NEUTROPHIL FUNCTIONAL DISORDER IN CHILDHOOD

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Abstract
Neutrophil functional disorders thought to be uncommon, yet important as a cause of morbidity and mortality in infants and children. During the first years of life, when the immune system is still not completely mature, when the viral infections are frequent and antibiotic overuse can damage and alter the immune response, the inadequate nutrition followed with iron deficient anemia and malnutrition can lead the child`s organism in state of immunodeficiency. Sometimes is difficult to distinguish at the beginning weather the cause of patient suffering from frequent infections is existing of primary immunodeficiency disorder or the cause of the immunodeficiency state is just from exogenous factors.
Fortunately, primary immune deficiencies are rare diseases and only 6–7% of all of them, due to the neutrophilic functional disorders. Unfortunately, many exogenous and environmental factors have influence to the immune system, and the percentage of secondary caused neutrophilic functional disorders is much higher and should be considered when children are investigated for immunodeficiency. So, when to suspect neutrophil functional disorder? The hallmarks for diseases related to the neutrophilic functional disorders are discussed in this article.

Key words: neutrophil, phagocytosis, NBT test, Neutrophil function, Neutrophil functional disorder, malnutrition.

Innate immunity
In the constant contact with a great number of living and non-living, infectious and non-infectious agents, human organism has created refined mechanism for protection from them. Non-specific immunity, "natural resistance" or innate immunity is the first line of defense against foreign substances and pathogenic microorganisms. It is an immediate, non-specific defense that does not involve immunologic memory of pathogens. A lack of immunologic memory means that the same response is mounted regardless of how often a specific antigen is encountered. Because of the lack of specificity, the actions of the innate immune system can result in damage to the body’s tissues.
Non-specific natural resistance is a complex system in which numerous internal and external non-specific responses/factors are included. Each of these factors has its place in the defense. However, only joint with other factors of defense, building one complex system of anatomic, physico-chemical, physiological, immunological, metabolic, endocrine and other numerous factors, as well as nutrition aspects, would enable optimum level of defense of the host [5, 20]. As a major actor, coordinator and immunomodulator in the center of these complex mechanisms is the macrophage with its ability for phagocytosis and secretion of great number of active substances, cytokines being in the first place, with whom is communicate with the other cells of the immune system. Macrophage appears in the role of antigen-presenting cell (APC) also, which starts, and includes the specific immune response, that means
that the macrophage is the main factor for the connection between the nonspecific and specific immunity [5]. Under the influence of different stimuli, macrophages enter a so-called process of activations, which is non-specific for the microorganism that has caused it. Once activated, the macrophage will kill all microorganisms equally. All nonspecific defense mechanisms together are the core of natural resistance, which is beforehand determined, standardized, with no memory and non-specific.

**Phagocytes**

White blood cells, leucocytes, were discovered for the first time in 1773 by William Hewson, but their main function was discovered more than one century later, in 1883, when Elya Metchnikoff noticed that during an inflammatory reaction, leucocytes ingest microorganisms. He named this process phagocytosis and the cells capable of phagocytosis, phagocytes. He described these cells as macrophages; "big eaters"; cells "professionals"; cells that guard, protect and clean the organism of foreign agents; cells that are involved in the first line of defense of foreign invaders. Today, more than one another century later is learned, that the phagocytes demonstrate just only one sequence of a complex orchestrated activities in the organism, as a response to injury or invasion by microbial pathogens, in which the neutrophils, highly specialized professional phagocytes, engulfed microbes, foreign agents and tissue detritus and debris [1]. Although neutrophils are mostly viewed as playing a beneficial role to the host, their improper activation may also lead to tissue damage during an autoimmune or exaggerated inflammatory reaction [9–11].

Phagocytosis is the earliest protective reaction from a foreign agent. Two types of phagocytic cells participate in the nonspecific defense of the host:

1. Polymorphonuclear leucocytes, (also known as neutrophilic granulocytes, or neutrophils), which mainly action is in the circulation with capability of diapedesis on the site of the inflammatory process. Phagocytosis in neutrophils is carried out with strong respiratory outburst when intracellular destruction of extracellular pathogens happens. Speaking about the phagocytic activity one think firstly of neutrophils, as a guardians wondering through the circulations and destroying everything that is foreign. They are armed with an impressive arsenal of bactericidal agents that allow these cells to play a vital role in host defense against invading pathogens.

2. Mononuclear cells – monocytes (neutrophilic Mo) and tissue macrophages (Ma) which basic activity is in the tissues where they are found in steady, non-stimulated condition. In these cells, phagocytosis is performed without respiratory outburst. They are responsible for intracellular pathogens, which are those pathogens that possess mechanisms for successful intracellular parasitism [3, 16].

**Development and Distribution of Phagocytic System**

Human myelopoiesis occurs early in fetal life, as early as 6–8 weeks of gestational age [17]. Phagocytes from a 20-week gestational-age fetus were found to have active phagocytic and respiratory burst activity. Granulocytes are produced in the bone marrow and released into the blood and tissues where they act as the first line of defense in host resistance and wound healing.

Phagocyte progenitor cells arise from pluripotential hematopoietic stem cells in the bone marrow. These precursors can give rise to mature phagocytes, including polymorphonuclear granulocytes and mononuclear phagocytes, under the influence of colony stimulation factors (CSFs) such as SCF, GM-CSF, G-CSF, and IL-3[22]. The proliferation and maturation phases of granulocytes (neutrophils) in bone marrow require 6–10 days. During that period polymorphonuclear precursors develop primary (azurophilic) granules, followed at latter stages by secondary (specific) granules and a smaller pool of storage granules, all of which distinguish them from mononuclear phagocytes. Metamyelocytes and band form neutrophils do not replicate but represent the main storage pool of neutrophilic precursors. Mature neutrophils remain in the marrow space for several days before they are released into the circulation. The intact phagocyte function is due to intrinsic maturation but can be modulated by environmental factors resulting alteration of phagocytic activities. Some microbial products, endotoxins, some drugs (lithium, some antibiotics), infections (especially viral infections), cortico-
steroids, cells products (lactoferrin, prostaglandin E) and complement fragments (C3e, C3d, and g) can cause inhibited or accelerated release of mature granulocytes from bone marrow into the blood. Once released, granulocytes in the circulation have a half-life of 6–8 hours. In spite of this short half-life, granulocyte numbers in the blood are normally maintained between 3000 and 6000 cells/mm$^3$. This baseline rate of production can be increased to a trillion cells per day during acute infection or other severe stress, but then, the neutrophil may survive less than an hour [3, 14, and 22].

### Phagocytosis in the newborn

The phagocytosis in the newborn is almost equal to that in the adults, but still with the limited capacity, as the phagocytes from full-term newborns, still do not have complete functional activity.

The number of circulating neutrophils increases sharply after birth, reaching a peak by 12 to 14 hours and declining by 72 hours to level similar to those found in adults. Although neonatal neutrophils ingest and kill bacteria about as well as adult neutrophils, the capacity is limited for their accelerated production as a response to infection. The capacity of neonatal neutrophils to move is also reduced and the adherence to the site of infection is diminished. The diminished neutrophil storage pool, the reduced production of neutrophils in response to infection, the impaired delivery of these cells to the site of infections, have all been implicated in the pathogenesis of overwhelming infection in the human neonate [3, 16]. The neonate is at significant risk for infections, due to pyogenic bacteria, viruses, and certain intracellular organisms [7, 15, 20].

The newborn is indeed partially passively protected with the transplacentally transferred maternal IgG antibodies, but, it is not able for production of highly specific IgG and IgA antibodies, that makes him insufficient for specific immunity.

### Phagocytosis in infants and smaller children

After the first month of birth, during the infancy, neutrophils are capable for phagocytosis, in the same manner as the adults. The innate immunity, or the natural resistance, or the nonspecific immunity is carrying the main responsibility for defense and protection of the infant and small child under the three years of age from the infective agents.

Specific, acquired immunity will developed and mature during the first three years of life, under the indispensable influence of antigens stimulations (infections, immunization) and other factors [7, 15, 20]. Nutrition in this period (breastfeeding!) is an important determinant of the immune response, and the malnutrition is the most frequent cause for secondary immunodeficiency [7, 19].

### Neutrophil Function

Today is known that neutrophils use highly sophisticated and complex mechanisms to perform their role in immune defense and inflammation [10]. They participate in antimicrobial host defense both as the first line of innate immune defense and as effectors of adaptive immunity. They are short-lived cells that usually die while performing their antimicrobial function. Because their primary role is the localization and elimination of invading microorganisms at any expense, a simplistic view of neutrophils being not more than "dumb" suicide killers has prevailed for a long time. A major wave of discoveries during the 1990s and early 2000s made immunologists begin to appreciate the amazing complexity and sophistication of neutrophil functions [9]. It became evident that neutrophils release cytokines and contribute to orchestrating the immune/inflammatory response. The whole neutrophils activities, is called "signal neutrophil pathway" which represent complex chronologic series of events, so called "end stage events", followed one by one, but each of them can be the last goal of this activity. For only one minute the neutrophil will recognize the foreign agent and the neutrophil activation is going to start and then going on with attraction and adhesion on the endothelial cells of the blood vessels. Adhesive glycoproteins on neutrophils including selectins, as well as integrins, direct them to specific locations and promote their adhesion and subsequent migration. Selectins are involved in granulocyte rolling in the blood circulation, whereas integrins mediate firm adhesion and granulocyte extravasation. In response to chemoattractants such as C5a, IL-8, PAF, leu-
kotrienes, and fMLP, granulocytes reorganize their cytoskeleton and migrate into tissues and inflammatory sites where they interact with target organisms. This is followed by phagocytosis, respiratory burst activity; intracellular killing of the microorganism and degranulation. Phagocytosis is a very complex process which is accomplished in two phases of ingestion and digestion, when intracellular destruction and degranulation of the agent occurs. Numerous intracellular enzyme systems and microbicidal mechanisms participate in this process. Defects or unregulated activation in any aspect of the above phagocytic responses may result in immunodeficiency diseases with associated infections or serious inflammatory disorders [14]. The most common disorder is chronic granulomatous disease (CGD), a rare disorder characterized by absent or reduced function of the respiratory burst, which produces oxygen free radicals important for intracellular killing.

**Genetic determination**

Having in mind that entire neutrophil’s defense is a cascade of reactions which include differentiation of granulocytic lineage, entire neutrophil’s activation and function, and modulation under the action of numerous cytokines, it could be concluded that the basis for this kind of response is genetically determined. Today are discovered numerous genes and their products that controlled the neutrophil function. Even the smallest punctiform change, on the gene level, will result in breaking the natural resistance and onset of a disease/infection. Certainly, great defect in neutrophil’s function will be incompatible for life. Inherited immune disorders may occur in any stage of the activity of neutrophils, resulting with primary immune deficiency diseases.

**Primary Neutrophil Functional Disorders**

Qualitative neutrophil function defects may be divided into defects involving leukocyte adhesion deficiency (LAD1, LAD2), movement and other disorders of chemotaxis (Hyper IgE Syndrome), disorders of opsonizations and ingestions, respiratory burst-dependent bactericidal activity (Chronic Granulomatous Disease, Myeloperoxidase deficiency, Glutathione Synthetase deficiency, severe Glucose-6-Phosphate Dehydrogenase deficiency), and degranulation (Chediak Higashi Syndrome, Specific Granule Deficiency). The understanding of primary immunodeficiency diseases (PID) in general is changing, shifting from the conventional towards more complex and not only immune system defined. As with other PID, the recent progress in molecular biology over the last decade made an influence of our better understanding of the nature of phagocytes defects [21].

The primary immunodeficiency diseases (PID) in general, are rare diseases. The true incidence and prevalence are unknown, although the incidence has been estimated to be 1:10000 births. The primary neutrophil functional disorders are rare primary immunodeficiency disorders. Fortunately, only 6–7% of all primary immune deficiency disorders due to neutrophils dysfunctions.

**Secondary Neutrophil Functional Disorders**

Unfortunately, many exogenous and environmental factors have influence to the immune system, and the percentage of secondary caused neutrophil functional disorders is much higher. Many intrinsic and extrinsic factors contribute to defects in neutrophil function at different stages, resulting in recurrent infections and delayed wound healing. The ability of the immune system to prevent infection and disease is strongly influenced by nutritional status of the host especially during the childhood. In fact, malnutrition is the most common cause of immunodeficiency in the world. Poor overall nutrition can lead to inadequate intake of energy and macronutrients as well as selected micronutrient deficiencies. These nutrient deficiencies can cause immunosuppression and dysregulation of immune responses. Specifically, nutritional deficiencies can impair phagocyte function in innate immunity and cytokine production. Impairment of this response can compromise the integrity of the immune system, thereby increasing one’s susceptibility to infection. Protein-energy malnutrition (PEM), also called protein-calorie malnutrition, is caused by insufficient intake of protein and/or energy, is more common in developing nations but is also present in certain subgroups in industrialized nations. Secondary PEM is more common in developed countries, often occur-
ring in the context of a chronic disease that interferes with nutrient metabolism, such as inflammatory bowel disease, chronic renal failure, or cancer [19].

Nonspecific immunity and the activity of cytokines which have an important role in various steps of immunogenic mechanisms are influenced by iron deficiency anemia. Iron is an essential component of hundreds of proteins and enzymes that are involved in oxygen transport and generation of reactive oxygen species that kill pathogens. Neutrophils have a reduced activity of the iron-containing enzyme, myeloperoxidase, which produces reactive oxygen intermediates responsible for intracellular killing of pathogens. Iron deficiency anemia due to nutritional deficiency is not just a disease of developing countries, but it can also be seen in developed countries. Worldwide, over 40% of the children have iron deficiency anemia [4].

From practical point of view, the physician encountering a malnourished child who suffers from frequent, recurrent and prolonged infections, should suspect that the problem may be laid in the defect in the neutrophil response. This particularly concerned the newborn, infant and small child, as the nonspecific immunity is the main part of the immune system that protects him from infections, since the adaptive or acquired immune system will mature with the time. In this period, the viral infections are frequent, and some of them, like EBV, CMV and HIV, can directly damage the immune system by infecting the immune cells/neutrophils themselves. Antibiotic overuse, given with the aim to cure, actually kills the normal bacterial flora that promotes immune function and increases strength of the other pathogen bacteria through bacterial mutation developing antibiotic resistance.

So, during the first years of childhood, when the immune system is still not completely mature, when the viral infections and antibiotic overuse candidame and alter the immune response, the inadequate nutrition followed with iron deficient anemia and malnutrition, lead the child organism in state of immunodeficiency. Sometimes is difficult to distinct at the beginning weather the patient suffering of frequent infections has primary immunodeficiency disorder or the cause of the immunodeficiency state is just of exogenous nature.

When to Suspect Neutrophil Function Disorder?

Patients with decreased neutrophils counts or with neutrophils functional defects have recurrent or even fatal infections as well as impaired wound healing. Moreover, evidence has shown that aberrant phagocyte activation can contribute to untoward complications of inflammation. Nevertheless early recognition is important in order to decrease the morbidity and mortality that can be associated with them. Patients with neutrophil functional disorders usually present early in life with recurrent bacterial or fungal infections.

The hallmarks for all diseases related to the neutrophil functional disorders are the development of: 1. Recurrent, repeated, frequent infections – usually starting in infancy. 2. Prolonged course and inclination to chronicity 3. Poor reaction to antibiotic therapy [1, 2, 11, 20, 22].

These infections tend to be superficial, involving the skin and mucous membranes (especially of the respiratory and urinary system) and are most characteristically caused by bacteria staphylococcus aureus and many different Gram-negative bacteria, as well as fungi of the type Candida and Aspergillus. Besides these common characteristics in the clinical picture, each of these disorders has its own features and specifics, which could more or less bring us to establish right diagnosis if we recognize them. Chronic granulomatous disease (CGD) is probably the most common inherited disorders of neutrophil dysfunction and should be included in any differential diagnosis of recurrent infection in children [3, 13]. Abnormal aspects of host response, such as lack of fever, local inflammation without pus, an indolent presentation and an attenuated inflammatory response should immediately alert the clinician to the possibility of a neutrophil defect.

A thorough history of illness is imperative to a directed evaluation for suspected immunodeficiency of any type. The time of onset of symptoms can provide clues to the underlying defect. Infants with frequent infections from birth to three months of age are likely to have maternal antibody present, therefore defects in the immune system at this age are much more likely to be due to severe defect of the other part of the immune system, combined
immunodeficiency or neutrophilic dysfunction. Aside from the timing of the infection episodes, other important features implicating a neutrophilic disorder include a history of delayed umbilical cord separation or poor wound healing [3, 15].

Certain types of infections, like recurrent severe cellulitis, gingivitis, or rectal abscesses, occurring at any stage of childhood signal a possibility of primary dysfunction of neutrophils. Specific infection locations, such as omphalitis or osteomyelitis, should raise the suspicion of phagocytic cell abnormalities. Unexplained liver abscess, fungal pneumonia, or infection with unusual microorganism, such as Serratia species, Burkholderia cepacia, Nocardia, Aspergillus nidulans or nontuberculous mycobacteria should always raise a suspicion of neutrophilic disorder [3, 14]. Infections could be with common childhood pathogens, but with unusual severity, and then again that should be also characteristic for the disorders with neutrophil dysfunction. Family history is vital in evaluation of suspected immunodeficiency. Possible consanguinity, pattern of inheritance, early infant deaths, autoimmune diseases or connective tissue diseases in the family, is the information that should be obtained.

**Laboratory evaluation**

As soon as anamnesis with a careful history is obtained and detailed physical examination is performed, reasonable laboratory evaluation with few routine tests can be done. The initial screen should always include a complete blood count with evaluation of the peripheral smear. The number of leucocytes in peripheral blood should be quantitatively determined, with special attention to the number of neutrophils. Granulocytopenia is likely the most frequently encountered disorder of the phagocyte system. Neutrophilia, although most commonly associated with acute infection, is a common finding in LAD-1. Sometimes neutrophil counts are in excess of 100 000 with this disorder. The smear should be inspected for cellular morphology and granule staining also. Since the children are mainly with acute infections, their inflammatory responses is further monitored with reasonable algorithmic approach to the laboratory. Therefore, by analyzing the inflammatory response, (SR, acute reactive proteins), total serum protein level, albumin fraction, ratio albumin/globulin, oligo-elements, serum iron, hemostasis with factors of coagulation, the condition of the immune system of the patient could be indirectly obtained. Other examinations of the immune system (level of serum IgA, IgG, IgM, IgE, components of the complement system) can be added depending on the created opinion. Anyhow, it is better to perform the right test at the beginning, than entire battery of immune defect tests. Careful consideration of the clinical and microbiologic presentation usually indicates the right path to pursue.

When there is suspicion for existence of disordered function of neutrophils, especially killing defect of neutrophilic cells, which should be suspected if a child has recurrent staphylococcal abscesses or gram-negative infections, evaluation by screening tests measuring the neutrophilic respiratory burst spontaneously and after stimulation should be performed. Spontaneous and stimulated NBT-test (nitro-blue-tetrazolium test) is used for estimation of the oxidative metabolic response during phagocytosis. The most reliable and useful test of this type is a flow cytometric assessment of the respiratory burst using nitrobluetetrazolium (NBT) dye test, or rhodamine dye lately. When low values of NBT-test are obtained, which point out to disorder of neutrophil function (main characteristic for chronic granulomatous diseases), then further more subtle examinations are necessary to define their molecular cause [6].

Leucocyte adhesion deficiencies can be easily diagnosed by flow cytometric assay of blood neutrophils, using monoclonal antibodies to CD11, CD18 (LAD 1) or to CD15 (LAD2) [2, 13].

**Treatment of Neutrophil Function Disorders**

The treatment includes antibiotic therapy (for therapeutic or prophylactic aims) and surgical interventions and drainage or debridement of infected sites. There is no substitute for the right drug, and that requires knowing the pathogen. Because the spectrum of infection in these diseases may range over several microbiologic pathogens, empiric therapy is to be discouraged in favor of firm diagnoses [18].
Prophylactic antibiotics, antifungal agents and cytokines are highly successful in treating chronic granulomatous disease, and appear to be useful in some other immunodeficiency as well. BCG vaccination should be avoided in individuals with CGD, as well as in their newborn close relatives until the defect is ruled out. In general, attenuated viral vaccines are not contraindicated in individuals with primary neutrophil disorders, as antiviral cell-mediated immunity is intact.

A molecular diagnosis should be done whenever possible. The expanding knowledge of genotype-phenotype relationships suggests that not all defects, even those within the same gene, are created equal [18].

The future of the primary immunodeficiency lies in the cytokine therapy, enzyme substitution therapy as well as transplantation of bone marrow. Progress in stem cell transplantation and gene therapy will have a major impact on the long-term prognosis in these patients by providing techniques to permanently correct these disorders in the future [8, 14].

The approach and treatment of the secondary neutrophil disorders concern the corrections of the malnutrition or iron deficient anemia in the patients that luckily, lead to reparation of the neutrophil function. Rational use of antibiotics is imperative for keeping health immune system, especially the neutrophil function during the childhood.

REFERENCES

Резиме

ФУНКЦИОНАЛНИ НАРУШУВАЊА НА НЕУТРОФИЛИТЕ ВО ДЕТСТВОТО

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Се смета дека болестите што настануваат поради нарушување во функцијата на неутрофилите не се чести, но, сепак, се довољно важни причини за морбидитетот и морталитетот во доениачката и во детската возраст. Во текот на првите години од животот, кога имунот систем сè уште не е комплетно зрел, кога вирусните и инфекции се чести и преку мерното користење антибиотици може да го оштети и видоизмени имунот одговор, несоодветната исхрана следена со појава на анемија поради недостаток на железо и/или малнитрија, може да го одведат детскиот организм во состојба на имунодефициенција. Некогаш е тешко да се разграничи уште на почетокот дали причината кај еден пациент кој страда од чести инфекции е поради постоение примарен имунодефицит или причината за имунодефициентна состојба е јатрогено условена.

За срека, примарните имунодефициенци се ретки болести, а само 6–7% од сите нив се должат на неутрофилните функционални нарушувања. За жал, многу надворешни фактори и фактори од околината влијаат на имунот систем, така што процентот на секундарно причинетите неутрофилни функционални нарушувања е многу повисок, па треба да се мисли на нив кога се врши процена на имунот систем.

Кога да се постави сомнение за постоение на нарушување во функцијата на неутрофилите?

Во овој труд се дискутирани главните знаци што указуваат на болестите врзани за нарушувањата во функцијата на неутрофилите.

Ключни зборови: неутрофил, фагоцитоза, НБТ-тест, неутрофилна функција, функционални нарушувања на неутрофилите, малнитрија.