Mini Review

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The Important Roles of C4d in Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of various autoantibodies and the injury of multiple systems. It is well known that the complement system plays an important role in the pathogenesis of SLE. Complement activation product C4d is a marker of the classical complement activation and hence can be used to reflect the tissue injury as well as therapeutic effects. Besides its important role in antibody-mediated rejection, C4d has been increasingly recognized as a potential biomarker in SLE. Here we will summarize the recent advances of C4d in SLE.

Keywords: Complement; C4d; Systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is one of the most clinically and serologically diverse autoimmune diseases. Currently, the diagnosis of SLE is mainly based on the classification criteria proposed by American College of Rheumatology [1,2]. The European league against rheumatism has proposed some useful recommendations for monitoring patients with SLE in clinical practice and observational studies [3]. Traditionally the presence of anti-double stranded DNA (anti-dsDNA) and hypo-complement in serum have been used to diagnose SLE, and their quantitative changes have been further used to reflect the disease activity [4,5]. However, some researchers found them less useful [6-8].

Complement system activation has linked more intimately to autoimmune diseases and been playing a great role in the pathogenesis of SLE [9,10]. Complement activation products may be also correlated with SLE. C4d, an ultimate product during complement C4 activation, with unknown function, had been recognized as a biomarker for its stability and strong association with antibody-mediated rejection in the 1990s [11]. In the last twenty years, the potential importance of C4d in SLE has been gradually understood (Table 1).

Type of C4d	Sample source	Potential clinical applications	References
Peripheral free C4d	Plasma	Monitor disease activity; predict disease flare	[12,13]
	Serum	Monitor disease activity	[14]
	Plasma	No correlation with SLE during pregnancy	[15]
C4d binding to blood cells	E-C4d	Diagnose SLE; monitor disease activity	[18,20,31]
	R-C4d	Monitor disease activity	[24]
	P-C4d	Diagnose SLE; monitor disease activity; marker for venous thrombosis in SLE; associated with all-cause mortality and ischemic stroke	[25-28]
	T/B-C4d	Diagnose SLE; monitor disease activity	[29-31]
C4d deposition in tissues	Placentas	Adverse events during pregnancy	[35-37]
	Renal tissue	Related to the presence of microthrombosis and histopathological classification of lupus nephritis	[38,42]
		No relationship with disease activity, histological activity and chronicity of lupus nephritis	[41,43,44]

Table 1: The clinical applications of C4d in SLE. E-C4d, erythrocyte-bound C4d; R-C4d, reticulocyte-bound C4d; P-C4d, platelet-bound C-4d; T/B-C4d, T/B lymphocyte- bound C4d.

Peripheral Free C4d in SLE Patients

As early as 1988, plasma levels of complement split product C4d were determined by laser nephelometric technique by Senaldi G et al. They found that C4d levels and C4d/C4 ratios were elevated in SLE patients compared to controls and furthermore C4d levels and C4d/C4 ratios were also correlated with lupus disease activity. The same trend was observed with C3d and C3d/C3, however failing to discriminate between patients with mild activity and moderate/severe activity. Nevertheless, C4 and C3 showed no correlation with disease activity in this study [12]. A prospective study of 86 SLE patients showed that the mean levels of complement split products, Ba, Bb, C4d, C5b-9 were significantly higher in patients with the very severe disease activity than those in patients with mild or moderate disease activity. Elevated C4d was most sensitive to subsequent flare form the analysis of the disease course in SLE patients with different stages of diseases activity [13]. Serum C4d detected by quantitative microassay plate enzyme immunoassay has also been shown to be more sensitive than C3, C4 and C5b-9 in lupus patients with moderate-to-severe disease activity [14].

Some researchers demonstrated that complement split products Ba, Bb and SC5b-9, but not C4d were significantly elevated in pregnant SLE patients with flared SLE compared to patients with stable SLE during pregnancy [15]. Interestingly, a study of healthy women at various stages of normal pregnancy showed that C4d concentrations were significantly increased in pregnant women than in non-pregnant women, accompanied by a significant reduction in C4 [16]. Ba can also be elevated in non-SLE patients with preeclampsia. However normal CH50 had the merit of distinguishing non-SLE patients and SLE patients. During the course of normal pregnancy in some SLE patients, a decline in serum C3, C4, and CH50 may occur. Whether such falls represent decreases in the overall synthesis of complememt or activation was not sure. Concurrent elevations of complement split products may possess the value of differentiating a lupus flare from non-SLE disease of pregnancy [15].

C4d Binding to Blood Cells in SLE Patients

Proteolytic fragments generated from complement activation contain thioester bonds and are capable of attaching to circulating cells [17]. For the past decade, researchers have finished a series of studies on complement activation products, especially C4d binding to circulation cells, such as erythrocytes, reticulocytes, platelets and lymphocytes. Erythrocyte-bound C4d (E-C4d) and complement receptor 1 were simultaneously detected by flow cytometry in SLE patients, patients with other diseases and healthy controls. Significantly high levels of E-C4d and low levels of erythrocyte-bound complement receptor 1 were found in SLE patients. Combined measurement of the above two molecules had high diagnostic sensitivity and specificity for lupus [18]. But there were some reports with the opposite conclusions. High expression of E-C4d and E-CR1 were also found in patients with primary antiphospholipid syndrome [19].

In a multivariable analysis of patients with SLE, E-C4d was significantly associated with the Systemic Lupus Activity Measure (SLAM) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) after adjusting for serum C3, C4 and anti-dsDNA antibodies [20]. Study on E-C4d from Taiwan demonstrated the same results and found that E-C4d had limited value in monitoring disease activity in SLE patients with hemolytic anemia [21].

Deposition of complement fragments C4d on erythrocytes *in vitro* can lead to significant decrease in both membrane deformability and flickering of erythrocytes. Calcium-dependent cytoskeletal changes and production on nitric oxide in red blood cells were observed in C4d-decorated RBCs [22,23].

One longitudinal observation showed that levels of C4d on reticulocytes were changed in relation to the clinical course in individual patients and may serve as biomarkers of disease activity in patients with SLE [24].

C4d can also deposit on platelet surface. Level of platelet-bound C4d (P-C4d) was found to be highly specific for SLE and significantly associated with positivity for lupus anticoagulant and anticardiolipid antibodies. P-C4d was also associated with SLEDAI [25]. Increased complement deposition on platelets was observed in SLE patients with a history of venous thrombosis compared to those without. Some vitro studies also demonstrated increased P-C4d deposition in the serum from patients with lupus coagulant, venous thrombosis or antiphospholipid (aPL) antibody syndrome increased as compared to those without this manifestation [26].

The deposition of C4d on platelets may lead to platelet activation. It was proven by significant increases in 14C-serotomin release assay reflecting platelet activation [27]. Furthermore, the deposition of C4d on platelet was also associated with the presence of IgG aPL, IgG anti-β2 glycoprotein 1 antibodies and arterial thrombotic events in SLE patients with positive aPL [27].

Kao AH reported that P-C4d was associated with all-cause mortality (hazard ratio 7.52, 95% confidence interval (CI) 2.14-26.45, p=0.002) and ischemic stroke (odds ratio 4.54, 95% CI 1.63-12.69, p=0.004) in patients with SLE. SLE Patients with positive P-C4d were more likely to have vascular events compared to those with negative P-C4d (35.7 vs. 18.2%, p=0.001) [28]. Besides, P-C4d is associated with acute ischemic stroke and stroke severity [29]. Therefore, P-C4d may be used as a prognostic biomarker to identify a subset of SLE patients with a worse clinical prognosis.

T/B lymphocyte-bound complement activation products have been reported to be as biomarkers for diagnosis of SLE, mainly in Caucasian and Chinese patients. T-C4d and B-C4d showed high diagnostic sensitivity and specificity for SLE [30,31]. T-C4d and B-C4d were also significantly associated with SLE disease activity as measured by SLEDAI [31]. A multicenter cross-sectional study showed that an assay panel combining anti-dsDNA, ANA, anti-MCV, E-C4d, B-C4d was more sensitive and specific for the diagnosis of SLE [32].

C4d Deposition in Tissues in SLE Patients

Murine model of antiphospholipid syndrome demonstrated that complement activation played an important role in fetal and placental injury in the presence of antiphospholipid antibodies (APAs) [33-35]. In human patients with positive APAs, C4d also demonstrated a similar association with tissue injury. There was evidence showing increased C4d deposition in the trophoblast cytoplasma, trophoblastic cell and basement membrane, and extracillous trophoblasts in patients with APAs compared to control patients [36]. Diffuse C4d staining at the feto-maternal interface was present almost exclusively in patients with SLE and/or APS and was related to intrauterine fetal death [37]. Dramatically increased immunohistochemistry staining of C-4d was statistically associated with low-placental weights and low birth weight in SLE patients [38].

Presence of C4d in murine and human placenta is strongly related to adverse fetal outcome in the setting of SLE and APS. C4d might be utilized as a biomarker to predict the autoantibody-mediated fetal loss in SLE and APS and evaluate the subsequent risk for intrauterine growth restriction and disease control during the gestation period in these patients.

C4d is now one of the core diagnostic parameters to identify antibody-mediated rejection. The deposition of C4d in lupus nephritis was different from that in antibody-mediated renal rejection. A small proportion of LN patients (6.81%) with positive C4d on peritubular capillaries showed higher SLEDAI score and higher frequency of hypocomplementemia compared to those with negative C4d. In contrast with acute antibody-mediate rejection, the staining pattern of C4d was granular deposition and the detection of C4d along peritubular capillaries was accompanied by deposition of IgG and C1q or C3 [39].

The intensity of glomerular C4d staining was found to be significantly related the presence of microthrombosis and the detection rate of IgG anti-β2GPI antibodies [40]. C4d deposition was not correlated with serum aPL status [41].

A recent study showed that glomerular C4d deposition was correlated with the histopathological classification of lupus nephritis. A diffusely intense and coarsely granular pattern of C4d deposition in all glomeruli was detected in class V membranous lupus nephritis [42]. The higher proportion of glomerular C4d staining was related with higher activity index score [43]. However several studies showed that glomerular C4d deposition was correlated with neither disease activity of SLE nor histological activity and chronicity of lupus nephritis [42,44,45] .

Conclusions

C4d binding to circulating cells may be of merit of diagnosing and monitoring SLE. Platelet-bound C4d may predict the thrombosis risk in SLE patients. The modified function of C4d-bounded blood cells in SLE patients needs to be further investigated. C4d deposition in placenta was related with antibodies-mediated tissue injury and adverse pregnant outcome. C4d deposition in renal biopsy samples could be a parameter for membranous glomerulonephritis and a biomarker to identify patients with lupus nephritis who are at risk of thrombotic microangiopathy. The use of C4d has been more and more applied in SLE and we expected stronger evidence in future.

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