Parameters of cytokine regulation as additional criterion of immunodiagnosis

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Aim. The work goal was to search more informative parameters of cytokine system for laboratory diagnosis in oncological and non-oncological pathology. Methods. The concentrations of some cytokines and chemokines (IL-4, 6, 8 and raIL-1), interferones (IFN-alpha, gamma), (VEGF) have been investigated. Results. Outcoming levels of investigated cytokines have exceeded the norm mostly in patients with malignant tumor of brain. The serum level raIL-1 increased in patients with lung cancer and a decrease of this indicator was detected by the end of treatment. Concentration of IL-8 in blood serum, initially increased, in majority of them, 82%, remained at the previous level by the end of investigation; the averaged level IL-6 was slightly increased and decreased by the end of investigation approximately reaching the norm limits. Serum level raIL-1 increased in 70% of patients with polytrauma and decreased by the end of treatment. Indicators IL-6 and IL-8 in the same patients initially increased and slightly decreased by the end of investigation. Concentration of VEGF in patients with high level malignancy (G3) comprised 285.9 ± 27.2 pg/ml with a slight increase by the end of investigation while in low level malignancy a reliable decrease in its content was detected. The serum level VEGF in patients with polytrauma increased by the end of investigation, thus in majority of investigated patients with favorable illness process statistically sufficient decrease was detected. Conclusions. The results obtained will help to select much more informative biomarkers that have diagnostic and prognostic importance.

Keywords: cytokins, immunodiagnostics, tumors, polytrauma, sepsis.

Introduction. Taking into consideration that cytokines play a principal role in the regulation of immune response, yet the literature data about cytokine secretion in different pathologies are scanty and enough controversial, the understanding of cytokine synthesis mechanisms may provide possibilities for its selected correction [1]. However, the present literature information about anti-inflammatory cytokines in oncological diseases is mostly controversial and interpreted in different ways by different authors [2–4]. It is known, that the tumor cells may be a production source of different cytokines which may be used to strengthen the own growth [5–7]. In the blood of patients with oncological diseases, the production of various inhibitors, such as cytokines and their activity are coming into view [7, 8].

Presently, the diagnostic value of measurable concentration of IFN-alpha and gamma in blood is established. FNO-alpha and IL-1 have similar effects on cell function and usually are classified together with inflammatory cascade, as far as the initiating cytokines frequently are correlated with the indicators of severity of pathological process.

Definition of serum-born concentration of IL-1, 2, 4, 6, 8, 10, FNO-alpha presently is offered not only for «standard» immunologic examination but also for differentiation of septic complication phase [9–12]. As regards to IL-8, there are data on its important role in various infectious and traumatic processes [12–14]. Thus,
as per data presented by Kozlov [15], especially the dis-
balance of cytokines but not the general level of hyper-
cytokinemia is characteristic of pathogenesis of diffe-
rent pathology, such as functional condition of CD4
cells tested by cytokine profile – the functional activity of
TH1 subpopulation is confirmed by IFN-gamma pro-
duction, while the activity of TH2 subpopulation is con-
firmed by IL-4 [11] production. In this way, cytokines
may become potentially new serum-born markers for
pathology with poor prognosis.

Therefore, the work goal is to search more informa-
tive parameters of cytokine system for laboratory diag-
nosis in oncological and non-oncological pathology.

Material and methods. The concentration of some
cytokines and chemokines (IL-4, 6, 8 and raIL-1), in-
terferones I and II types (IFN-alpha, gamma), angioge-
nesis factor (VEGF) involved in regulation of compen-
sator immune response in 137 patients with malignant
tumor in various localization (lung cancer, ovary can-
cer, urinary bladder cancer, brain tumors) as well as in
83 patients with polytrauma and septic complications
have been investigated. The investigation of cytokine
status has been conducted at the admission during 1–2
days and through a week after end of treatment.

To determine the capability on production of cytoki-
nes, the lymphocytes at concentration of 2 mln/ml sepa-
rated from peripheral blood have been cultivated in
medium RPMI-1640 by 10 % of embryonic fetal serum
and phytohemagglutinin (PHA) at dose of 10 μg/ml du-
ring 72 h. The obtained supernatants have been kept at
–20 °C before testing.

The concentrations of cytokines in blood serum and
in cultivation liquid have been examined by ELISA
«Diaclone» (France) and «Vector-Best» (Russian Fe-
deration). An average arithmetical variation line (M)
have been determined by Student (т) criterion. The le-
vels of serum and induced of cytokines, in vitro, have
been confirmed by Pearson’s statistical processing.

Results and discussion. According to preliminary
results the initial levels of studied cytokines statistically
insignificantly exceeded the norm in most patients with
brain malignant tumors. During the treatment there are
no changes in the indices as raIL-1 and IL-6 and IL-8,
with the exception of 2 cases. Lack of significant chan-
ges in serum concentrations of cytokines studied in the-
se patients, is perhaps a sign of growing depletion of an-
titumor immunity, as a result of reactivity of the immu-
ne system. In the patients who have lung cancer, the
serum level of raIL-1 was higher in 52 % of cases (1732 ±
15), and there was a tendency of decrease of this in-
dicator. As to IL-8 and IL-6 indices of those patients,
the concentration of former in the blood serum that was
initially high (1.5 times) remained on the same level by
the end of the research, whereas the average level of IL-
6 was slightly higher during the I investigation in 70 %
of cases and decreased by the end of research, almost
getting to the normal level. Taking into consideration
published data, the production of IL-8 increases when a
patient has benign tumor, perhaps, the change of serum
levels in its dynamics may be a criterion of immuno-
diagnostics of a patient having lung cancer.

RaIL serum was raised in 70 patients with poly-
trauma, and by the end of treatment tended to reduce this
figure. As for the indicators 6 and 8 in the same pa-
tients, the concentration of the first serum was originally
raised in more than half of them, as in several studies
reduced by the end of the research, while the average
level of IL-8 was initially raised in 80 % of cases (56.2 ±
4.9), and also reduced by the end of the study, almost
reaching the normal range. In the literature there is an
evidence that low concentrations of raIL-1 are associa-
ted with a worse prognosis, perhaps, explains places wi-
without restriction or activity of endogenous IL-1, indu-
cing the state similar to septic shock. Increasing con-
centrations of IL-6 and IL-8, is perhaps a marker of tis-
ue destruction, an increase in the serum concentration
of raIL-1 antagonist may be an additional sign of deve-
loping inflammation.

Study on serum concentration VEGF in the blood
of 58 patients having cancer of bladder showed consid-
erable dependence between increased serum level of the
marker and level of tumor malignance as well as how
the process is spread. So, the concentration of VEGF in
the patients with higher level of malignance (G3) was
285.9 ± 27.2, slightly decreasing by the end of the stu-
dy (217.2 ± 22.7), whereas in those having low level of
tumor malignance there was a reduce in its consistency
by the end of the research (respectively 247.5 ± 12.3 and
107.5 ± 14.3).

Serum level of VEGF was considered among 24 pa-
tients with polytrauma – during I study the concentra-
tion of VEGF was 183.8 ± 15.2, statistically rising by the end of the study (296.2 ± 26.7), and the majority of patients with favorable process of disease had a statistically remarkable reduction in serum concentration VEGF – 147.5 ± 12.3, whereas the patients with poor prognosis during the first research had serum level of 102.23 ± 7.2, having almost the same level throughout the whole period of study (respectively, 111.2 ± 9.4 and 85.7 ± 11.4).

We carried out a correlational analysis of serum levels and induced in vitro cytokines to identify similarities and differences, especially violations of their synthesis, in order to find the ways how to influence their regulation under different pathological conditions, and to monitor the dynamics of their changes. According to the results, in patients with malignant tumors a positive correlation (statistically significant) in serum levels of studied cytokines was noted between γ-IFN and α-IFN, between γ-IFN and α-TNF, and between α-TNF and IL-4. In patients with polytrauma and sepsis statistically significant positive correlation was in concentrations of serum between raIL-1 and IL-8, between γ-IFN and α-TNF, and between IL-6 and IL-8.

For in vitro cytokine production in the dynamics of treatment in patients with malignant tumors there was revealed positive correlation between γ-IFN spontaneous and induced, and IL-4 induced and spontaneous.

In patients with polytrauma and sepsis, positive correlation between in vitro cytokine production was noted for γ-IFN spontaneous and induced, and α-TNF induced and spontaneous. IL-4 did not correlate with any of the other cytokines and with each other (spontaneous and induced levels).

The results obtained will encourage the selection of the most informative biomarkers of diagnostic and prognostic significance with regard to the return of the disease, metastasis, the possibility of purulent septic complications for a differentiated approach to immunodiagnosis and immunotherapy in various pathological conditions.

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Параметри цитокінової регуляції як додатковий критерій імунодіагностики

Резюме

Мета роботи полягалася в пошуку інформативних параметрів цитокінової системи для лабораторної діагностики при нулінній і непухлинній патології. Методи. Вивчені концентрації IL-4, 6, 8, rαIL-1, rIFN-альфа, rαγ-ІФН і VEGF у хворих з нуліннію і непухлинною патологією. Результати. Вихідні рівні досліджуваних цитокінів перевищували норму у більшості хворих з із хліцованими пухлинами мозку. У хворих на рак легені рівні raIL-1 у сироватці крові спочатку були підвищені і наприкінці лікування спостерігалася тенденція до зниження цього показника. Концентрація IL-8 у сироватці крові, збільшена на початку, у 82% пацієнтів знижувалася на пооперативному рівні наприкінці дослідження, тоді як середній рівень IL-8-за незначного підвищення спочатку під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою і знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політраумою i знижувався під кінець дослідження знижувався, майже схожо норми. Сироватковий рівень raIL-1 виявився збільшенням у 70% хворих з політро
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