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Predictive value of serum cytokine levels in chronic myeloid leukemia patients

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ABSTRACT

Serum samples taken at diagnosis in 28 chronic myeloid leukemia patients were tested for the presence of 20 cytokines by a magnetic bead-based Bio-plex immunoassay. According to complete cytogenetic remission achieved at 12 months of treatment, patients were divided into groups with either optimal or non-optimal outcome. Patients with increased cytokine levels tended to react optimally to the therapy more frequently than those others. TGF-β3 was a notable exception; its levels were significantly higher in patients with non-optimal outcomes. Further analysis enabled us to define two combinations of cytokine cut-off levels – namely low TGF-β3 and either high IL-8 or high MCP-1–each of which corresponded to therapy outcome better than either Sokal or EUTOS scores.

1. Introduction

The knowledge on the cytokine activities in CML patients is one of the preconditions for understanding the immunology of this disease and for the future development of its immunotherapy. In the past a number of papers was published reporting on the serum cytokine levels in chronic myeloid leukemia (CML) patients [1–4]. They were concerned on selected cytokines in usually small and inhomogeneous groups of patients. We have started several studies aimed at clarifying the possible role of these immune factors in the development and course of this disease. Recently we have studied serum levels of 20 cytokines in a group of chronic myeloid leukemia (CML) patients. The sera tested were obtained from the patients at diagnosis, prior to the start of any therapy, and then after achieving hematological remission. The results indicated that in most patients' serum levels of the majority of the tested cytokines dropped highly significantly after achieving hematological remission (manuscript in preparation).

Prognostic scoring systems have been developed for risk stratification of patients with CML. The Sokal score has been used as the primary predictive means for CML [5]. It recognizes three patient risk groups (high, intermediate; and low risk, HR, IR and LR) with significantly varying overall survival (OS) probabilities. More recently EUTOS, another scoring system, has been established [6] recognizing two patient risk groups (HR and LR). It predicts complete cytogenetic remission at 18 months in imatinib mesylate (IM)-treated patients and is able to identify two groups with significantly different OS [7]. Our aim was to show whether the extent of cytokine production at the time of diagnosis had a predictive value on therapeutic outcome and compare it with these two scoring systems.

2. Materials and methods

2.1. Patients

A total of 28 patients (16 males, 12 females) aged 18 to 81 years (median 56; mean 55 years) were tested. All patients underwent treatment with tyrosine kinase inhibitors (TKI) (23 with IM,

Abbreviations: CCyR, complete cytogenetic response; CML, chronic myeloid leukemia; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-monocyte-colony stimulating factor; HR, high risk; IL, interleukin; INF, interferon; IR, intermediate risk; LR, low risk; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; NO, non-optimal; O, optimal; OS, overall survival; PD-1, programmed cell death protein-1; TKI, tyrosine-kinase inhibitors; TNF, tumor necrosis factor.

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4 with nilotinib, and 1 with dasatinib) and were followed for up to 30 months. Before being enrolled into this study, none of the patients had undergone prior therapeutic transplantation or interferon α treatment, and none of them progressed into an accelerated phase or blast crisis over the course of TKI therapy. Six IM-treated patients received second-line therapy (3 nilotinib and 3 dasatinib); 5 of them because of therapy failure, 1 because of an IM-toxicity reaction. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Institute of Hematology and Blood Transfusion. Routine hematological, cytogenetic, biochemical, and molecular tests were performed on all patients. All were found to be Philadelphia chromosome positive (Ph+) and all achieved hematological remission in the course of treatment. For the present study the evaluation of response to TKI treatment was completed at 12 to 30 months after the start of treatment. In patients with complete cytogenetic response (CCyR; Ph^+ metaphases = 0%) the outcome was designated optimal (O), and it was considered non-optimal (NO) if CCyR could not be demonstrated. For control purposes we included two types of sera from healthy subjects in the present experiments, namely (i) 9 individual sera from blood donors, and (ii) pool of sera obtained in 20 blood donors. Thus 10 control samples were tested in parallel with sera from CML patients.

2.2. Detection of cytokines in serum samples

Cytokine concentration was measured with cytokine magnetic bead-based assays: Bio-Plex Pro™ Human Cytokine 17-plex containing microspheres for IL-1β, Il-2, IL-4, IL-5. IL6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-18, G-CSF, GM-CSF, INFγ, MCP-1, MIP-1β and TNFα (M50-00031YV, Bio-Rad) and the Bio-Plex Pro™ Human TGFβ-plex (171-W4001M, Bio-Rad) containing microspheres for TGF-β1, TGF-β2 and TGF-β3 detection according to manufacturer protocols. Results were analyzed by Bio-plex Manager software (version 6.0). Duplicate values of 8 four-fold dilutions of known standards were used to construct a 5-parameter logistic curve. Average values of the samples tested were mapped to the curve to interpolate concentration levels. Resulting values were expressed in pg/ml.

2.3. Statistical methods

For statistical analysis of the cytokine levels, a non-parametric Mann-Whitney test was applied, and medians have been plotted in the figures. Kaplan-Meier analyses were performed to assess event free survival (EFS) and cumulative incidence for achievement of CCyR and major molecular response (MMR; BCR-ABL1 levels ≤0.1%). Data were analyzed with a Log-rank Mantel-Cox (Chi-Square) test. For EFS analysis, an event was defined as follows: TKI discontinuation, disease progression, or loss of response according to a TIDEL-II protocol [8]. A significance level of P = 0.05 was used. Graph Pad Prism version 3 (Graph-Pad Software, San Diego, CA, USA) was employed for data visualization and analysis.

3. Results

3.1. Comparison of cytokine levels in healthy controls and in CML patients at diagnosis

We compared the cytokine levels of 28 CML patients at diagnosis with their clinical outcome. The criterion for either optimal (O) or non-optimal (NO) outcome was the achievement of CCyR at 12 months after the start of the treatment. Based on this classification, 21 patients belonged to the O group and 7 patients to the NO group. Table 1 presents ranges and median levels of 20 cytokines in healthy controls, in CML patients at diagnosis, and additionally the cytokine ranges of the same patients divided according to their

Table 1
A range of 20 cytokine levels in sera of healthy donors and CML patients at diagnosis.

	Healthy donors (n=10)	Patients at diagnosis (n=28)	HD vs Dg	Optimal outcome (n=21)	Non-optimal outcome (n=7)	O vs NO
IL-1β	1.09 (0.33 – 3.49)	5.64 (0.43 – 27.83)	P=0.0047**	6.19 (0.54 – 17.97)	2.31 (0.43 – 27.83)	P=0.2224
IL-2	0.01 (0.01 – 19.65)	15 (0.01 – 315.4)	P=0.0057**	16.00 (0.01 – 315.4)	11.07 (0,01 – 105.1)	P=0.3097
IL-4	0.68 (0.04 – 6.71)	3.19 (0.23 – 13.47)	P=0.0149*	3.20 (0.86 – 7.82)	2.89 (0.23 – 13.47)	P=0.1847
IL-5	1.21 (0.09 – 37.23)	9.84 (0.6 – 30.88)	P=0.0039**	10.93 (1.92 – 30.88)	6.15 (0.60 – 12.67)	P=0.0800
IL-6	1.59 (0.1 – 18.36)	21.31 (0.1 – 276.6)	P=0.0018**	23.7 (0.51 – 276.6)	14.3 (0.1 – 109.5)	P=0.2651
IL-7	5.59 (2.12 – 19.63)	23.61 (4.45 – 118.8)	P=0.001***	25.99 (4.45 – 118.8)	21.39 (4.67 – 30.99)	P=0.4260
IL-8	21.4 (7.32 – 63.85)	29.91 (12.58 – 68.65)	P=0.0267*	33.62 (12.58 – 68.65)	29.46 (13.66 – 30.24)	P=0.1055
IL-10	5.89 (0.1 – 58.47)	23.0 (0.1 – 809.0)	P=0.0674	26.56 (0.1 – 809)	11.47 (0.1 – 34.25)	P=0.0894
IL-12	13.83 (0.01 – 1 008)	104.5 (5.88 – 1 530)	P=0.0052**	109.7 (5.88 – 1 530)	79.43 (7.73 – 162.4)	P=0.2224
IL-13	1.06 (0.01 – 15.53)	8.46 (0.85 – 27.31)	P=0.0026**	9.59 (1.22 -25.68)	5.98 (0.85 – 27.31)	P=0.4738
IL-17	0.01 (0.01 – 83.37)	80.05 (0.01 – 200.6)	P=0.0011**	81.23 (0.01 – 200.6)	62.7 (0.01 – 144.5)	P=0.5066
G-CSF	11.91 (0.01 – 261.4)	44.92 (6.76 – 116.2)	P=0.012*	45.77 (14.8 – 116.2)	38.83 (6.76 – 89.74)	P=0.3670
GM-CSF	0.01 (0 – 98.26)	147.3 (0 – 2243)	P=0.0397*	151.8 (0 – 2 243)	24.42 (0 – 541.8)	P=0.7272
IFNγ	3. 0 (1.0 – 1 032)	294.1 (3.0 – 1 696)	P=0.0109*	334.6 (64.68 – 702)	222.5 (3.00 – 1 696)	P=0.1115
MCP-1	36.63 (19.20 – 85.85)	72.27 (15.17 – 151.4)	P=0.0727	76.87 (17.76 – 151.4)	49.42 (15.17 – 87.74)	P=0.0438*
MIP-1β	303.5 (156.0 – 1 740)	570.8 (242.6 – 1 387)	P=0.0549	594.2 (242.6 – 1 203)	529.1 (265.7 – 1387)	P=1.000
TNFα	4.01 (0.1 – 24.84)	16.57 (1.51 – 138.2)	P=0.0035**	17.52 (2.75 – 108.8)	12.44 (1.51 – 138.2)	P=0.1678
TGF-β1	12 982 (1 006 – 36 256)	41 592 (224 – 116 522)	P=0.0046**	53 156 (224 –116 522)	22 394 (15 523 – 97633)	P=1.000
TGF-β2	1 552 (179 – 2 040)	1 990 (103 – 3 264)	P=0.0069**	1 949 (103 – 2 623)	2 033 (1 569 – 3 264)	P=0.4903
TGF-β3	485 (70 – 826)	526.5 (0 – 3 167)	P=0.4968	474 (0 – 3 167)	861 (208 – 1 438)	P=0.0438*

Abbreviations: HD, healthy donors; Dg, patients at diagnosis; O, patients at diagnosis with optimal outcome; NO, patients at diagnosis with non-optimal outcome; Medians and ranges of cytokine concentrations in pg/ml are listed. P values were derived from Mann-Whitney tests. A significance level of P = 0.05 was used for all statistical tests. Grey indicates no significance.