Usefulness of the soluble membrane attack complex to determine dermatomyositis clinical activity and treatment response

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Abstract

Introduction: Dermatomyositis is an autoimmune disease and the most common idiopathic inflammatory myopathy. During patient follow-up, determining biochemical parameters is required in order to assess for disease activity and treatment efficacy.

Objective: To determine the relationship between the degree of activation of the complement system through the soluble membrane attack complex (C5b-9), dermatomyositis clinical activity and its variations with conventional treatment.

Method: Forty-five patients with active and inactive dermatomyositis were studied. Chemical parameters and clinical severity were established and correlated with ELISA-determined C5b-9 serum levels. **Results:** There was positive correlation between dermatomyositis cutaneous and muscular activity and C5b-9 serum levels, which was lower than with traditional biochemical markers. In the case of treatment response, C5b-9 showed significant reduction, similar to clinical severity; with biochemical parameters, the reduction was not significant at one month of treatment with systemic steroids. **Conclusions:** Serum levels of C5b-9 are higher in patients with dermatomyositis than in healthy subjects; dermatomyositis active and inactive cases were determined by means of their measurement. They can be a reliable parameter of therapeutic response, more accurate than muscle enzymes measurement, particularly creatine phosphokinase.

KEY WORDS: Dermatomyositis. Activity. Complement system. C5b-9. Creatine phosphokinase.

Introduction

Dermatomyositis is an autoimmune disease included in the group of idiopathic inflammatory myopathies.¹ It is a disease with common complications deriving from visceral involvement or from progression or exacerbation of the process.² In recent decades, attempts have been made to identify serological markers that facilitate dermatomyositis diagnosis,³ and that also assess clinical response to different treatment regimens and, as a consequence, that allow prognosis of the disease to be established.

The parameters used for diagnosis (muscular strength, muscle enzymes, histological findings and electromyography), although useful, are not sensitive or specific enough to assess treatment response,³ therefore, other options related to the pathogenesis of

the disease have been studied in order for clinical correlation to be established (for example, associated with muscle injury, with endothelial lesion, with the immune phenomenon, with cytokines, with adhesion molecules, among others); however, none has obtained a sufficient degree of correlation to replace traditionally used biochemical and electrophysiological markers.^{2,3}

Muscle damage in dermatomyositis has been observed to be related to complement-mediated microangiopathy,^{4,5} with membrane attack complex or C5b-9 deposits being described in the muscular microvasculature of patients with the disease,⁵ at different degrees of severity and with positive gradient, with its potential usefulness being translated into determining the degree of disease activity.

C5b-9 is a complex of proteins that bind to each other to form pores through the membrane, which results in cell death.⁶⁻⁸ Several studies have shown deposits of this protein complex in the skin, muscle fibers and in arterioles

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and capillaries in biopsies of patients with dermatomyositis.⁹⁻¹¹ The measurement of complement activation product levels is a measure of the catabolic state and, consequently, it is more accurate for establishing the degree of activation thereof.

So far, there are only few studies focused on the analysis of patients with dermatomyositis and, for this reason, we conducted an investigation to determine the relationship between the degree of activation of the complement system through the C5b-9 soluble complex, dermatomyositis clinical activity and its variations with the treatment.

Methods

The study was carried out between 2005 and 2011 and was approved by the Committee of Ethics in Research with registry code DI-06-109-3-61. All patients and healthy controls gave their consent to participate in the study. Forty-five patients of the female gender between 28 and 46 years of age and who met Bohan and Peter^{12,13} criteria were included:

- Group 1: patients with recent diagnosis, untreated and with active disease.
- Group 2: patients with inactive disease, with or without maintenance treatment.

A group of healthy subjects matched by age with the two previous groups in a 1: 1 ratio was included.

Patients were assessed at the moment of inclusion to the study, always by the same researcher. Disease severity was determined using data obtained from the medical records, by interrogation and physical examination using the Disease Activity Evaluation Scale (MYOACT), established by the International Myositis Assessment and Clinical Studies Group.¹⁴ The MYOACT score was recorded as muscular, cutaneous and total. Patients with overlap syndrome or dermatomyositis varieties other than type 2 were excluded.

Patients considered with inactive disease were to maintain a score of 0 on MYOACT (muscle, cutaneous and total) for at least one month. Patients who did not meet this criterion were considered to have active disease.

Clinical data were obtained when the patient agreed to participate in the study and included age, time of disease evolution and the MYOACT score in its three modalities (cutaneous, muscular and total). Blood samples were obtained (10 mL) for the measurement of creatine phosphokinase as a biochemical marker of dermatomyositis, as well as serum C5b-9. For the controls, blood samples were obtained from 45 healthy women, matched by age (26 to 45 years of age), from the hospital blood

bank. The obtained blood sample was used to determine creatine phosphokinase and C5b-9 serum concentration by the ELISA technique, using a commercial kit of human serum C5b-9 (MyBiosource, San Diego, CA, USA). All samples, both from cases and controls, were simultaneously analyzed and in duplicate, with patient clinical status and identity being unknown. The definitive value of levels for each case was obtained by calculating the mean of the two obtained values.

After the sample was taken in patients with active disease, treatment was started with prednisone at 1 mg/kg/day for 4 weeks, with new blood sample collection.

Statistical analysis was carried out with Fisher's exact test for categorical variables' comparison and Mann-Whitney rank test for comparison of numerical variables. Correlation coefficients were established with Spearman's correlation. To compare quantitative variables averages, Student's t-test (between groups) and paired t-tests (before and after) were used. Data were analyzed with SPSS, version 22.0. A p-value < 0.05 was considered to be statistically significant.

Results

Patient clinical and biochemical characteristics are presented in table 1. Overall mean disease duration was 8 months (range: 2 to 17 months). At study inclusion, 28 patients had active disease (group 1) and 17 inactive disease (group 2).

Serum C5b-9 concentration values, as well as creatine phosphokinase levels are shown in table 1. Significant differences are observed in C5b-9 levels between cases with active disease and those with inactive disease ($p = 0.008$) and controls ($p = 0.001$) (Fig. 1). Creatine phosphokinase levels also showed significant differences between the three groups.

Serum C5b-9 correlation coefficients with disease severity yielded a positive correlation, not so with creatine phosphokinase levels; similarly, the latter parameter showed low correlation with clinical severity (Table 2).

Twenty-eight patients newly diagnosed with dermatomyositis were treated with prednisone at 1 mg/kg/day. Serum C5b9 levels at the beginning of the study (67.45 ± 18.35) were significantly higher than those measured at the conclusion of treatment (17.33 ± 8.43 , $p = 0.001$) (Fig. 2). Creatine phosphokinase levels did not show significant reduction at one month of treatment, although clinical parameters of activity (MYOACT) had a significant reduction after treatment with systemic steroids (Table 3).

Table 1. Clinical characteristics of patients with dermatomyositis

	Active disease (n = 28)		Inactive disease (n = 17)		p
	n	%	n	%	
Clinical characteristics					
Interstitial pulmonary disease	5	18	0	0	0.001
Dysphagia	18	64	0	0	0.001
Raynaud's phenomenon	8	28	0	0	0.001
Mechanic's hand	12	43	0	0	0.001
Arthritis	6	21	0	0	0.001
	Mean±SD		Mean±SD		
Demographic data					
Age at onset (years)	34.16 ± 15.23 (21-66)		32.33 ± 14.55 (19-65)		ns
Duration of symptoms until the moment of the study (months)	8.12 ± 6.56 (2-17)		12.55 ± 8.85 (4-16)		ns
Creatine phosphokinase levels at the moment serum C5b-9 was measured	1675 ± 1375		1.33 ± 0.85		0.001
MYOACT disease activity score					
Total	6.5 ± 2.25		0		0.001
Muscular activity	6.3 ± 1.85		0		0.001
Cutaneous activity	4.3 ± 1.55		0		0.001

MYOACT = Myositis Disease Activity Assessment, SD = standard deviation, ns = not significant.

Figure 1. sC5b-9 serum levels in patients with dermatomyositis with active and inactive disease and in healthy subjects.

Discussion

C5b-9 serum levels in patients with dermatomyositis were higher than in healthy subjects; in addition, determining dermatomyositis active and inactive cases was possible with this parameter. Another important aspect is that serum C5b-9 can be a reliable parameter of therapeutic response, even more accurate than measuring muscle enzymes, particularly creatine phosphokinase.

Elevated serum C5b-9 levels in patients with dermatomyositis confirm complement activation in the pathogenesis of

the disease and tend to be higher in patients with active rather than inactive disease and in healthy controls. In a previous work by Campo et al.,¹¹ C5b-9 serum levels were observed to be correlated with disease severity, although the results didn't allow to evaluate treatment response, even when it is possible to distinguish patients with active and inactive disease. In our study, in addition to observing a good correlation between clinical activity and biochemistry of the disease and the marker, as well as its ability to distinguish between active and inactive disease, it was useful to assess treatment response, and thus it could be useful to determine the prognosis.

Table 2. Correlation between C5b-9 serum levels, creatine phosphokinase and clinical markers in patients with dermatomyositis

	C5b-9 levels	Creatine phosphokinase	Total MYOACT
C5b-9 levels	1	0.122, $p = 0.063$	0.433, $p = 0.001$
Creatine phosphokinase	0.122, $p = 0.063$	1	0.231, $p = 0.05$
Total MYOACT	0.433, $p = 0.001$	0.231, $p = 0.05$	1

Correlations were performed with Pearson's test; statistical significance with $p < 0.05$. MYOACT = clinical markers.

Table 3. C5b-9 serum concentration, creatine phosphokinase and MYOACT before and after one-month treatment with systemic steroids in patients with dermatomyositis

Variable	Before treatment	One month after treatment	p
C5b-9 serum levels (ng/mL)	67.45 $\pm$ 18.35	17.33 $\pm$ 8.43	0.001
Creatine phosphokinase (mg/dL)	1675 $\pm$ 1375	1427 $\pm$ 1239	0.049
Total MYOACT	6.5 $\pm$ 2.25	1.7 $\pm$ 1.3	0.001

MYOACT = Myositis Disease Activity Assessment

Figure 2. sC5b-9 serum levels after one month of treatment with systemic steroids.

C5b-9 is an inflammatory mediator related to the pathophysiology of many diseases. Evidences show that C5b-9 is an indicator of clinical severity of diseases it has been associated to, including systemic lupus erythematosus and dermatomyositis and, therefore, blocking this inflammatory mediator in many inflammatory disease models contributes to reduce its severity.¹⁵

In our study, we observed a positive correlation between C5b-9 serum levels and signs of disease activity and, similar to observations reported by Campo et al.,¹¹ this complement activation parameter showed no correlation with traditional activity markers, specifically creatine phosphokinase.

In dermatomyositis, muscle enzymes are released as a result of muscle fiber lesion, and thus the temporal correlation between their increase and the development of muscle weakness is important. In C5b-9 serum levels,

elevation can precede muscular activity and possibly cutaneous activity, and its levels are maintained during disease activity and are correlated with severity.

The primary antigenic target in dermatomyositis is the endomysium capillaries endothelium. These antibodies elicit C3 activation, which in turn causes the formation of C3b, C4b and of C5b-9 fragments, which are detectable in the course of the disease both in serum and the capillaries, and are even observed in muscle tissue prior to inflammatory or structural changes. These deposits are important to induce muscle fiber destruction.¹⁶

Another mechanism to explain the relationship between C5b-9 and the development of dermatomyositis is based on membrane attack complex activation, which causes an excess of Ca^{2+} that gives rise to mitochondrial overload and mitochondrial transmembrane potential loss, thus resulting in an energy crisis within the cells, while ion pumps try to

reestablish Ca^{2+} homeostasis; cytochrome C release into the cytoplasm and caspase activation trigger apoptosis. This suggests the crucial role of C5b-9 in NALP3 inflammasome activation, by means of which it amplifies the inflammatory response.^{17,18} The NLRP3 inflammasome plays a key role in the development of dermatomyositis,¹⁹ which demonstrates that C5b-9 activation may precede the development of clinical, biochemical and immune manifestations of the disease.

One limitation of our study is that it was not possible to compare C5b-9 serum levels with skin and muscle deposits thereof, which is why our study could serve as a basis for others to assess whether such a correlation exists. We assume it does, since, in previous studies, muscle biopsies of patients with dermatomyositis were observed to show C5b-9 deposits in endothelial cells.²⁰ In addition, C5b-9 deposits were observed in injured skin biopsies of patients with the disease, not so on unaffected skin, suggesting that it would also be involved in the pathogenesis of skin lesions.²¹

On the other hand, given that this study included one single center, the findings require confirmation in other hospitals.

Current treatments against the complement, especially eculizumab, specifically targeting C5b-9 or alternate pathways, are under development.¹⁵

In conclusion, complement system activation product levels quantification is a tool for clinical control of patients with dermatomyositis, since it allows to distinguish between active and inactive disease and adequately correlate with treatment response.

On the other hand, it is necessary to determine whether C5b-9 serum levels might serve to distinguish other types of myositis.

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