ALLERGY TO COW'S MILK PROTEINS

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Rezumat: *Alergia la proteinele din laptele de vacă este definită ca reacția imunologică, mediată sau nemediată IgE, la proteinele din laptele de vacă. Afectează populația pediatrică în primii 2 ani de viață, după această vârstă instalându-se toleranța la proteinele din laptele de vacă. Sistemul imun intestinal este răspunzator de inițierea reacției alergice intestinale prin stimularea secreției de IgAs şi inițierea toleranței orale. Toleranța orală indusă reprezintă strategia de prevenire a reacției alergice intestinale. Prezența epitopilor legați de proteinele din laptele de vacă măresc capacitatea de recunoaştere antigenică şi constituie baza imunoterapiei orale. Simptomatologia este nespecifică. Diagnosticul pozitiv se bazează pe datele furnizate de istoricul familial, dieta de eliminare, testul de provocare şi dozarea IgE totale şi specifice. Tratamentul constă în administrarea de formule terapeutice. Alimentația naturală rămâne gold-standardul de prevenire a alergiei la proteinele din laptele de vacă. Prognosticul este favorabil, cu remisiune după vârsta de 3 ani.*

Keywords: *allergy, milk cow`s proteins, oral tolerance*

Abstract: *Allergy to cow's milk proteins is defined as the immunologic reaction, mediated or non-mediated, towards proteins from cow's milk. It affects infants up to the age of 2; after this age, tolerance to cow's milk proteins sets in. The intestinal immune system is responsible for initiating the intestinal allergic reaction, by stimulating the secretion of Ig As and initiation of oral tolerance. Induced oral tolerance represents the strategy of prevention of an intestinal allergic reaction. The presence of epitopes bound to cow's milk proteins increases the capacity of antigenic awareness and represents the basis for oral immunotherapy. The symptomatology is unspecific. The positive diagnosis is based on data from the family history, elimination diet, the provoking test and determining total and specific IgE levels. Treatment consists of administering therapeutic formulas. Natural diet remains the gold standard of cow's milk protein allergy prevention. The prognosis is favorable, remission occurring after age 3.*

INTRODUCTION

Definition. Terminology. Cow's milk protein allergy (CMPA) is defined as IgE-mediated or non-mediated immunologic reaction, towards one or more proteins from cow milk. Cow's milk protein intolerance (CMPI) differs from CMPA as far as pathogenesis (non-immunologic reaction towards CMP) as well as evolution and prognosis are concerned.

Epidemiology. Most authors estimate a 5-15% prevalence of CMPI and a 2-7.5% prevalence of CMPA. The rates vary due to the action of several factors: age, nutrition, genetic factors, cultural factors, diagnosis criteria used. Sicherer et. al.[2] (Feb. 2010) report an CMPA- incidence of 1.9-2.8% during the first two years of age; the rate drops to 0.3% after age 3. After this age (sometimes after age 5), tolerance towards CMP sets in; the rare cases found in older children and adults are in fact multiple food allergies. Approximately 0.5% of breast-fed infants develop CMPA[3]; the low rate is due to the low concentration of CMP in breast-milk (100,000 times lower than in cow's milk).

Etiopathogenesis. Food allergens are glycoproteins, 10-40 kDa in size, which are water-soluble, heat-, acid- and enzyme- resistant. The most important food allergens are: beta-lacto-globulin (from cow's milk whey), 7S globulin-vicilin (from soy), Ara h_1, Ara h_2 and Ara h_3 (from hazelnuts). In cow's milk, more than 20 proteic fractions have been identified. Whey (20%) is represented by beta-lactoglobulin and alfa-lactalbumin, and casein (80%), by the alfaS_1, alfaS_2, beta, gamma, kappa fractions. Beta-lactoglobulin is responsible for 60-80% of CMPA cases; casein proteins are low allergenic, due to their flexible (non-compact) structure.

CMPA is associated in 30-50% of cases with soy-protein allergy and secondary lactose intolerance.

Intestinal immunity. Physiopathological data.

The intestinal immune system is directly responsible for the onset of intestinal allergic reactions. The intestinal absorption of the food allergen depends on the immunitary state of the intestinal mucosa[4]. The maturity of the intestinal mucosa, the presence of the Peyer patches, the normal intestinal microflora and the absence of intestinal inflammation and infection maintain the immunocompetence of the intestinal mucosa.

The intestine has defense mechanisms that neutralize, disintegrate and inhibit the absorption of the food allergen. IgA and M and the lymphocytes of the intestinal epithelium and lamina propria act as the immunologic barrier, whereas gastric acid, intestinal mucus, lactoferrin, the normal microflora, the hepatic filter constitute the non-immunologic barrier[6].

The regulatory T-lymphocytes insure the balance

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between the Th_1 lymphocytes (via the gamma-IFN and IL_2 which stimulate cell-mediated immunity and phagocytosis) and the Th_2 lymphocytes (via IL_4, IL_5, IL_{10}, IL_{13} that stimulate the humoral immune response). Studies show that the allergic reaction is the consequence of an inadequate regulatory response[7].

At the intestinal level, the food allergen can be absorbed in 3 ways:(4, 8)

- the trans-cellular path (which represents the major means of absorption), either directly (maintaining the antigen intact) or indirectly (after prior degradation of the antigen);
- the para-cellular path and
- the direct path via the M-cells (minor path of absorption).

Once absorbed, the food allergen is taken over by the antigen-presenting cells (from the epithelium and the lamina propria) and transferred to the Peyer patches. Here, the antigen is presented to the GALT, which has the role of protecting the host against intestinal pathogens (by stimulating Ig As secretion) and of preventing intestinal allergic reactions (by initiating oral tolerance).

Oral tolerance. The new-born is confronted with a physiologically insufficient oral tolerance (OT), which is part of the normal maturation process. After the first month, the oral tolerance has the tendency to normalize due to the contribution of growth factors from colostrum (that contribute to the maturation of the intestinal mucosa and maintaining normal permeability) and of breast milk (that supplements the insufficient Ig As production from the intestine)[9, 10].

Under some pathological circumstances - cellular or humoral immunity suppression or intestinal inflammation (leading to increased permeability of the intestinal mucosa) – OT is blocked or becomes insufficient.

Induced oral tolerance (self tolerance) is known to be part of the strategy of preventing allergic reactions. In 1829, Dakin[11] showed that the North American native population protected themselves from the cutaneous allergic reaction induced by urushiol (an oily substance found in poison ivy) by repeatedly consuming poison ivy. Wells[12], in his research from 1911, demonstrated the existence of self tolerance in mice that had been repeatedly exposed to eggs, and were thus protected from anaphylaxis when intravenously injected with egg proteins.

In 2001, Bennet et. al[13] demonstrate the existence of two mechanisms of inducing oral tolerance, based on the amount of the food allergen. For a small dose of allergen, the regulatory cells are suppressed. The mutant gene $FOXP_3$ blocks the Th_1, Th_2 response and is responsible for the IPEX syndrome, with X-linked inheritage and whose clinical signs are enteropathy, atopic dermatitis and food allergies. In the case of high doses, anergy and clonal deletion by inhibition of IL_2 takes place.

A series of factors can influence the development of oral tolerance: allergen properties, the means of exposure, and genetic factors.

It is known that soluble food allergens are more tolerogenous and, although most food allergens are soluble, it seems that the way that they are processed changes their solubility.

Oral exposure to food allergens stimulates OT, as opposed to cutaneous exposure, which inhibits it. Strid[14] et. al., in 2005, have demonstrated that the epicutaneous and epidermal exposure to proteins from hazelnuts inhibit OT for hazelnuts, in mice with existing OT, by stimulating Th_2 and a consecutive rise of IL_4 and IgE.

Li X(15), in 1999, carried out a study on the mice species C3H/HeSn, AKR/j and BALB/c which had been injected with $Arah_2$ DNA for 3-5 weeks. The C3H/HeSn species developed anaphylaxis, whilst AKR/j and BALB/c hadn't developed anaphylaxis, due to the high level of IgG2a (but not IgG_1 and IgE). Another study [16] (Morafo, 2003), carried out on the C3H/HeJ and BALB/c species, showed that the intragastric exposure to cow's milk and hazelnuts induced anaphylaxis only in the C3H/HeJ species (87% to cow's milk and 100% to hazelnuts), because high levels of IL_4 and IL_{10} were found in the splenocytes of this species (whereas in the BALB/c species, only high levels of gamma-INF were found).

In 2001, Chatchatee[17] discovered the existence of the epitopes bound to kappa-casein (8 bound to IgE and 4 to IgG) and beta casein (9 bound to IgE and IgG). The author showed that epitopes increase the capacity of allergenic awareness in patients that had been systematically exposed to allergens. The presence of epitopes plays a predictive role in persistent allergy and is the base of oral and sublingual short-term immunotherapy.

Physiopathology

Three types of hypersensitivity reactions are involved in CMPA [18]:

- type I, immediate hypersensitivity, IgE-mediated, which is responsible for high-risk symptoms (anaphylactic shock, urticaria, angioedema etc.);
- type III, hypersensitivity by circulating immune complexes, non- Ig E- mediated, which is responsible for the symptoms that occur 2-3 hours after the allergenic exposure;
- type IV, cell-mediated, late hypersensitivity; the prototype of this kind of reaction is the coeliac disease.

Clinical signs and symptoms

The symptoms can occur within minutes from the ingestion of a small amount of milk (a few drops).

There is not even a single patognomonic symptom for CMPA. Often, various symptoms (with no apparent cause) are associated with CMPA.

Given these circumstances, early diagnosis and adequate treatment become indispensable to later normal weight development.

Given the diversity of symptoms, the clinical picture of CMPA can be classified in 4 categories:

- gastro-intestinal symptoms are the most frequent (50-80%); in order of frequency of occurrence there are regurgitations (16-42% of patients with CMPA suffer from associated gastro-esophageal reflux disease), vomiting, diarrhea, constipation (perianal erythema), stools with blood-strings (with consecutive iron-deficiency anemia);
- cutaneous symptoms (20-40%), atopic dermatitis, angioedema, urticaria (not related to infections or drug-use); the presence of dermatitis increases the risk of sensibility to CMP 4 times and to egg-proteins 8 times;
- respiratory symptoms (4-25%), not related to infections;
- general symptoms (abdominal colics, persistent discomfort – longer than 3 hours a day, more than 3 days a week, for more than 3 weeks).

Life-threatening symptoms are immediate (glotic edema, severe bronchoconstriction and anaphylactic shock) and late, which interfere with growth and development (enteropathy with protein-loss, severe exsudative atopic dermatitis with protein-loss).

Clinical forms

Acute forms of CMPA are IgE dependant, whilst chronic forms are non-Ig E dependant. Mild and moderate forms show as atopic dermatitis, generally in breast-fed infants, and severe forms manifest either acutely, with life-threatening symptoms or late symptoms (failure to thrive, growth faltering).

Positive diagnosis is based on data from personal and family history, symptomatology (even uncharacteristic),

elimination diet (good clinical evolution after a diet), provoking test (reoccurrence of the symptoms after re-exposure to CMP), determination of total and specific cutaneous and seric IgE levels (there is a 58.8% concordance between these two for commercial products and 91.7% for fresh foods).

Family history yields very useful information: a parent with atopia increases the risk by 20-40%, a sibling with atopia, by 25-35% and both parents with atopia increase it by 60%[20].

The provoking test

It is carried out under medical supervision, initially, in the hospital. The test is carried out after 2-4 (6) weeks of elimination diet, under the condition that the symptoms are remitted.

In certain situations (acute and severe onset, untreatable diarrhea and MPC), the test is only carried out after the age of 12-18 months. Delactosed formulas are used, due to the 50% rate of association with secondary lactose intolerance. Thus, the first day 1 ml (according to some authors even one drop, the dose varying according to the severity of the disease) is applied on the hand or the lips. If there is no reaction (if a reaction occurs, the test is stopped), every 30 minutes increasing doses are administered (5, 10, 50, 100 ml etc.), until the dose according to age is reached. The subject is kept under surveillance for two more hours and then released; the test is continued at home for the next 1-2 weeks.

Treatment

If CMPA is suspected, the patient undergoes elimination diet for 2-4 weeks (with the exception of severe cases that require emergency medication). Elimination diet is suited for breast-fed, mixed-fed, artificially-fed infants as well as those who receive diversified food[19].

For breast-fed infants, the diet actually addresses the mother, who should avoid allergenic foods (milk, eggs, hazelnuts, fish, wheat) during breastfeeding. We specify that complete elimination of these foods may result in an important imbalance of the mother's diet. If the symptoms disappear, the mother will resume the intake of one of these foods every week; if the symptoms reoccur, the mother will avoid the respective food during the whole period of breastfeeding. A calcium-supplement (1000 mg/day) is also necessary for the mother, during breastfeeding.

In the case of severe atopic dermatitis and important growth faltering, natural food intake can be excluded, and the infant receives therapeutic formula.

Mixed-, artificially- and diversified- fed infants will receive therapeutic formula (which is tolerated by 90% of CMPA patients). The first choice formula is extensively hydrolyzed and contains omega 3 fatty acids, oligopeptides (smaller than 2 kDa in size); it does not contain lactose.

There are situations (rejection due to its unpleasant taste, persistency of symptoms, MPC), when use of the extensively hydrolyzed formula is discontinued, and an amino acid-based formula is introduced.

Diversification should be undertaken using the following guidelines:

- the food first introduced is rice and only after the age of 6 months;
- at the age of 7 months, vegetables are introduced (carrots, potatoes) and at the age of 8-10 months, fruits are brought in (apples, pears, bananas, peaches, plums, apricots);
- at the age of 10 months cereals are introduced (corn, rye, oats, wheat) and at the age of 12 months, meat is introduced (lamb, pork, turkey, beef);
- cow's milk is introduced only after 12 months;
- eggs are introduced after age 2-3 and hazelnuts, walnuts, fish after 3-4 years of age.

Prognosis

It is favorable, with an 80-90% rate of remission after age 3, when tolerance to CMP sets in. The probability of becoming tolerant depends on the age of the patient and the specific IgE level at the time of the diagnosis. Patients whose cutaneous or seric tests were negative develop tolerance much faster than those with positive tests.

The failure of treatment with extensively hydrolyzed formulas is due to residual allergens contained in them. There is no evidence of failure of treatment with amino acid- based formulas (if the symptoms persist during treatment, the diagnosis should be reconsidered).

Prophylaxis of CMPA

Natural food intake represents the gold standard in CMPA prevention, under the following conditions: a mixed diet should contain hypoallergenic formulas and the mother must avoid foods with allergenic potential during breastfeeding.

An artificial diet with cow's milk is recommended only after age 1, and diet diversification should be done under special circumstances (avoiding too early diversification, before the age of 4 months).

The CMPA patient must be monitored periodically (at 2 months, 4 months, 1 year, 2 years, 3 years); during these check-ups, clinical evaluation and cutaneous and seric allergic testing should be carried out.

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