positivity, response to induction therapy), RFS and OS were evaluated separately in both clinical situations.

Statistical evaluation was done by a professional statistician using software R 3.5.3 (R Core Team 2019). The values were expressed as mean ± SD. $p < 0.05$ with Bonferroni–Holm correction were considered statistically significant.

RESULTS AND DISCUSSION

At diagnosis of B-ALL, we found significantly higher levels of sIL-2Rα, sIL-6R, sTNFR-1, sTNFR-2 and significantly lower levels MMP-9 in comparison with CR ($p < 0.001$ in all cases) (Table 1).

Levels of sTNFR-1 correlated with sTNFR-2 at diagnosis and in CR ($p < 0.001$). In CR, sIL-2Rα correlated with sIL-6R, sTNFR-1, sTNFR-2 ($p < 0.001$) and sIL-6R correlated with sTNFR-2 ($p < 0.001$).

BCR/ABL positive patients had higher levels of sIL-2Rα at diagnosis (r = 0.484; $p = 0.014$), but not in CR (Figure). Correlations of cytokines with other acknowledged prognostic factors in B-ALL did not reach statistical significance. Correlation coefficients are shown in Table 2 and Table 3.

In our cohort, CR after one cycle of induction therapy was achieved in 88% of patients (12% of patients achieved CR after additional cycle of therapy), 1-year RFS was 74% and 1-year OS was 86%. Serum levels of evaluated cytokines were not associated with achievement of CR after one cycle of induction therapy, RFS or OS. The correlation coefficients are shown in Table 4 and Table 5.

Cytokines are an important part of leukemia microenvironment [10]. Biochip array technology enables simultaneous quantitative detection of multiple related cytokine immunoassays (in parallel) from a single sample and will have great value in personalized and precision medicine [11]. Previously, we published our experience with evaluation of multiple cytokines and adhesion molecules by biochip array technology in acute leukemias [8, 9, 12, 13]. In this study, we measured a different set of cytokines, containing soluble cytokine receptors and MMP-9, at diagnosis and in CR of B-ALL. Our results show that serum levels of all evaluated cytokines are significantly altered in newly diagnosed B-ALL, reflecting activity of the disease. Overexpression of cytokine receptors is obvious in active acute lymphoblastic leukemia (ALL), but declines after chemotherapy with achievement of CR.

Several analyses in various cohorts of patients have been made to evaluate prognostic information rising from baseline cytokine levels [14, 15]. In our study, we found statistically significant correlation between BCR/ABL positivity and sIL-2Rα levels at diagnosis. BCR/ABL positivity belongs to negative prognostic factors in ALL [16]. However, the outcome of these patients has dramatically improved with the introduction of tyrosine kinase inhibitors (imatinib and other agents) [17]. So far, we did not find any significant correlations with response to first induction therapy, RFS or OS. Further studies with a larger spectrum of cytokines and a longer follow-up will be needed.

We hope that further research in this field will bring new findings and these markers would serve as independent predictors of outcome or novel therapeutic targets in ALL.

Table 1. Serum levels of cytokine receptors and MMP-9 at diagnosis and in complete remission of B-ALL

Cytokine receptors and MMP	At diagnosis	CR
sIL-2Rα [μg/L]	1.33 ± 2.04	0.06 ± 0.08
sIL-6R [μg/L]	2.32 ± 1.70	1.25 ± 0.80
sTNFR-1 [μg/L]	0.91 ± 0.43	0.43 ± 0.21
sTNFR-2 [μg/L]	0.95 ± 0.82	0.37 ± 0.37
MMP-9 [μg/L]	25.36 ± 25.04	50.60 ± 34.34

Note: The difference between concentration at diagnosis and in CR was significant for all studied cytokine receptors and MMP-9 ($p < 0.001$).

Table 2. Coefficients of correlation between cytokine receptors and MMP-9 levels and prognostic factors at diagnosis of B-ALL

Cytokine receptors and MMP	Age	WBC	HR IF	BCR/ABL	LDH
sIL-2Rα	0.235	0.407	0.304	**0.484**	-0.033
sIL-6R	0.144	0.158	0.251	0.295	-0.186
sTNFR-1	0.256	-0.012	0.058	-0.018	-0.311
sTNFR-2	0.246	0.114	0.228	0.221	-0.276
MMP-9	0.176	0.225	0.123	0.200	-0.272

Notes: WBC – white blood cell count; LDH – serum lactate dehydrogenase; HF IF – high risk immunophenotype.

Table 3. Coefficients of correlation between cytokine receptors and MMP-9 levels and prognostic factors in CR of B-ALL

Cytokine receptors and MMP	Age	WBC	HR IF	BCR/ABL	LDH
sIL-2Rα	0.265	0.168	0.136	0.091	0.019
sIL-6R	0.194	0.061	0.219	0.128	0.041
sTNFR-1	0.519	0.008	0.241	0.146	0.063
sTNFR-2	0.493	0.085	0.343	0.305	0.023
MMP-9	0.177	-0.108	-0.025	0.008	0.305

Notes: WBC – white blood cell count; LDH – serum lactate dehydrogenase; HF IF – high risk immunophenotype.

Table 4. Coefficients of correlation between cytokine receptors and MMP-9 levels at diagnosis and response to first induction therapy and survival in B-ALL

Cytokine receptors and MMP	CR after 1st IND	RFS	OS
sIL-2Rα	0.062	0.239	0.253
sIL-6R	0.161	0.086	0.149
sTNFR-1	-0.153	-0.030	0.246
sTNFR-2	-0.072	0.071	0.249
MMP-9	-0.034	0.107	0.171

Table 5. Coefficients of correlation between cytokine receptors and MMP-9 levels in CR and response to first induction therapy and survival in B-ALL

Cytokine receptors and MMP	CR after 1st IND	RFS	OS
sIL-2Rα	-0.065	-0.150	-0.239
sIL-6R	0.091	0.017	-0.227
sTNFR-1	-0.183	-0.142	0.040
sTNFR-2	-0.006	-0.071	-0.015
MMP-9	0.116	-0.072	0.083

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The work was supported by a long-term organization development plan 1011 (FMHS, Hradec Kralove) and by MH CZ — DRO (UHHK, 00179906).

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ОЦІНКА РІВНІВ ЦИТОКІНОВИХ РЕЦЕПТОРІВ У СИРОВАТЦІ ДОРОСЛИХ ХВОРИХ НА ГОСТРУ ЛІМФОБЛАСТНУ ЛЕЙКЕМІЮ З ПОПЕРЕДНИКІВ В-КЛІТИН ТА ЇХНЯ АСОЦІАЦІЯ З ПРОГНОСТИЧНИМИ ФАКТОРАМИ І ВИЖИВАНІСТЮ

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Мета: Оцінити рівні деяких цитокінових рецепторів у сироватці крові дорослих хворих на гостру лімфобластну лейкемію з попередників В-клітин (В-ГЛЛ) та їх асоціацію з визнаними прогностичними факторами, безрецидивною виживаністю (БРВ) та загальною виживаністю (ЗВ). ***Матеріали та методи:*** Загалом у дослідження було включено 42 дорослих пацієнти з В-ГЛЛ, серед яких 19 були BCR/ABL-позитивними. Рівні розчинного рецептора α для IL-2 (sIL-2Rα), розчинного рецептора для IL-6 (sIL-6R), розчинного рецептора для TNF-α типу I та II (sTNFR-1, sTNFR-2) та матриксної металопротеїнази-9 (MMP-9) визначали за допомогою технології білкових мікроматриць під час встановлення діагнозу та за повної ремісії (ПР). ***Результати:*** На момент встановлення діагнозу В-ГЛЛ ми виявили значно вищі рівні sIL-2Rα, sIL-6R, sTNFR-1, sTNFR-2 та значно нижчі рівні MMP-9 порівняно з ПР ($p < 0,001$ у всіх випадках). BCR/ABL-позитивні пацієнти мали більш високий рівень sIL-2Rα на момент встановлення діагнозу ($r = 0,484$; $p = 0,014$). Не було виявлено асоціації між рівнями досліджуваних цитокінів у сироватці крові й досягненням ПР після одного циклу індукційної терапії, БРВ або ЗВ. ***Висновок:*** Рівень усіх досліджуваних цитокінів у сироватці крові хворих на В-ГЛЛ суттєво змінюється, що відображає активність захворювання. Не було виявлено істотних кореляцій з відповіддю на першу індукційну терапію, БРВ або ЗВ. Потрібні подальші дослідження з більш тривалим спостереженням.

Ключові слова: цитокін, розчинний рецептор цитокіну, sIL-2Rα, прогноз, виживаність, гостра лімфобластна лейкемія.