

Increased monocyte chemoattractant protein 1 (MCP-1/CCL-2) serum level in acute myeloid leukemia

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Acute myeloid leukaemia (AML) is an aggressive malignancy with accumulation of blasts in bone marrow. Myeloblasts can entry into peripheral blood stream and secondary localize in extramedullary sites. The regulation of this process has not been clearly explained so far, but interactions between some chemokines and their specific receptors could be one of the mechanisms responsible for such kind of migration. Monocyte chemoattractant protein 1 (MCP-1/CCL2) is the chemokine which could be involved in this process. The aim of the study was to evaluate plasma level of CCL2 in patients with AML. Plasma samples from 65 adult patients with AML taken before chemotherapy and in complete remission were measured by enzyme linked immunoassay to evaluate CCL2 levels. Control group consisted of 15 healthy subjects. In AML patients mean baseline CCL2 level (\pm SEM – standard error of measurement) was significantly higher than in normal control: $365,26 \pm 5,62$ pg/ml vs $265,56 \pm 5,48$ pg/ml respectively ($p < 0.01$). We demonstrate increased mean CCL2 plasma level in untreated patients with AML. Significantly lower plasma level of CCL2 was observed in patients with M4 and M5 AML subtypes according to FAB classification. In AML group chemotherapy did not reduce CCL2 plasma level.

Key words: MCP-1/CCL2, acute myeloid leukaemia, chemokines

Chemokines are small proteins which play an important role in regulation of development, differentiation and recruitment of leukocytes in immune and inflammatory reactions [1, 2]. Some chemokines influence interleukin production, angiogenesis and through paracrine and autocrine mechanisms can stimulate growth and expansion of diverse tumours [3-6]. On the base of a cysteine motif family chemokines has been divided into four subgroups: C, CC, CXC and CX3C [7]. Monocyte chemoattractant protein-1 (MCP-1, CCL2) is one of the most representative members of CC chemokines (called also β chemokines) – group with two residues of Cys juxtaposed within their amino-terminus. CCL2 contains 76 amino acid residues (molecular mass 8,7 kDa). CCL2 shows chemoattracting action for monocytes, natural killer cells (NK) and memory T lymphocytes, and activates basophiles and dendritic cells [reviewed in 3]. CCL2 exerts its action through chemokine receptor CCR2. A variety of cell types produce CCL2 in inducible way, these include: endothelial cells, monocytes and fibroblasts [8]. On the other side it has been reported

that certain tumor lines produce CCL2 constitutively [9-11]. Elevated levels of CCL2 have been found in active phase of multiple myeloma and non-Hodgkin's lymphomas [12]. The role of CCL2 is also known in some chronic diseases, such as: transplant rejection [13], pulmonary sarcoidosis and pulmonary fibrosis [14], atherosclerosis [15], Behcet's disease [16] and atopic dermatitis [17].

Acute myeloid leukaemia (AML) is an aggressive malignancy with overall leukaemia free survival lower than 50%. In AML there is an accumulation of blasts in bone marrow. Leukaemic blasts can entry into periphery blood stream and secondary localize in extramedullary sites. The regulation of this process has not been yet clearly explained so far, but interactions between some chemokines and their specific receptors could be one of the mechanisms responsible for such kind of migration. The production of chemokines by AML cells has not been investigated precisely but there is a correlation between concentration of CCL2 and monocyte migration [18, 19].

The purpose of this study was to evaluate the plasma level of CCL2 in patients with acute myeloid leukemia.

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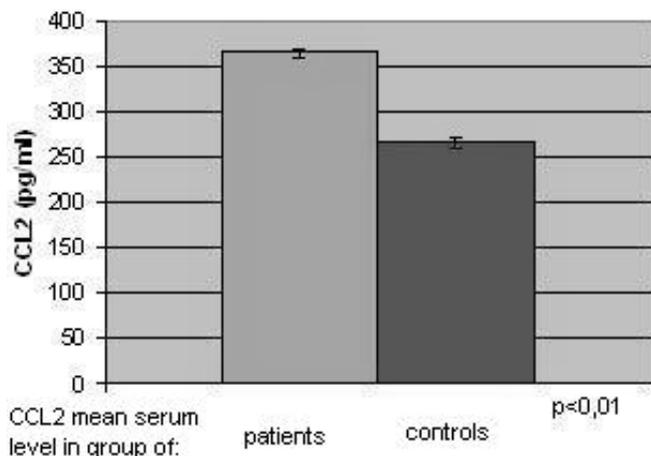


Figure 1. Mean baseline CCL2 serum level in AML patients and controls

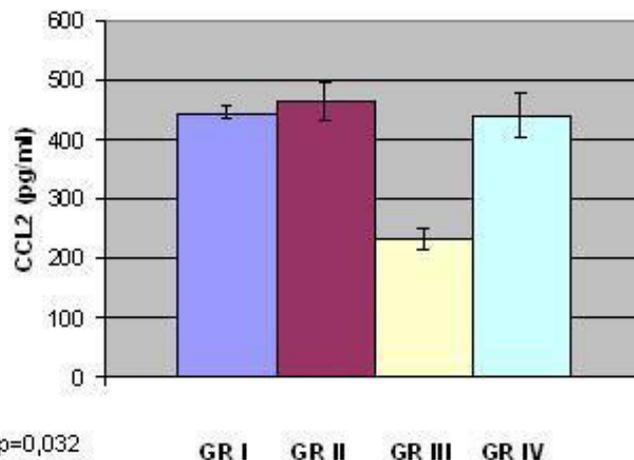


Figure 2. CCL2 serum levels in groups of patients with following AML subtypes: group I – M0, M1, M2; group II – M3, group III – M4, M5; group IV – M6, M7

Materials and methods

Plasma samples from 65 adult newly diagnosed patients (28 females and 37 males), median age 42 years (range 21-83 years) with AML referred to our institution were taken at the diagnosis, before chemotherapy was administered. In addition 19 out of 65 patients were analysed again after achieving complete remission. To define AML subtype according to standard criteria FAB classification was applied. Control group was consisted of 15 healthy sex- and age-matched individuals: 10 women and 5 men aged 32-71 years (median age 40). CCL2 serum concentrations were measured by the quantitative sandwich enzyme immunoassay technique using ELISA kit (R & D System, Minneapolis, USA).

Statistical analysis was performed with a commercially available package STATISTICA, version 7.0. The results were presented as a mean (\bar{X}) ± one standard error of measurement (SEM). The distribution of data was examined using the Shapiro-Wilk test. Differences between analysed parameters were examined using non-parametric U-Mann-Whitney test or ANOVA Kruskal-Wallis test. Correlation between variables were tested using the Spearman correlation coefficient "r". A p-value less than 0,05 was considered statistically significant.

Results

In AML patients mean baseline CCL-2 serum level was significantly higher than in the normal control: $365,26 \pm 5,62$ pg/ml vs $265,56 \pm 5,48$ pg/ml respectively; $p < 0.01$. (Figure 1)

According to FAB classification the AML patients were divided into 4 groups: group I consisted of M0, M1, M2 patients ($n=31$); group II: M3 patients ($n=5$); group III: M4, M5

patients ($n=25$) and group IV: M6 and M7 patients ($n=4$). Mean CCL2 serum concentration in respective groups was: $445,25 \pm 10,8$ pg/ml – group I; $464,94 \pm 33,33$ pg/ml – group II; $234,01 \pm 16,95$ pg/ml – group III; $441,03 \pm 35,43$ pg/ml – group IV. Serum level of CCL2 was significantly lower in group III than in group I; $p=0.032$. (Figure 2)

In AML group CCL2 values did not differ significantly before chemotherapy and after achieving CR ($492,06 \pm 6,18$ pg/ml vs $379,85 \pm 8,08$ pg/ml).

Basing on the white blood cells count (WBC) at the time of AML diagnosis patients were also divided into 3 groups: group 1 with $WBC < 4,5$ G/l ($n=23$), group 2 with normal WBC between 4,5 G/l and 10 G/l ($n=7$) and group 3 with WBC higher than 10 G/l ($n=35$). In 1 group serum level of CCL2 was higher than in other groups. There was statistically significant difference in CCL2 concentration between groups: 1 vs 2 ($590,52 \pm 20,67$ pg/ml vs $240,63 \pm 17,79$ pg/ml; $p < 0,01$) and group 1 vs 3 ($590,52 \pm 20,67$ pg/m vs $237,67 \pm 6,43$ pg/ml; $p < 0,001$).

There was negative correlation between count of WBC in AML patients and serum concentration of CCL2. (Figure 3)

The count of blast cells in peripheral blood was negatively correlated with level of CCL2. No correlation between percentage of myeloblasts in bone marrow and CCL2 concentration has been observed.

Considering the median of CCL2 serum level ($241,09$ pg/ml) as a value dividing the group of AML patients, two following subgroups were formed: I - under the median value of CCL2 ($n=33$) and II – over the median value of CCL2 ($n=32$). In group I (where concentration of CCL2 was lower than $241,09$ pg/ml) there was the prevalence of M4 and M5 AML subtypes ($n=22$) and in group II there was the prevalence of M0, M1, M2 ($n=21$). Mean leukocytosis was significantly

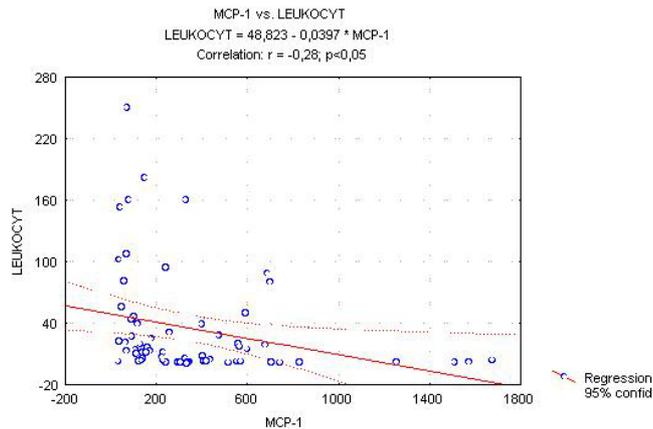


Figure 3. Correlation between the count of WBC in AML patients and serum level of CCL2

higher in group I comparing to the II group (48,56 vs 19,28 G/l respectively; $p < 0,01$). (Figure 4)

In group I the count of blast cells in peripheral blood was significantly higher than in the II group ($42,42 \pm 1,86$ vs $16,06 \pm 0,96$; $p < 0,05$). (Figure 5)

On the contrary to peripheral blood, there was no significant difference observed between groups I and II in count of bone marrow blasts.

No correlation between level of CCL2 and clinical outcome and survival in AML patients was found.

Discussion

Interactions between chemokines and their receptors mediate recruitment of adequate cell subpopulations necessary in process of host defence and inflammation [2, 20]. It is known that chemokines play an important role in biology of different tumor types and they are involved in malignancy in various ways. Chemokines can promote and/or inhibit the tumor growth and progression, dependently on the setting in which chemokine expression occurs. CCL2 is one of the CC chemokines, which displays different types of activity on cancers. CCL2 can be produced by various types of stromal cells (monocytes, endothelial cells, fibroblasts) and also by tumor cells. CCL2 activates cytostatic function of monocytes against tumor cells. CCL2 has been shown to be involved in malignancy in different ways. On one side CCL2 can be secreted by tumor cells and on the other – tumor cells themselves can induce CCL2 production by stromal and endothelial cells. Mononuclear cells stimulated by CCL2 secrete growth and survival factors, which promote the tumor progression or facilitate angiogenesis by providing angiogenic factors (i.e. vascular endothelial factor – VEGF) [3, 8, 21]. Monocyte chemoattractant protein 1 can influence the tumor growth in both: paracrine and autocrine manner. In the study performed by Ueno et al. high levels of CCL2 were found in patients

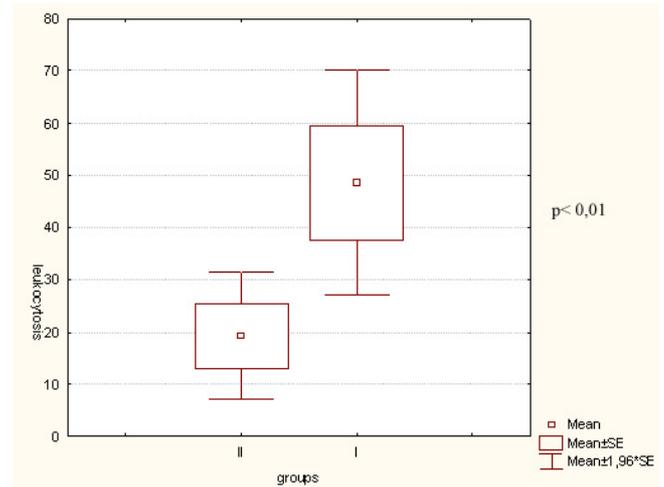


Figure 4. Leukocytosis in two groups of patients: I – with CCL2 serum level $\leq 241,09$ pg/ml and II – with CCL2 serum level $> 241,09$ pg/ml

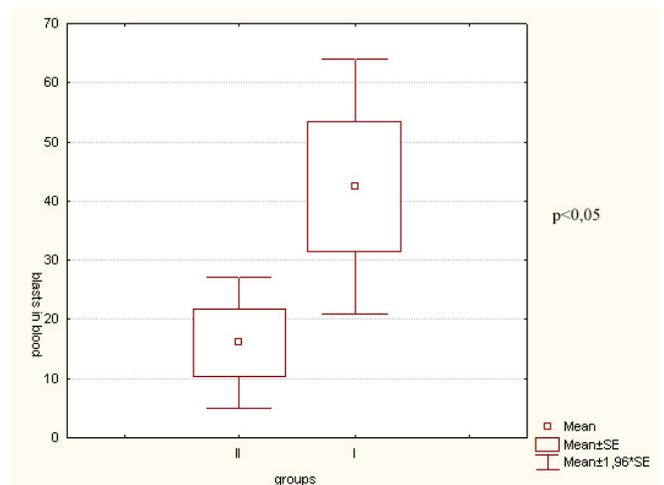


Figure 5. Blast cells count in peripheral blood in two groups of AML patients: I – with CCL2 serum level $\geq 241,09$ pg/ml and II – with CCL2 serum level $> 241,09$ pg/ml

with breast cancer and expression of this chemokine correlated with early relapse of the disease [5]. CCL2 gene is expressed during early stages of melanoma and is also produced in metastatic lesions [22, 23]. Urinary levels of CCL2 correlate with tumor stage and grade in patients with bladder cancer [24]. Lucciani et al. have shown that CCL2 is an additional cytokine involved in pathogenesis of Hodgkin's disease and showed expression of CCL2 in some solid tumors (lung, breast, thyroid, and ovary carcinomas) [10]. Study conducted by Vande Broek has indicated CCL2 as a chemokine which is responsible for myeloma cells homing to the bone marrow microenvironment [25]. The association between the involve-

ment of central nervous system in acute lymphoblastic leukaemia and high level of MCP-1 in cerebrospinal fluid has been recently proved [26].

As it has been previously shown that acute leukaemic cell lines were able to produce different amounts of CCL2 [18], we wanted to evaluate the level of this chemokine in patients with AML. Continuing study in which it has been elucidated that CCL2 was an important factor in the migration of monocytes to leukaemic cells but did not increase monocyte-mediated cytotoxic effects, Legdeur et al. investigated the ability of AML cells to produce CCL2 [19]. In addition, the migration of monocyte towards blasts from AML patients was examined. In the study 15 newly diagnosed AML patients were included. In result, blasts from all patients produced CCL2, and in 12 cases this chemokine induced migration of monocytes towards blasts from AML patients.

In our study we found CCL2 level significantly higher in patients with AML than in control group. What is interesting, there was statistically significant higher CCL2 serum level in AML patients with M0, M1 or M2 subtypes than in patients with M4 or M5 FAB subtypes of AML. In the study of Cignetti et al. 25 cases of AML were analysed *in vitro* for production of some chemokines, including CCL2, and expression of their receptors [27]. Obtained data demonstrated that production of CCL2 differed depending on degree of AML blast maturation. More differentiated AML cells (M4 and M5) produced bigger amounts of CCL2 and expressed its specific receptor CCR2. AML-M4 and M5 blasts migrated in response to self-produced CCL2. According to authors, this could be an explanation of possible mechanism by which blasts accumulate into extramedullary sites (such as skin, spleen, gingival mucosa), typical for these subtypes of AML. As it is known, CCL2 is produced by fibroblasts, monocytes, endothelial cells, as well as by blast cells. In our study the serum level of CCL2 was higher in less differentiated AML subtypes (M0, M1, M2). What is important to notice, our work was performed *in vivo*. Decreased level of CCL2 in serum of patients with high number of blast cells in peripheral blood is possibly the effect of monoblasts migration from bone marrow towards chemokine in blood and other localizations (where CCL2 concentration is increased). In this context, the migration of blast cells to extramedullary sites, typically involved in M4 and M5 AML subtypes, may be favoured by production of chemoattractant factors for leukaemic cells (including CCL2) at the site of infiltration.

Concluding, we demonstrate increased mean CCL-2 plasma level in untreated patients with AML. Lower plasma levels of CCL2 were observed in M4 and M5 AML subtypes.

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