Revisão / Review

Haematological alterations in protein malnutrition
Alterações hematológicas na desnutrição protéica

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*Protein-calorie malnutrition (PCM) is usually found in children, the elderly, patients suffering from neoplasia or chronic disease, patients undergoing chemotherapy, or even patients under parenteral nutrition. PCM presents a wide spectrum of signs and symptoms that are a result of not only the cause(s) that led to malnutrition, but also of the different degrees of protein or carbohydrate deficiency. Here we present data obtained from observational and experimental epidemiological studies that suggest that malnourished individuals frequently present a greater susceptibility to infection with high morbidity and mortality indices. Data both found in literature and obtained by our group evidence that malnutrition modifies the organism's defence processes, impairing lympho-haematopoietic organs and modifying immune response. The haematological alterations in malnutrition, such as leucopoenia and hypoplasia, are described, with an emphasis on the results in experimental protein malnutrition obtained by our group. In particular, the structural and ultra-structural alterations of bone marrow, spleen and thymus; functional alterations such as the reduction of cell migration and spreading, phagocytosis, bactericidal and fungicidal activity as well as alterations in the production of reactive oxygen species are discussed. The implications of modifications of the haemopoietic environment in malnutrition states are still obscure, however, they seem to be responsible for inefficient haemopoiesis, especially inefficient myelopoiesis, and they seem to be irreversible over the short-term. Rev. bras. hematol. hemoter. 2004;**26**(1):49-56.*

Key words: *Malnutrition; bone marrow; marrow hypoplasia; anaemia; leucopoenia.*

Introduction

Protein restriction modifies physiological responses and may induce cellular lesion. However, differences as to the extent and timing of damage exist: tissues with a high protein turnover are affected before those which present a low protein turnover. Hence, mechanisms involved in the proliferation, differentiation and death of the cell can become altered in malnutrition affecting differently each tissue that makes up the organism.[1,2]

The elevated and constant demand of haemopoietic tissue for protein leads to the condition that both

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qualitative and quantitative alterations that may be found when protein malnutrition is installed.

Malnutrition

Protein-calorie malnutrition (PCM) also known as protein-energy malnutrition is defined by the World Health Organisation (WHO) as being "pathological conditions that are a result of a lower ingestion, in various proportions, of protein and calories", which occur more frequently in children with less than five years of age and is commonly associated with infection.[3]

Protein-calorie malnutrition is still the most common type of malnutrition: approximately 800 million people in the world present some kind of malnutrition.[2,4,5,6] Under 5-year-old children, especially those in developing countries, present a deficiency of more than two standard deviations in relation to the WHO/NCHS body weight standards for their age (193 million), to height standards (230 million) and to weight to height ratio standards (50 million).[6,7,8]

Protein-calorie malnutrition is usually found in new-born children who present a lower weight than average for their gestational age, patients under parenteral nutrition, individuals with alimentary diseases such as anorexia nervosa and bulimia, patients with neoplasia or chronic diseases as well as in patients under radical diets.[9-15]

In Brazil, the distribution of malnutrition indices is irregular. In the North and Northeast regions there are high indices of malnutrition, varying between 23% and 27.3%, similar to those of poor African countries. In the South and Southeast regions of Brazil, however, the prevalence of malnourished children is as low as 8% to 9%.[16]

Malnutrition may originate from the deficiency or absence of any nutrient. The establishment and severity of a state of malnutrition depend on the cause, intensity and duration of nutritional deficiency. It can be caused, primarily, by an inadequate diet or, secondarily, by deficiency in gastrointestinal absorption and/or deficient ingestion or increase in demand, or even, by an excessive excretion of nutrients. Also, several forms of malnutrition may occur simultaneously,[17] taking into account that a deficiency status caused by quantitatively and/or qualitatively insufficient diets may occur, which lead to the several types of malnutrition.

Depending on the degree of PCM various clinical forms of this syndrome may occur, which may lead to difficulties when comparing cases. PCM can appear under several clinical forms, with the most characterised forms being *Kwashiorkor* and *Marasmus*.[2,18,19,20] Even though these forms are clinically different, the physiopathology of these two syndromes can frequently be superimposed, and it is common that *Marasmus* be characterised as *marasmatic-kwashiorkor* or a clinical form of *Kwashiorkor* without oedema.

Epidemiological and experimental observations prove that malnourished individuals, especially children, present a higher susceptibility to infectious processes and higher morbidity and mortality indices.[21,22]

The malnutrition-infection complex can be viewed under two aspects: malnutrition altering the defence mechanisms of the individual and infection aggravating the previously installed deficient nutritional status or even the triggering of this deficient nutritional status by the disease itself.[23-26] So, malnutrition can facilitate the invasion of the agent and favour its proliferation in the organism, as well as increasing the chances of a secondary infection occurring and modifying the evolution and prognosis of the disease.[27,28]

Haemopoiesis

Blood, as a tissue is characterised by ***(i)*** its high rate of renewal, taking into account that its mature cells present a relatively short lifetime in circulation;[29] and ***(ii)*** its flexibility and ability to adapt to different physiopathological conditions.

Physiologically, in human adults, haemopoiesis occurs in the bone marrow. The constant production of cells depends on the microenvironment of the marrow, an organised structure that regulates the physiology of the haemopoietic stem cells.[30,31,32]

The microenvironment is constituted by haemopoietic cells in different states of maturation, stromal cells (reticular cells, macrophages, endothelial cells, adipocytes), by an extracellular matrix (ECM) and by soluble substances,[33,34,35] in what is a compartmentalised, dynamic structure, that in addition to supplying parenchymal support for the haemopoietic cells, provides a "biochemical environment" that is fundamental for their proliferation, differentiation and maturation.[33] So, regulatory factors that make up inductive microenvironments are supposed to exist.[36,37,38] The term "inductive microenvironment" is applied more specifically to the factors that act in association with the stroma, rather than to factors that act at a distance and which would have a permissive role as opposed to a regulatory role.[39,40]

It is believed that the inductive microenvironment may control haemopoiesis through the production and local secretion of cytokines by stromal cells, permitting the co-localisation of cytokines or even, by direct stimulus through direct contact with the cell.[36,41,42] The cellular interactions are mediated by different groups of receptors present on the cell surface, which are known as adhesion molecules.

These molecules influence various processes such as cellular growth and differentiation, the formation of junctions and cell polarity.[43] The most studied components of bone marrow extracellular matrix are collagens I and III, fibronectin, laminin, vitronectin and thrombospondin.

Haemopoiesis is influenced by several stimuli that act at different levels in the process. For this reason, cell-cell and cell-stroma interactions that occur in both the haemopoietic inductive microenvironment and other locations must be considered.[37,44] As should the action of the different growth factors and cytokines, hormonal action - especially that of the estrogens, androgens, thyroid hormones, corticosteroids and epinephrine, plasmatic and cellular mediators of the inflammatory response and, obviously, the nutritional state of the individual.[45,46,47]

One can see that haemopoiesis is a complex and highly regulated phenomenon which depends on ***(i)*** the existence of primitive cells with a proliferative capability; ***(ii)*** the regulatory action of growth factors; ***(iii)*** the components of the microenvironment and ***(iv)*** the architectural structure of this microenvironment.[48,49,50]

Concepts acquired on the effect of human protein privation have largely been based on information obtained from severely malnourished individuals, suffering multiple nutritional deficiencies, or from patients with chronic systemic diseases in which the effects of the disease and its therapy are difficult to distinguish from those of nutritional deficiency. Data obtained in animals have, as we see it, allowed a partial evaluation of the production, mobilisation and function of phagocytes. We must remember that typically, information about each of these aspects are obtained from studies which use animals of different species, sex and age, which are fed qualitatively and quantitatively different diets, and are under the most varied inflammatory and infectious stimuli. Hence, particularly with respect to haemopoiesis, the existing data in literature are contradictory.

Malnutrition and Erythropoiesis: Anaemia

The haemopoietic tissue, like all tissues that present a high rate of renewal and cellular proliferation, has a high demand for nutrients. The sheer need for protein by the process of haemopoiesis could in itself justify the occurrence of anaemia and leucopenia which are frequently encountered in malnourished individuals.

PCM is a syndrome in which anaemia together with multivitamin and mineral deficiency may be present. According to Vilter (1975), children with typical PCM present normochromic, normocytic anaemia, with haemoglobin levels that lie between 8 and 10 g/dL and normal medullary erythropoiesis, or a discretely hypo-plastic marrow with fatty infiltration.[51]

The lack of iron has been considered as being the main cause of anaemia in malnutrition.[52] However, other authors have found normal iron serum levels with an increase of transferrin saturation[53] and normal serum ferritin levels, with the bone marrow presenting normal or elevated iron deposits. Liver biopsies obtained *post-mortem* from individuals suffering from *Marasmus* presented high levels of iron.[2,54]

Fondu et al (1978), working with children from Zaire suffering from PCM, concluded that anaemia was due to a reduction in the mean life of erythrocytes, which in the population studied was 18 days. This suggests that an increase in erythrocyte fragility was due to a decrease in selenium and vitamin E.[55] Furthermore, these same authors suggested that amongst the causes for anaemia in PCM there is the adaptation of the organism to the reduction of the demand for oxygen and also to chronic infections, which are frequently present.

Anaemia, in experimental malnutrition presents a decrease in iron incorporation and in the number of reticulocytes,[56] and furthermore, an interruption of the maturation process of erythroblasts. Studies[57,58,59] demonstrate that in rats, a decrease in erythropoietin occurred due to the reduced ingestion of protein. Data from our laboratory evidence that the anaemia found in adult mice, submitted to protein malnutrition is not due to iron deficiency, as the serum concentration of iron in these animals was found to be high, as were the levels of transferrin saturation and ferritin concentration in the bone marrow, liver and spleen.[60]

The analysis of medullary erythropoiesis in malnourished animals reveals a reduction in primitive erythroid cell count (yet unpublished data) and similar to Aschkenasy (1975), we found that the maturation of erythroblasts became altered.[57] Taking into account that we have excluded the haemolytic nature of this anaemia, our results suggest that the alterations in proliferation and maturation may be due to the impairment of the haematopoietic inductive environment.[48, 61]

Malnutrition and Leucopoiesis

Leucopenia and leucocytosis are situations that have been described in literature, as occurring in malnutrition, especially in human beings, as it is usually accompanied by infectious processes or chronic disease.[57,62,63,64]

Studies have often presented conflicting evidence, which can be the result of the presence of several different deficiencies, which are often associated to different pathological processes.[2,57] The first reports on blood leucopenia in protein malnutrition were made by Kornberg et al (1946).[65] Even though leucocyte response is variable, there is evidence that situations in which malnutrition is not accompanied by other diseases, leucopenia is always present.[55,66] Hypoproteic diets or those with an inadequate composition of aminoacids have a neutropenic[67] and eosinopenic[68] effect.

Endotoxins alter cell mobilisation not only dislocating "marginal" granulocyets, but also modifying the influx into the maturation compartment.[45,69] The apparent difficulty in leukocyte response in bacterial infections in PCM is, according to Suda et al (1976) due to a reduction in the reserve compartment of the bone marrow, and not due to the primary impairment of leukocyte mobilisation from blood to tissue.[70] However, data regarding cell mobilisation are also divergent.[71]

Goyal et al (1981) reported a reduction in poly-morphonuclear leukocyte adhesion to nylon in patients suffering from *Marasmus.*[72] Harris et al (1985) found in neutrophils obtained from new-born rats of malnourished mothers a decrease in chemotaxis and adhesion.[73] In mice submitted to PCM there was a reduction of cellularity in the peritoneal cavity after an intraperitoneal administration of glycogen[50,74] and BCG (unpublished data), as well as an *in vivo* reduction in leukocyte migration.

De la Fuente et al (1992) observed, *in vitro,* an increase in the adhesion of peritoneal macrophages obtained from malnourished mice,[75] however, Borelli et al. (1998) found a reduction in adhesion as well as in the expression of fibronectin.[61,76,77]

Severe malnutrition produces several cellular effects and the results of some studies indicate a loss or reduction in cell proliferation in several organs.[78,79]

Leucopenia may be due to alterations in the cell cycle, even though Aschkenasy (1975) reported a bone marrow with normal cellularity.[57]

Ortiz & Betancourt (1984) described the relationship between protein deficiency and the reduction in cell proliferation in several organs.[78] Suda et al (1976) reported that in basal conditions, cell kinetics in malnourished animals was normal, in spite of the reduction of the bone marrow pool, however, when under inflammatory stimulation, the neutrophilic response was less intense, perhaps due to the reduction in the bone marrow reserve compartment.[70]

Diet may be considered as being a modulator of the immune system,[10] and there have been reports that in malnutrition there may be significant alterations of several aspects of immunity, especially, the impairment of T-cell-dependant response, phagocytosis, the complement system and cytokine synthesis.[79-84]

Chandra (1977) found, in malnourished children, a reduction in lymphoid blastic transformation percentage in response to mitogens.[84] Cell proliferation in bone marrow and spleen of malnourished mice is diminished, and a smaller number of pluripotential cells is found.[56] The production of myeloid cells is impaired.[50,56,70]

Olmos et al (2001) evaluated, in animals the influence of malnutrition on cell cycle, and found a decrease in the number of viable nucleated cells in the bone marrow and an alteration in the mitosis index.[85]

Borelli et al (1995) observed *in vivo* and Borsatto (1999) observed *in vitro* that malnourished animals have a lower production of granulo-monocytic progenitors, suggesting that this is one of the factors responsible for the hypoplasia observed *in vivo* in these animals.[50,86]

Protein malnutrition induces structural alterations in lymphoid organs, especially in thymus-dependant areas.[2,20,49,63,87] Protein deficiency leads to lymphopenia, thymus, spleen and lymph node involution, which is particularly intense in the thymus and spleen.[4,57, 68,71,81] Aschkenasy (1966b) reported atrophy of the thymus and lymph organs, with a pronounced reduction in cellularity, especially in thymus-dependant areas.[68] It must be remembered that in other species, organs other than the bone marrow have a hematopoietic functions.[88]

The different lymphocyte populations seem to be affected by malnutrition differently: in thymus-dependant areas, there is a reduction in the number of T-lymphocytes, especially the $CD4^+$ population, whereas the number of B-lymphocytes in the spleen, lymph nodes and blood remain normal.[89]

According to Frayn (1986), lymphopenia is a result of the reduction in cell proliferation which in turn can be a direct consequence of the lack of protein or elements like iron, zinc and copper or due to hormonal imbalance involving adrenaline, insulin, thyroxin or cortisol.[90]

Existing data on humoral response are conflicting, which makes definitive conclusions hard to reach.[2] The concentrations of IgA, IgG and IgM can be, according to some authors, increased,[3,91] or normal or decreased.[92] IgE is found at a higher concentration in malnourished children.[93]

Gross & Newberne (1980) have considered that, even though immunoglobulin concentrations may seem to be normal in malnutrition, functional studies involving B-lymphocytes indicate that the type and intensity of a response to various antigens is altered.[63] According to Bounous et al (1985), this may be due to, in quantitative terms, a shy production of antibodies or even, the production of antibody molecules with low specificity.[94] The authors observed that alterations in the quantity and quality of protein in diets modifies the humoral response in mice, thus reflecting the functional alterations suffered by B-lymphocytes.

Literature describing the cellular immune response in malnutrition is broad, however, inconclusive. The functional evaluation of T-cells by means of blastic response to mitogens, delayed hypersensitivity reactions, rejection to grafts and the production of lymphokines is variable.[66,92,95-99] Thus, if on the one hand, we have a group of researchers reporting the occurrence of depression of the cellular response,[66,96,100] on the other we have studies whose results are the opposite, revealing that in protein malnutrition the cellular response becomes stimulated.[101,102]

As to the production of cytokines in states of malnutrition, there is also no consensus in literature. Results obtained in malnourished human beings are still controversial regarding the production of IL1 by macrophages.[100,103] Data obtained in literature suggest that the ability of the organism to respond to exogenous IL1 depends on the nutritional conditions of the individual.[97] Grimble & Silk (1990) observed that there was a reduction in the capability of monocytes to synthesise cytokines.[104] Other authors observed a reduction in the production of IL-1, in children and adults with severe protein deficiency.[105,106] On the other hand, Bradley et al (1990) found normal concentrations for IL-1 and TNF-α in malnourished women,[107] whilst Vaisman & Hahn (1991) found an increase in the synthesis of TNF-α in anorexic patients and patients with a severe degree of malnutrition.[108]

Studies on the production of cytokines indicate a decrease of Interleukin 2.[109] This decrease could be one of the mechanisms by which malnutrition would induce a lack of immunocompetence.[110]

Malnutrition and the Hemopoietic Environment

Few are the studies that associate malnutrition and the extracellular matrix (ECM). Lyra et al (1993) evaluated the effects of malnutrition on children's thymus and observed an increase in the density of the ECM, which was considered as being responsible for thymocyte depletion.[111] Reif et al (1993) observed that the components of the ECM decreased, however, the relation protein/tissue did not present a significant difference, suggesting that the main effect was that of fatty pervasion with hepatic steatosis.[112] Alxelsson et al (1990) noted bone mineralisation, inferring that malnutrition leads to the synthesis of proteoglycans of a higher molecular weight leading to the inhibition of calcification.[113]

We have found, in protein malnutrition, bone marrow hypoplasia[50] with histological evidence of extracellular matrix alterations.[48] Xavier (1999) found, in malnourished mice, an absolute hypoplasia of the myeloid tissue, which was directly proportional to the degree of malnutrition, with an emphasis on the depletion of the erythroid and granulo-monocytic components. At more severe degrees of malnutrition, he also found the depletion of the megacaryocytic component and a blockage of maturation.[49] As to the marrow stroma, a progressive sinusoidal dilation, occupying a large part of the marrow compartment was observed. The population of adipocyte-like cells was apparently maintained unaltered throughout the development of malnutrition. Vituri et al (2000), when quantitatively analysing the ECM obtained from bone marrow of malnourished mice, found alterations in the proportion of ECM proteins, especially fibronectin, thrombospondin and laminin, a fact that, in our opinion, may be contributing to the hypoplasia observed.[114] This hypoplasia was partially reversed in previously malnourished animals that then received basal chow. Nevertheless, the restitution of cellularity occurred in a haphazard fashion, with a loss of the topographic relationship between the different lineages, and furthermore, the haematopoietic cells presented aberrant morphological characteristics, describing a histopathological status of dysplasia.[49] These animals presented pancytopenia of peripheral blood (unpublished data). Hence, the implications that the modifications suffered by the microenvironment involved in cellular proliferation impose in a state of malnutrition remain obscure. However, they seem to be responsible for inefficient haematopoiesis, especially inefficient myelopoiesis, and are apparently irreversible in the short run (unpublished data).

In our point of view, malnutrition modifies *per se* the defence mechanisms of the organism, altering haematopoiesis at different levels, impairing the organism's homeostasis and modifying both the specific and nonspecific immune responses, including the inflammatory response.

Resumo

*Desnutrição protéico-calórica (DPC) é geralmente encontrada em crianças, idosos, pacientes com neoplasias ou doenças crônicas, pacientes submetidos à quimioterapia ou à nutrição parenteral. A DPC apresenta uma variedade de sinais e sintomas que são um resultado não apenas da(s) causa(s) que provocam a desnutrição, mas também de diferentes graus de deficiência de proteínas e carboidratos. Nesta revisão, nós apresentamos resultados a partir de estudos epidemiológicos observacionais e experimentais, que evidenciam que indivíduos desnutridos freqüentemente apresentam uma maior susceptibilidade para infecções com índices elevados de morbidade e mortalidade. Dados obtidos a partir da literatura e do nosso grupo evidenciam que a desnutrição modifica os processos de defesa do organismo, prejudicando órgãos linfo-hematopoéticos e alterando a resposta imune. As alterações hematológicas na desnutrição, tais como leucopenia e hipoplasia, foram descritas, com ênfase em relação aos resultados em desnutrição protéica experimental obtidos pelo nosso grupo, especialmente as alterações estruturais e ultra-estruturais da medula, baço e timo; alterações funcionais, como a redução da migração celular, da capacidade de espraiamento, da fagocitose, da atividade bactericida e fungicida, bem como alterações na produção de espécies reativas de oxigênio. As implicações das modificações do ambiente hematopoético em estados de desnutrição são ainda obscuras, contudo, parecem ser responsáveis pela hematopoese ineficiente, especialmente pela mielopoese ineficiente, e parecem ser irreversíveis em curto período de tempo. Rev. bras. hematol. hemoter. 2004;**26**(1):49-56.*

Palavras-chave: *Desnutrição; medula óssea; hipoplasia medular; anemia; leucopenia.*

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