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C3 and C4 complement system components as biomarkers in the intermittent atopic asthma diagnosis

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Abstract

Objective: To analyze serum C3 and C4 complement system components with a view to their possible utility as biomarkers of intermittent atopic asthma.

Methods: Serum levels of the C3 and C4 complement components were assayed in 70 children aged from 3 to 14 years and with a history of "wheezy chest." After 2 years' outpatients follow-up and after application of inclusion and exclusion criteria, the children were divided into two groups: 40 children with intermittent atopic asthma and 30 children without asthma. None of the children in either group were treated with inhaled or systemic corticosteroids or long-acting bronchodilators. The two groups had similar ages according to Student's *t* test. The C3 and C4 component test results followed a normal distribution and were therefore compared using Student's *t* test with significance set at $p < 0.05$.

Results: The results for the group with intermittent atopic asthma were significantly elevated for C3 in 85.0% of the children, for C4 in 87.5% of the children, for both C3 and C4 in 72.5% of the children, and for either C3 or C4 in 97.5% of the children, when compared with the results for the children without asthma from the same age group.

Conclusions: We observed an increase in the serum levels of the C3 and/or C4 components of the complement system in the majority of the patients with intermittent atopic asthma studied here, when compared with the results for children in the same age group without asthma. We conclude that the presence of elevated C3 and/or C4 complement components could represent a biomarker for diagnosis of intermittent atopic asthma.

J Pediatr (Rio J). 2011;87(6):512-6: Asthma, complement system, biomarker.

Introduction

Asthma affects more than 300 million people worldwide[1] and is the most common chronic disease in childhood. According to data from the Brazilian Unified Health System (Sistema Único de Saúde, SUS), in 2004 approximately 200 thousand children up to the age of 14 years were admitted to hospital with diagnoses of asthma, making it the fourth-ranked cause of admissions in the service.[2]

When SUS emergency services see patients with bronchospasm crises, they recommend outpatients follow-up at specialized services. More and more health

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professionals have been giving this advice and it has been increasingly accepted by patients. However, during the intervals between acute crises, it is not always possible to distinguish between patients with and without asthma, particularly if they have intermittent forms. As a result, we are interested in the possibility of identifying new markers for intermittent asthma.

A asthma is defined as a chronic inflammatory disease characterized by hyperreactivity of the lower respiratory tract and by variable limitation to airflow, reversible spontaneously or with treatment and manifesting clinically in the form of repeated episodes of wheezing, dyspnea, tight chest and coughing, in particular at night and in the morning on waking.[2]

The complement system consists of more than 30 plasma and cell surface proteins that interact with each other and with other immune system components. Its activity is highly regulated and it generates products that destroy infected cells or microorganisms. The products of complement system protein activation have a number of different biological effects. The anaphylatoxins and chemoattractant components C3a, C4a and C5a can contribute to inflammatory processes, recruiting leukocytes, increasing vascular permeability, stimulating bronchoconstriction and causing degranulation of mast cells.[3-7]

Studies of bronchoalveolar lavage samples from patients with asthma have detected high levels of the anaphylatoxins C3a and C5a.[8] In samples from asthma patients, variations have been observed in regions of genes that code for C3 molecules[9] and for the C3 receptor.[10] These findings suggest that activation of the complement system may be one of the factors involved in development of the respiratory dysfunction associated with the inflammatory process of asthma.

In view of recently-published findings on the role of the complement system in asthma, the significance of this disease and the difficulties involved in diagnosing intermittent asthma, the objectives of this study were to quantify C3 and C4 complement system components in the serum of children with a history of bronchospasm, classify these children as either intermittent asthma sufferers or not and compare the levels of C3 and C4 serum components between the 2 groups.

Patients and methods

This study was conducted at an Allergy Clinic in conjunction with an Immunology Laboratory. A total of 70 children aged 3 to 14 years were recruited over a 1-year period and then followed-up for 2 years. In the majority of cases, patients had been referred by emergency service because of "wheezy chest." At the start of outpatients follow-up, in addition to patient history and physical examination, the following supplementary tests were also requested: full blood test, stool parasitology, urine sedimentation test, chest x-ray, pulmonary function tests, immediate hypersensitivity skin tests (prick test), serum immunoglobulin assays and a variety of tests for differential diagnoses, depending on the patient's history and physical examination findings, such as iontophoresis, gastroesophageal reflux tests, tests for autoimmunity, serology to rule out infectious processes and purified protein derivative (PPD) tests.

Additionally, C3 and C4 serum complement component levels were assayed in serum at the start of outpatients follow-up. Before the laboratory test was performed, serum was separated and stored at -4 ºC for up to 5 days. The C3 and C4 assays were conducted using radial immunodiffusion plates (Dade Behring, Marburg, Germany) and all readings were taken by the same professional.

Once supplementary test results were in and outpatients follow-up was complete, 70 patients with a history of bronchospasm and ages ranging form 3 to 14 years were selected, excluding children who had been given immunosuppressors/transfusions/immunizations/immunotherapy during the previous 3 months, those who had had surgery recently, had associated diseases, malnutrition or bacterial or parasite infections.

Next, the 70 patients were separated into 2 groups: 40 children with intermittent atopic asthma and 30 children without asthma. Diagnoses of intermittent asthma were confirmed on the basis of patient history, physical examination and pulmonary function tests compatible with this diagnosis (rare symptoms, nighttime waking, exacerbations, but no severe exacerbations, $VEF_1 \geq 80\%$ of expected, according to the IV Brazilian Asthma Management Directive)[2]. These 40 patients also had positive immediate hypersensitivity skin test results, in line with their clinical history and a personal or family history of atopic disease, leading to a final diagnosis of intermittent atopic asthma. The other 30 children did not exhibit signs or symptoms of asthma or other atopic diseases during the 2-year follow-up period and were retrospectively diagnosed as having suffered reactive reaction bronchospasm.

Both groups were within the same age range.

In addition to these 70 children, other patients were not considered for this study, since they did not fully meet the inclusion criteria for the study. These patients had other diagnoses such as gastroesophageal reflux, congenital malformations, cardiac diseases, Löeffler syndrome and celiac disease.

None of the 70 children recruited were treated with medication during follow-up because the primary diagnostic hypothesis was intermittent asthma.

Analysis of data and ethical considerations

The complement assay results had a normal distribution. Numerical variables were evaluated using Student's *t* test. Results were defined as statistically significant when $p < 0.05$. The confidence interval for verification of sample validity was determined using Epi-Info.

This research was submitted to and approved by the Institutional Ethics Committee (protocol 207/10).

Results

Forty of the patients referred by Emergency Services because of a "wheezy chest" and followed-up for 2 years at the Allergy Clinic were diagnosed with intermittent asthma (diagnostic criteria described above) and 30 patients were retrospectively diagnosed with reaction bronchospasm.

The controlled asthma patients were distributed across both sexes, 25 males and 15 females, and had ages varying from 3 to 14 with a mean of 7 years. Fifteen of the children without asthma were female and 15 were male, with ages varying from 3 to 14 with a mean of 8 years. Age distribution was similar for the two groups with no difference according to Student's *t* test.

The results were tabulated.

Table 1 shows the arithmetic means and standard deviations for serum levels of the C3 and C4 components for the 40 atopic asthma patients and the 30 children without asthma.

The intermittent atopic asthma patients had elevated C3 and C4 component levels that were statistically significant ($p < 0.05$) in comparison with the levels observed for the children without asthma.

Table 2 lists the age of each child and their serum C3 and C4 complement component levels with means and standard deviations for both groups. It will be observed that C3 levels were elevated in 85% of the patients with atopic asthma and C4 levels were elevated in 87.5% of the asthma group, when compared with the range of normality already determined and adopted by the Immunology Laboratory. Serum C3 and C4 levels for the children without asthma were all within the preestablished range of normality.

The confidence intervals calculated for statistically elevated levels in the serum of children with asthma were from 61.8 to 90.2% for elevated C3, from 58.8 to 77.5% for elevated C4, from 44.8 to 77.5% for both elevated C3 and C4 and from 78.1 to 98.3% for either elevated C3 or C4, in relation to the levels observed for the serum of healthy children without asthma.

Table 1 - Arithmetic means (mg/dL) ± standard deviations of serum levels of C3 and C4 components of the complement system for 40 children with intermittent atopic asthma and 30 healthy children without asthma, both groups with ages varying from 3 to 14 years

	C3	C4
Asthma	143.6*±28.8	30.2*±9.8
Free from asthma	98.9±12.3	18.4±3.5

Student's *t* test ($p < 0.05$).
* Significant difference between asthma patients and control group.

Discussion

The data observed in this study demonstrate a significant increase in the serum levels of C3 and/or C4 complement components in the majority of patients with intermittent atopic asthma, when compared with the levels observed in healthy children without asthma from the same age group. The C3 and C4 levels observed in the serum of healthy children were within the preestablished range of normality used by the immunology laboratory. The confidence interval was narrow, indicating that this was a representative sample.

Several of the patients initially studied were not included in this study in an attempt to ensure that the only variable under analysis was intermittent atopic asthma.

None of the patients studied here had been given medication, which is in accordance with the treatment criteria laid down for intermittent asthma in the IV Brazilian Asthma Management Directive.[2] No other groups of asthma patients were included because they would have needed inhaled or systemic corticosteroids, and long-acting β adrenergics. This was done in an attempt to avoid possible interference with the results by medications.

The C3a and C4a fragments have marked inflammatory and hyperreactivity effects and could contribute to the pathogenicity of asthma. Elevated production of fragments C3a and C4a leads to an increase in total C3 and C4, since the "a" fractions, with the exception of C2a, have a lower molecular weight and remain free in plasma, whereas "b" fragments, once more with the exception of C2b, have greater molecular weights and continue the complement cascade: C1qrs, C4b, C2a, C3b, C5b6789.[7] Therefore, it is increases in C3a and C4a fragments that is primarily responsible for increased plasma C3 and C4 component levels. Furthermore, assaying the subfractions of C3 and C4 is expensive, making them less applicable as possible biomarkers of asthma. This is why serum C3 and C4 complement components were quantified in this study.

Sensitization and progression of asthma are influenced by the balance between the function of mesenchymal cells and components of innate and adaptive immunoresponse.[11,12] Innate response is gaining more and more attention in the

study of the pathogenesis of asthma,[13-15] in particular the role of the complement.

Najan et al.,[16] analyzed 64 patients with moderate to severe asthma aged from 1 to 12 years and found elevated C3 serum levels, assayed by radial immunodiffusion, in the majority of the patients they studied. According to these authors, it is possible that the elevated C3 levels are the result of the action of cytokines involved in asthma pathogenesis. It was also demonstrated that the cytokines IL-4 and IL-13, involved in asthma,[17,18] induce RNA expression for C3 in human epithelial cells.[19] Hasegawa et al.[10] found an association between proteins of the complement system

Table 2 - Serum levels of C3 and C4 complement components in 40 intermittent atopic asthma patients and 30 children without asthma

Asthma patients				Free from asthma			
Patient	**Age (years)**	**C3 (mg/dL)**	**C4 (mg/dL)**	**Patient**	**Age (years)**	**C3 (mg/dL)**	**C4 (mg/dL)**
1	3	130.2*	25.6*	1	3	81.6	15.1
2	3	133.9*	24.4*	2	3	104.2	14.8
3	3	121.6*	38.0*	3	4	98.1	19.6
4	3	110.3	28.3*	4	4	110.3	20.4
5	3	133.9*	22.0	5	4	110.3	20.0
6	4	140.2*	31.0*	6	4	104.2	18.2
7	4	129.0*	24.0*	7	5	72.9	15.5
8	4	180.0*	37.8*	8	5	116.5	11.8
9	4	129.0*	24.0*	9	5	90.2	15.6
10	5	135.6*	14.8	10	5	91.1	14.2
11	5	133.9*	18.5	11	6	100.5	13.6
12	5	115.5	55.5*	12	6	119.3	21.7
13	5	116.5	25.6*	13	6	98.0	23.0
14	5	146.7*	22.0	14	6	90.5	16.5
15	5	135.6*	29.6*	15	6	95.0	21.8
16	5	127.7*	28.3*	16	7	98.1	15.1
17	5	130.2*	25.5*	17	7	86.9	17.3
18	5	115.5	56.3*	18	8	85.0	18.5
19	5	187.0*	43.4*	19	8	91.9	23.3
20	6	154.0*	49.4*	20	9	84.3	13.4
21	6	135.6*	25.6*	21	9	97.2	18.7
22	7	117.0	18.5	22	10	108.2	21.0
23	7	122.8*	27.0*	23	11	101.3	21.8
24	7	135.6*	28.3*	24	12	98.1	15.1
25	7	164.7*	32.4*	25	12	98.1	17.3
26	8	194.0*	26.0*	26	13	116.5	23.1
27	8	122.8*	27.0*	27	13	109.6	21.8
28	9	142.3*	32.4*	28	14	110.5	23.7
29	9	127.7*	23.8*	29	14	78.2	22.3
30	9	135.6*	25.6*	30	14	119.8	16.5
31	10	220.8*	52.1*				
32	10	129.1*	25.6*				
33	10	118.5	24.7*				
34	11	122.8*	24.3*				
35	11	127.7*	36.6*				
36	12	194.0*	36.5*				
37	13	173.0*	25.2*				
38	14	180.0*	33.8*				
39	14	223.0*	36.5*				
40	14	154.0*	24.0*				
Mean:	7	143.6	30.2	Mean:	8	98.9	18.4
SD:	3	28.8	9.8	SD:	4	12.3	3.5

Mean = arithmetic mean; SD = standard deviation.
* Levels over the preestablished range of normality: C3 = 72.9 to 119.8 and C4 = 11.8 to 23.7 mg/dL.

and development of asthma: polymorphisms in the genes for C3, C5 and in the receptors for C3a and C5a are related with elevated IgE levels and with development of asthma in children and adults.[10] Children who live in homes with smokers have elevated serum C3 levels.[20]

Animal models have also contributed to the study of the complement in asthma. It was found that mice that are deficient in C3[21] or the C3 receptor[22] do not develop asthma. Blocking C3 receptors in mice inhibited the hyperreactivity and inflammation of airways that is seen in asthma.[23] Particulate materials, such as particles of diesel, induced C3 deposition in the bronchial epithelium of mice and bronchoalveolar lavage from mice exposed to pollutants contained high concentrations of C3.[24]

In this study we observed that the majority of patients with intermittent atopic asthma had elevated serum levels of C3 and/or C4, when compared with the normal levels observed in children of the same age group who are free from asthma. We conclude that there was an increase in C3 and C4 components in the patients who had intermittent asthma. These components could be biomarkers of intermittent atopic asthma in the patients studied here.

Considering the importance of asthma and its mortality and morbidity, more studies are needed that investigate the role that the complement system plays in the pathology of this disease.

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