

DINAMICS OF CYTOKINES PATTERN IN PATIENTS WITH PRESUMPTIVE AND DIAGNOSED AUTOIMMUNE DISORDERSClaudia Vlad¹ Coralia Bleotu^{2*} Veronica Lazăr³¹ District Emergency Hospital of Ploiesti, Romaia²S Nicolau Institute of Virology, Bucharest, Romania³Microbiology Department, Faculty of Biology, University of Bucharest, Romania**Article info****Abstract**Received: 01.03.2012
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The purpose of the study was to determine the dynamic of cytokinic pattern in patients with suspicion or diagnosed with autoimmune illnesses in different stages, and the potential of these biological assays as possible indicators in the diagnosis and the monitoring of these illnesses. The 41 patients were selected at the District Emergency Hospital and the Municipal Hospital of Ploiesti. Bioethics requirements were followed upon for patients selected for the study. The patients' ages varied from 21 to over 81 years old. The assay of IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, TNF- α was performed using the immunoenzymatic method ELISA (Enzyme-Linked ImmunoSorbent Assay). Low seric concentration of C3 and Ig A have been associated with elevated seric levels of CRP, fibrinogen, CIC, IL-1 β , IL-4 and IL-6 for the majority of patients used in the study. Very high seric levels of IL-1 β were registered in cirrhosis and hepatitis, diabetes, mushroom intoxications, CVA and hemorrhage. Patients intoxicated with mushrooms and patients with rheumatism, who also had an underlying disease, such osteoporosis and obesity, presented higher values of IL-1 β , IL-2, IL-4, IL-6, and IL-10. However, the determined titres for cytokines can be used in the diagnosis of some autoimmune disorders and in the monitoring of treatments, only in correlation with clinical and paraclinical complementary data.

Keywords autoimmune disorders, immunodepression, cytokinic pattern*Corresponding author e-mail address: cbleotu@yahoo.com**Introduction**

The immune function is essential to health condition [1,2] severe immunitary dysfunctions being incompatible with survival of the human organism [3]. The most frequent immunitary disfunctions are represented by hypersensitivity reactions, autoimmune reactions and immunodeficiencies [4], each of them being of several types and the consequence of varied causes [5]. It is known that in humans, the ageing of the immune system occurs after the age of 30 years [6] and is characterized by an increase in antibody production and implicitly the disruption of tolerance

towards *self* components [7]. The etiology of autoimmune diseases is not well deciphered [8] but it is assumed that environmental factors play an important part in the triggering of such diseases [9]. The purpose of the study was to determine the dynamic of cytokinic pattern in patients with suspicion or diagnosed with autoimmune illnesses in different stages, and the potential of these biological assays as possible indicators in the diagnosis and the monitoring of these illnesses.

Experiment Details

The study was done on a number of 41 patients. The patients considered for this study had different

immunodepression causing illnesses- cirrhosis, diabetes, intubation, septicemia, LED, hepatitis, on

dialysis; rheumatism, emergency conditions (with and without background illnesses). The determination of IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, TNF- α was performed using the immunoenzymatic method ELISA (*Enzyme-Linked ImmunoSorbent Assay*). The patients were selected at the District Emergency Hospital and

the Municipal Hospital of Ploiesti. Bioethics requirements were followed upon for patients selected for the study. The patients' ages varied from 21 to over 81 years old. The dominant age group was between 51 and 60 years old. Most patients selected for the study were from urban areas.

Results and Discussions

Elevated values of IL-1 β have been recorded in patients with diabetes, cirrhosis and hepatitis. These illnesses might have an autoimmune cause, and the elevated levels of this cytokinic marker, may represent an important index for establishing the diagnosis. IL-1 β contributes to the destruction of pancreatic β cells, in the case of diabetes. At the level of the hepatocyte, IL-1, just like IL-6, stimulates the production of acute phase proteins, and based on the local sequestration of other proteins, lowers the seric release of albumine. Elevated values of IL-1 β were found in patients intoxicated with mushrooms and patients with ulcer clinical syndrome. After intoxication with mushrooms, hepatic insufficiency occurs, associated with an elevated level of IL-1 β . It is known that IL-1 has elevated values when it comes to ulcers [5,10]. Elevated values of this cytokine have also been registered in patients with cerebrovascular accident (CVA) and hemorrhage. According to a previous study, in which seric levels of classical immunological markers were determined, in these patients elevated seric levels of Ig M were registered, due to the fact that IL-1 stimulates the proliferation of lymphocytes and the production of immunoglobulins [11,12,13]. IL-1 β has registered high values in patients with rheumatism which have osteoporosis as a secondary diagnosis and obesity. At the intra-articular level, IL-1 promotes the proliferation of synoviocytes, the formation of collagen deposits, the resorption of cartilage and the bone tissue, conditions that are associated with rheumatoid arthritis. In patients with rheumatism, elevated seric levels of IL-2 have been detected, compared to other groups of patients. All the patients with rheumatism in the study have been diagnosed with rheumatoid arthritis. IL-2 is the main cytokine with a key role in generating an efficient immune response, as well as a crucial regulator. IL-2 is

synthesized by the activated T CD4 lymphocytes. The T CD4 lymphocytes, while inactive, do not produce IL-2, but the Th 1 lymphocytes are activated after they recognize the antigens associated with the MHC (Major Histocompatibility Complex) class II molecules, producing IL-2. The stimulated lymphocytes proliferate, and at high density, they express the IL-2 receptors onto their surface. This is the way by which the clonal expansion of lymphocytes that have specifically recognized an antigen occurs. The interaction between IL-2 and its receptor starts the cells activation and proliferation. IL-2's synthesis is stimulated by IL-1, and IL-2 stimulates the synthesis of IL-4, IL-5 and IL-6. T lymphocytes are directly involved in the pathogenic mechanism of rheumatoid arthritis. In the synovial tissue, there are T lymphocytes that express MHC class II molecules. Whatever the stimuli, the inflammatory reaction begins in the joint, initiating a pathologic process that determines the progression of rheumatoid arthritis. Within the group of patients with rheumatoid arthritis in the study, IL-2 exhibited high seric levels in patients which have osteoporosis as a secondary diagnosis. It is well known that a bone demineralization process occurs in patients with rheumatoid arthritis, as a response to the immunological aggression. IL-4 presents elevated values in all studied groups. It is produced by several sets of lymphocytes, and has multiple effects on others. It has an autocrine way of action and determines the stimulation of producer cells also. It stimulates the expression of MHC class II molecules on the surface of B lymphocytes and the secretion of Ig E and Ig G1. In certain subcategories of these batches of patients, slightly elevated seric levels of Ig G have been additionally observed (for example: patients with cirrhosis and hepatitis, patients intoxicated with mushrooms, but also patients with cardiac and vascular

illnesses). Elevated levels of IL-4 could be explained by the fact that these patients present illnesses that generate immunodepression. High values have also been registered for patients with rheumatism and obesity as a secondary diagnosis, for patients with clinical ulcer syndrome, tachycardia and mushroom intoxications. In a previous study it has been shown that these patients have also displayed elevated seric levels of CIC (circulant immune complexes) and low Ig A values, demonstrating the predisposition of these patients to autoimmune diseases. All emergency patients with background illnesses, presented elevated levels of IL-4. According to our previous study, these patients also presented elevated values of CIC. Patients with CVA presented elevated seric levels of IL-4, while it is known that this cytokine is involved in the development of immunocompetent cells' function and the central nervous system's physiopathology. Compared to normal values, IL-6 presented elevated values for all the studied groups, and among them, a very high seric level has been registered for patients which came in the emergency room without background illnesses. IL-6 is a multifunctional cytokine, secreted by macrophages, T lymphocytes and endotelial cells that has synergic effects with IL-1. It is a mediator of the acute phase response, rapidly reaching detectible plasmatic levels, unlike many other cytokines. This response is a systemic anti-inflammatory reaction, consecutive to the infection or tissular lesion, and is characterized by fever, leukocytosis, increased vascular permeability and seric level of acute phase proteins. IL-6 stops the inflammatory process because it inhibits the synthesis of IL-1 and TNF-- α , concomitantly with the stimulation of IL-1RA synthesis. All batches of patients in this study concomitantly presented elevated seric levels of CRP (C reactive Protein). Some subcategories of batches have registered elevated values of fibrinogen (the patients with septicemia, intoxicated and the intubated ones, and with CVA). IL-6 presents very high values in patients with septicemia and the intubated ones. In normal physiological conditions, IL-6 is being produced in very low quantities in the body, but when a viral or bacterial invasion occurs, or when tissular destructions

happen it is rapidly and transitory induced. Slightly elevated levels have also been registered in patients on dialysis and with cirrhosis and hepatitis, once again demonstrating the hyperexpression of IL-6 in the pathology of a large number of illnesses, as well as in those with an autoimmune substrate. IL-6 presents elevated values for all patients with rheumatism. At the bone tissue level, IL-6 stimulates the formation of osteoclasts, favoring osteoporosis. IL-6 is considered the most important inducer of the hepatic synthesis of acute phase proteins. According to our previous study, these patients presented elevated seric levels of CRP and fibrinogen. Elevated seric levels have also been registered in patients intoxicated with mushrooms, once again confirming that this cytokine is also being synthesized in the case of intoxications as an acute phase mediator. Very high levels have been reported in patients with renal and biliary colic [14,15], demonstrating the involvement of IL-6 in numerous acute and chronic pathologic processes, and as well as in the autoimmune pathology. IL-10 presents elevated values for all the studied groups, and among those, a very high seric level has been registered in patients with rheumatism. Although Th 2 lymphocytes constitute de primary source of IL-10, it is produced by clones of Th 1 after stimulation with the specific antigen. IL-10 is the major anti-inflammatory and immunosuppressive cytokine, mediating the organisms' response to various pathogenic factors. Due to the fact that IL-10 can be produced in an autocrine manner, regulating its production by means of a negative feedback loop, IL-10 can regulate the immune response from the inside of the inflammatory focal point. The modulating role of the IL-10 is complex: on one hand, IL-10 inhibits the synthesis of cytokines associated with cellular immunity and an allergic type of inflammation, and on the other hand, it stimulates the immune cytotoxic and umoral responses. It is involved in a great number of illnesses, especially those with an autoimmune substrate. IL-10 participates in the appearance and progression of LES, and the elevated seric levels are correlated with antibody production. During our study IL-10 exhibited elevated values in all patients with rheumatism. IL-12 presented low values for all types of patients. IL-12 is implicated

in the initiation of the immune response, being considered the bridge that links unspecific defense reactions to the specific immune response. It stimulates the differentiation of unengaged Th lymphocytes in Th 1 lymphocytes, which mediate cell mediated immune response. Normal B lymphocytes do not synthesize significant amounts of IL-12. The immunodepressed group of patients, registered elevated values of TNF- α . TNF- α is produced not only by macrophages and monocytes, but also by lymphocytes, mastocytes, neutrophils, keratocytes, astrocytes, microglial cells, smooth muscle cells and some tumoral cellular lines.

Large quantities of TNF- α are released after the contact of macrophages, T CD 4+ lymphocytes and natural-killer cells with lipopolysaccharides, other bacterial products and IL-1. Determination of the seric level of TNF- α constitutes a marker for the systemic inflammatory response, associated with sepsis, traumatism and cardiac arrest. The level of TNF- α can be used as an indicator in the diagnosis of some immune illnesses and in their treatment monitoring, only in correlation with clinical and paraclinical complementary data.

Conclusions

The presumptive autoimmune disorder diagnosis is based on the clinical examination, while the confirmatory diagnosis and the monitoring of patients are especially based on periodical paraclinic exams, needing good sensitivity and specificity. The determination of the cytokinic pattern in patients susceptible or for those with autoimmune illnesses, diagnosed in different stages, has led to the establishment of certain correlations between different types of pathologic manifestations, immunological markers and certain cytokines as possible indicators in the diagnosis and monitoring of these illnesses. Low seric concentration of C3 and Ig A have been associated with elevated seric levels of CRP, fibrinogen, CIC, IL- 1 β , IL-4 and IL-6 for the majority of patients used in the study. Very high seric levels of IL- 1 β were registered in cirrhosis and hepatitis, diabetes, mushroom intoxications, CVA and hemorrhage. Patients intoxicated with mushrooms and patients with rheumatism, who also had an underlying

disease, such osteoporosis and obesity, presented higher values of IL- 1 β , IL-2, IL-4, IL-6, and IL-10. However, the determined titres for cytokines can be used in the diagnosis of some autoimmune disorders and in the monitoring of treatments, only in correlation with clinical and paraclinical complementary data. The correlation of the autoimmune condition's markers with the clinical profile of the illness has demonstrated the fact that autoimmune disorders present a wide spectrum of illness manifestations, ranging from subclinical to severe forms, with an ascending evolution, in successive, extensive acute stages, with unspecific symptoms. As the autoimmune condition evolves, the expression of immunological and cytokinic factors change in time, depending on age, sex, background disorder and the association of several autoimmune diseases, which demonstrates the deceiving character of the poly autoaggression, concerning the illness's manifestation both in an individual and at the population's level.

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