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Balance between interleukin-10 and interleukin-12 in adult cancer patients with or without infections

R. Kallio [a,*], H.-M. Surcel [b], A. Bloigu [b], H. Syrjälä [c]

[a] *Department of Oncology and Radiotherapy, Oulu University Hospital FIN-90220 Oulu, Finland*
[b] *National Public Health Institute, Oulu, Finland*
[c] *Department of Infection Control, Oulu University Hospital FIN-90220 Oulu, Finland*

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Abstract

Reliable markers for identifying infections in cancer patients on admission are lacking. The utility of the balance between interleukin (IL)-10 and IL-12 was analysed in this respect. The infection group ($n = 56$) had higher median serum levels of IL-10 (3.8 pg/ml; interquartile range (IQR) 1.7–11.4 pg/ml versus 1.8 pg/ml; IQR 0.6–4.6 pg/ml; $P = 0.005$) and IL-10 to IL-12 ratio (0.4; IQR 0.06–4.23pg/ml versus 0.05; IQR 0.02–0.31pg/ml; $P < 0.001$) than the non-infection group ($n = 36$). IL-10 and the ratio had the following figures of sensitivity (79%; 95% confidence interval (CI) 66–88 versus 39%; 95% CI 27–53), specificity (40%; 95% CI 12–74 versus 90%; 95% CI 56–100) and positive predictive value (88%; 95% CI 76–96 versus 96%; 95% CI 78–100) for identifying infections (56 cases with infection and 10 with neoplastic fever), and the corresponding area under curve (AUC) values for IL-10 and the ratio in identifying infections in general were 0.58; 95% CI 0.39–0.78 versus 0.64; 95% CI 0.46–0.82 and in bacteraemia 0.71; 95% CI 0.50–0.92 versus 0.75; 95% CI 0.58–0.93, respectively. Thus, IL-10 can be used as a screening method for identifying infections in cancer patients and the ratio of IL-10 to IL-12 for confirming the diagnosis.

Keywords: Cancer; Infection; Interleukin-10; Interleukin-12; Balance

1. Introduction

Lymphocytes recognise microbes by means of antigen-specific cell-surface receptors. The humoral immune response is mediated by B cell lymphocytes and the cellular immune response by T lymphocytes. T lymphocytes have traditionally been subdivided into CD4+ (T-Helper lymphocytes) and CD8+ (Cytotoxic T lymphocytes) by the type of antigen receptors. Both CD 4+ and CD8+ cells can be further differentiated into Type 1 (Th1) and Type 2 (Th2) cells, depending on the cytokine repertoire they secrete upon activation [1]. Th1 cells produce pro-inflammatory cytokines (interferon-gamma (INF-γ), interleukin-12 (IL-12) and IL-18) and are responsible for phagocyte-dependent protective host responses. Th2 cells secrete anti-inflammatory cytokines (IL-4, IL-6, IL-10, IL-13) and IL-5 and have been considered to favour phagocyte-independent protective host responses [2,3]. The outcome of the immune response depends on the net effect of pro-inflammatory and anti-inflammatory cytokines [4]. The presence of IL-12 at the time of T cell priming drives naive T cells to Th1 differentiation and the presence of IL-4 to Th2 differentiation [3].

IL-12 is secreted mainly by phagocytic cells, but also by monocytes and polymorphonucleated cells, which are essential regulators in the early phases of infection and inflammation [3]. IL-10, which is produced by mononuclear phagocytes, CD4+ T lymphocytes, B cells, keratinocytes and tumour cells, antagonises the activities of IL-12 [5,6]. IL-10 is also known as an immunosuppressive cytokine, because it deactivates macrophages and inhibits T cell responses [6].

Because of the opposite roles of IL-10 and IL-12 in the regulation of the cytokine network, the balance between these cyokines has aroused interest in different clinical settings [3], both in infections [7] and in oncology [8,9]. We were interested in whether this balance between IL-10 and IL-12 differs in cancer patients with and without infection, and if so, whether this ratio can be used to identify infections in cancer patients and to differentiate them from neoplastic fever.

* Corresponding author. Tel.: +358-8-315-2011; fax: +358-8-315-2452.
E-mail address: raija.kallio@oulu.fi (R. Kallio).

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2. Patients and methods

2.1. Study design for the identification of study groups

The study protocol was approved by the Ethics Review Committee of the Medical Faculty of the University of Oulu, Oulu, Finland. Between September 1996 and March 1998, 92 consecutive cancer patients suspected by the oncologist in charge to have infection and with Karnofsky performance scores higher than 40 were enrolled in this prospective study at the Department of Oncology and Radiotherapy, Oulu University Hospital. After oral and written informed consent from the participating patients, admission serum samples were obtained and stored at −70°C until analysis. Only one suspected episode of infection per patient was accepted. The patient population has been described in more detail elsewhere [14]. The patient data were afterwards analysed jointly by an oncologist and an infectious disease physician using the following definitions.

2.2. Definitions of the study groups

2.2.1. Infection group

A patient was considered to have bacteraemia if he or she had a clinical infection and a positive blood culture (3 had *Staphylococcus aureus*, and 1 each, *Escherichia coli*, *Pasterella multocida, Clostridium bifermentas*, another gram-negative anaerobic rod and mixed bacteraemia). The diagnosis of urinary tract infection required both symptoms and significant growth of bacteria $10^{4–5}$ colony forming units (cfu)/ml in the urine culture ($n=7$). The diagnosis of pneumonia was based on both respiratory symptoms and a pneumonic infiltrate that disappeared during the antibiotic treatment while the patient recovered ($n=15$). For other foci, distinct radiological or microbiological documentation and recovery during the antimicrobial treatment were required ($n=9$); 2 patients had sinusitis, in addition to which there were cases with infection at the insertion site of a central venous catheter, cholangitis, perirectal abscess, mediastinitis, pulmonary tuberculosis, erysipelas and *Herpes zoster* infection (1 each). In addition, the patients who had a clinical picture of infection and showed an unequivocal antibiotic response with the abatement of fever and decreasing C-reactive protein (CRP) values during the follow-up were considered to have infection, although no foci of infection could be demonstrated ($n=17$).

2.2.2. Non-infection group

The patients were considered to have neoplastic fever if they did not have any evidence of infection clinically or in the examinations performed. They did not respond to empirical antibiotic treatments, but typically showed a response to steroids, anti-inflammatory analgesics or radiotherapy ($n=10$). In addition, the non-infection group consisted of voluntary patients with different types of malignancy without clinical infection, who were randomly selected before their first course of chemotherapy ($n=26$).

26 of the 92 patients with suspected infection did not meet the study criteria because of simultaneous antibiotic and cancer treatments and were therefore excluded from the further analyses.

2.3. Cytokine analyses

Cytokine concentrations were determined by the enzyme immunoassay (ELISA) method using commercially available ELISA kits for IL-12 (Duoset, Genzyme Diacnostics, Cambridge, USA) and for IL-10 (PeliKine Compact™, CLB, Amsterdam, The Netherlands) according to the manufacturers' instructions.

2.4. Statistical analyses

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc. Chicago, IL, USA), and the confidence intervals were calculated with the CIA program. The calculations for continuous variables were performed with the Mann–Whitney U-test and those for non-continuous variables with the Pearson χ^2 test (or Fisher's Exact test when appropriate). A P value of <0.05 was considered significant. The diagnostic applicability of serum cytokine concentration was evaluated for 66 patients with suspected infection, using the area under a receiver operating characteristic (ROC) curve [10]. The optimal cut-off values for identifying infections were based on the Youden index, which indicates simultaneous sensitivity and specificity [11]. The ROC curves have been calculated by plotting the sensitivity versus 1−specificity for each possible cut-off value and then joining the points. The corresponding AUC values (area under the curve) were obtained by using the SPSS statistical software. The test is ideal if its AUC value is 1.0, while a value of 0.5 does not differ from that obtained by chance.

3. Results

The first endpoint of the study was to evaluate the possible differences between cancer patients with and without infection. Essential clinical data were comparable between the infection and non-infection groups (Table 1). The median admission concentrations of IL-10 were significantly higher in the infection group (3.8 pg/ml; interquartile range (IQR) 1.7–11.4 pg/ml) than in the non-infection group (1.8 pg/ml; IQR 0.6–4.6 pg/ml; $P=0.005$), while the median serum IL-12 concentra-

Table 1
Demographic data and underlying cancer and their stages in infection and non-infection groups of cancer patients

Variable	Infection group ($n=56$)		Non-infection group ($n=36$)		P value
	n	(%)	n	(%)	
Gender					NS
Male	35	63	23	64	
Female	21	38	13	36	
Mean age (S.D.) (years)	57	(16)	57	(13)	NS
Tumour type					NS
Lymphoma	23	41	11	31	
Lung cancer	7	13	13	36	
Breast cancer	6	11	3	8	
Gastrointestinal tract	7	13	3	8	
Urinary tract	4	7	1	3	
Other cancer	9	16	5	14	
Stage[a]					NS
I	4	7	0	0	
II	7	13	5	14	
III	12	23	9	25	
IV	30	57	22	61	

NS, non significant; S.D., standard deviation.
[a] 3 cases with glioblastoma multiforme (not staged) in the infection group.

tions were significantly lower ($P=0.007$) in the infection group (10.6 pg/ml; IQR 0–55.9 pg/ml versus 71.6 pg/ml; IQR 0.7–104.4 pg/ml). The IL-10 to IL-12 ratio differentiated clearly between the study groups (0.4; IQR 0.06–4.23 pg/ml versus 0.05; IQR 0.02–0.31 pg/ml; $P<0.001$).

After subdivision of the patients with local (stages I–II) or advanced disease (stages III–IV), the differences between the study groups remained statistically significant only in advanced disease for the median IL-10 concentration (4.5 pg/ml; IQR 1.8–11.7 pg/ml in the infection group and 1.8 pg/ml; IQR 0.5–4.5 pg/ml in the non-infection group; $P=0.011$), for IL-12 (15.8 pg/ml; IQR 0.0–55.0 pg/ml versus 71.6 pg/ml; IQR 2.2–106.0 pg/ml; $P<0.05$) and for the IL-10 to IL-12 ratio (0.40; IQR 0.06–3.3 versus 0.05; IQR 0.01–0.4; $P=0.001$).

The second endpoint of the study was to evaluate the utility of IL-10 and the IL-10 to IL-12 ratio in differentiating infections ($n=56$) from neoplastic fever ($n=10$) among 66 cancer patients with suspected infections using the area under (AUC) receiving operating characteristic curves (Fig. 1). In differentiating infections from neoplastic fever, IL-10 had a relatively high AUC value of 0.71 (95% confidence interval (CI) 0.50–0.92) in identifying bacteraemia, but not in demonstrating infections in general, 0.58, (95% CI 0.39–0.78) or non-bacteraemic infections, 0.56 (95% CI 0.35–0.76). The corresponding AUC values of the IL-10 to IL-12 ratio were slightly better for bacteraemia, 0.75 (95% CI 0.58–0.93), for infections in general, 0.64 (95% CI 0.46–0.82), and for identifying non-bacteraemic infections, 0.61 (95% CI 0.42–0.80).

To evaluate the ability of IL-10 and the IL-10 to IL-12 ratio to differentiate patients with infections from those with neoplastic fever, cut-off values of $\geqslant 1.4$ pg/ml for IL-10 and $\geqslant 1.44$ for the IL-10 to IL-12 ratio were used in the analyses. As shown in Table 2, the sensitivity of IL-10 (79%) and the specificity of the ratio (90%) were high. Both IL-10 and the ratio also had high positive predictive values (PPVs) (88 and 96%), but the negative predictive values (NPVs) remained low (25 and 21%). The accuracy (percentage of correct diagnoses) of IL-10 to identify infections was relatively high (73%).

4. Discussion

It is well known that cancer and its treatments disturb the host immune response and thereby expose the patients to a wide spectrum of infections [12]. The diagnosis of these infections is problematic, because cancer also induces the acute-phase response, and the traditional infection markers, such as CRP and erythrocyte sedimentation rate, are therefore not necessarily reliable [13,14]. We have recently demonstrated that the analysis of procalcitonin, a new marker of severe infections [15], is effective in discriminating bacteraemias in cancer patients, but not other infections [16],

Table 2
Utility of admission interleukin-10 (IL-10) and IL-10 to IL-12 ratio in identifying infections in 66 cancer patients with a suspicion of infection

Variable	Sensitivity	Specificity	PPV	NPV	Accuracy
IL-10 $\geqslant$ 1.4 pg/ml[a]	79 (66–88)[b]	40 (12–74)	88 (76–96)	25 (7–52)	73 (60–83)
IL-10/IL-12 $\geqslant$ 1.44	39 (27–53)	90 (56–100)	96 (78–100)	21 (10–36)	47 (35–60)

PPV, true positives/(true positives + false positives); NPV, true negatives/(true negatives + false negatives);
Accuracy, (true positives + true negatives)/(true positives + false positives + true negatives + false negatives)

PPV, positive predictive value; NPV, negative predictive value.
[a] The cut-off values were selected using the Youden index.
[b] 95% confidence interval.

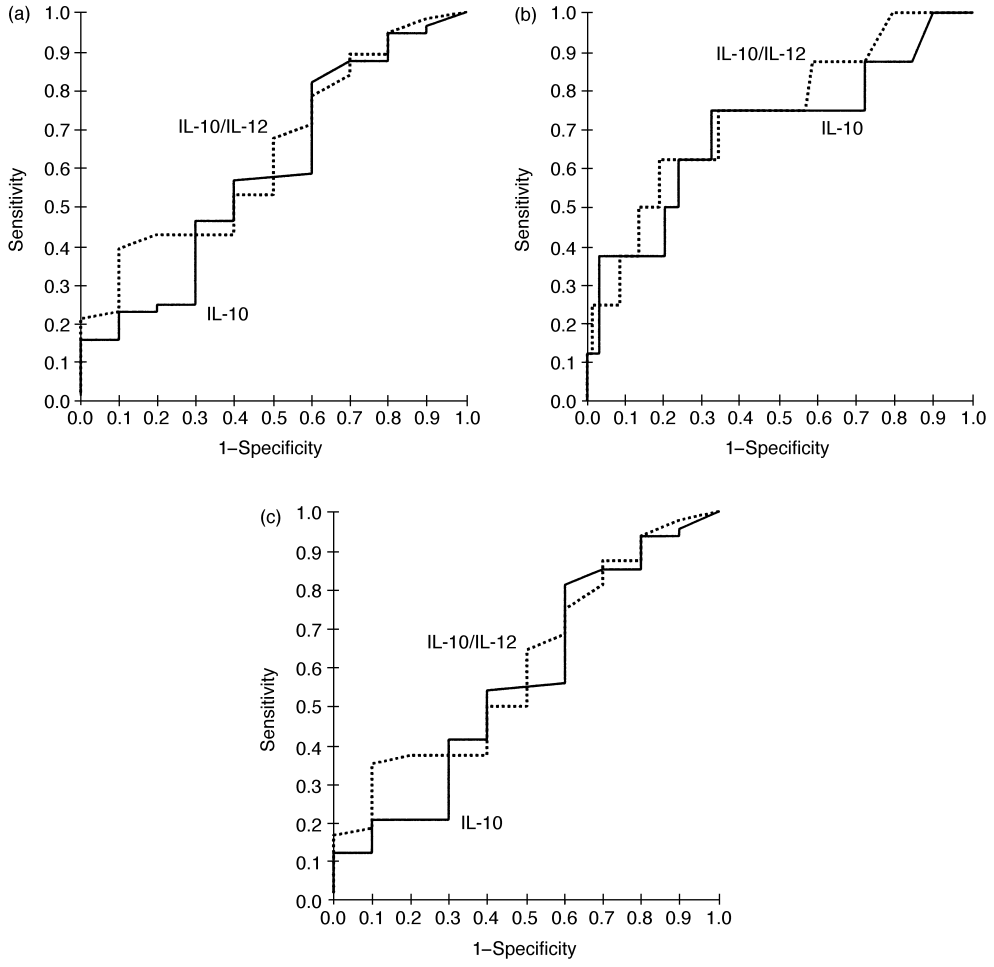

Fig. 1. Comparison of the area under the receiver operating characteristic curves for interleukin-10 (IL-10) and IL-10 to interleukin-12 (IL-12) ratio in the prediction of infection in 66 cancer patients with suspected infection: (a) discrimination of infections ($n=56$) from neoplastic fever ($n=10$); (b) discrimination of bacteraemic infections ($n=8$) from other patients with suspected infections ($n=58$); (c) discrimination of non-bacteraemic infections ($n=48$) from neoplastic fever ($n=10$).

which are, however, the most frequent and difficult diagnostic problems in oncology wards. The present study showed that both IL-10 (0.71) and the IL-10 to IL-12 ratio (0.75) were relatively good discriminators for bacteraemia. However, for the more common problem of identifying infections in general, the ratio (0.64) was clearly better than has been reported for CRP (0.42) and even slightly better than PCT (0.61), while IL-10 (0.58) alone was poorer than PCT in this respect [16].

In comparison with healthy controls, patients with different types of cancer have been found to have elevated serum levels of IL-10 correlating with the extent of disease [17,18]. Elevated IL-10 levels are also associated with different types of infection, such as gram-positive and gram-negative bacteraemias [19,20]. In this series, the tumour load did not statistically significantly influence the median concentrations of IL-10 within the two study groups (data not shown). However, the median concentrations of IL-10 were statistically higher in the infection group than in the non-infection group, and after subdivision of the patients into local (stages I and II) and advanced diseases (stages III and IV), the difference remained significant in advanced disease. Thus, we assumed that the differences in the IL-10 levels were due to the ongoing infection. According to our results, IL-10 had relatively high sensitivity (79%) and PPV (88%) to identify infections from neoplastic fever, though its specificity remained low (40%).

Because of the opposite roles of IL-10 and IL-12 in the regulation of the cytokine network, the balance between these cytokines has attracted interest in oncology [8,9] and in infections [7,21]. According to our results, the balance between IL-10 and IL-12 seems useful in the diagnosis of infections in cancer patients, having a high specificity and positive predictive value. In this pilot study, we were interested in the diagnostic potential of the studied cytokines in the diagnosis of infection in cancer patients. Larger populations of different histotypes of cancer are needed to confirm whether there are cancer-related differences in this respect.

In conclusion, IL-10, which is a sensitive infection marker, can be used as a screening method in identify-

ing infections in cancer patients, and the diagnosis of infection can be confirmed with the IL-10 to IL-12 ratio, which has a high specificity. Our results must be confirmed in a larger population together with a cost-benefit analysis.

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