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**Increased C1q, C4 and C3 deposition on platelets in patients with systemic lupus erythematosus – a possible link to venous thrombosis?**

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## Abstract

**Objective:** Patients with systemic lupus erythematosus (SLE) have an increased risk of developing vascular diseases (VD) such as myocardial infarction, stroke and venous thrombosis, which can only partly be explained by traditional risk factors. The role of platelets in this process has not been extensively studied. Platelet activation support complement binding to the platelet surface, and increased C4d has been seen on platelets in SLE patients as well as in non-rheumatic patients with stroke. In this study we investigated *in vivo* platelet deposition of the classical complement pathway components C1q, C4d and C3d in relation to VD in SLE patients. Furthermore, the ability of serum to support *in vitro* complement deposition on fixed heterologous platelets was analyzed. **Methods:** Blood from 69 SLE patients and age- and sex-matched healthy individuals was collected in sodium-citrate tubes and platelets isolated by centrifugation. Complement deposition on platelets was detected by flow cytometry. **Results:** We could demonstrate that SLE patients had increased C1q, C3d and C4d deposition on platelets as compared to healthy controls ( $p < 0.0001$ ). SLE patients with a history of venous thrombosis had increased complement deposition on platelets as compared to SLE patients without this manifestation ( $p < 0.05$ ). *In vitro* studies demonstrated that serum from patients with lupus anticoagulant, venous thrombosis or the antiphospholipid antibody syndrome supported increased platelet C4d deposition *in vitro* as compared to SLE patients without these manifestations ( $p < 0.05$ ). Our data support the hypothesis that platelet activation and the subsequent complement deposition on platelets are central in development of venous thrombosis in SLE. **Conclusions:** Altogether we suggest that complement deposition on platelets could reflect important pathogenetic events related to the development of venous thrombosis in SLE and might be used as a marker for venous thrombosis in SLE.

Key words: systemic lupus erythematosus, platelet, complement, cardiovascular disease, venous thrombosis

## Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by inflammation in several organ systems and increased risk in the development of vascular diseases (VD) such as myocardial infarction (MI), stroke and venous thrombosis<sup>1</sup>. The increased risk for VD is not solely explained by traditional risk factors, but clearly also SLE-related factors are present. Venous thrombosis in SLE is often associated with the presence of antiphospholipid (aPL) antibodies<sup>2</sup>. Complement activation seems to play an important role in the pathogenesis of aPL-syndrome (APS) since C3- and C5-deficient mice are protected against APS<sup>3</sup> and treatment with anti-C5 antibodies could prevent APS in mice<sup>4</sup>. Furthermore, even in humans, C2-deficient individuals with anti-cardiolipin antibodies seem to be protected from venous thrombosis<sup>5</sup> suggesting a role for classical pathway complement activation in APS-mediated thrombosis formation. We and others have demonstrated that SLE patients have increased platelet activation, which could contribute to the increased risk for both venous and arterial VD<sup>6,7</sup>. Surface expression of different complement components, including C1q, C4 and C3, could be seen on platelets in certain human diseases as well as in *in vitro* studies<sup>8-13</sup>. Several different hypotheses of how complement components interact with platelets have been proposed, and most of the theories require platelet activation. Common activators of platelets include immune complexes, which are frequently seen in SLE patients, shear stress due to atherosclerosis and perhaps inflammatory cytokines including interferon (IFN)-alpha<sup>6, 12, 14</sup>. Platelet C1q deposition has been described *in vitro* upon platelet activation and it has been suggested that C1q binds directly to chondroitin sulphate<sup>8</sup>. Platelet

C4d deposition has been described in SLE<sup>11</sup> and also in patients with acute ischemic stroke without rheumatic disease<sup>10</sup>. Recently, Peerschke *et al* demonstrated that complement fixation *in vitro*, especially C4d, on immobilized heterologous platelets is increased in SLE patients with arterial thrombosis<sup>13</sup>. C3 has been described to bind to the platelet surface without proteolytic activation<sup>9</sup>, and another study demonstrated that C3b could interact with P-selectin and activate the complement system<sup>15</sup>. Thus, there might be several different mechanisms of how complement components could bind to platelets.

In this study we have investigated *in vivo* platelet deposition of the classical complement pathway components C1q, C4d and C3d in relation to VD in SLE patients. Furthermore, to investigate if platelet and complement activation is a prerequisite for complement deposition on platelets, the ability of serum to support *in vitro* complement deposition on fixed heterologous platelets was analyzed. We could demonstrate that SLE patients had increased levels of C1q, C3d and C4d on their platelets *in vivo* and this was especially pronounced in patients with a history of venous thrombosis. *In vitro* platelet C4d deposition was not increased in SLE patients in general when compared with healthy controls. However, within the SLE cohort, increased C4d deposition was observed in patients with APS, lupus anticoagulant (LAC) or venous thrombosis. Altogether we suggest that complement deposition on platelets could reflect important pathogenetic events related to the development of venous thrombosis in SLE and might be used as a novel marker for venous thrombosis in SLE.

## Methods

### *Patients*

SLE patients (n=69) were recruited during their normal visit to the clinic and a selection of patients with a history of VD was made with 45% of the patients having a history of VD as been described previously <sup>6</sup>. VD is defined as a history of either MI, arterial thrombosis (12/13 with cerebrovascular incidents), or venous thrombosis (pulmonary embolism or deep venous thrombosis) as defined by the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index <sup>16</sup>. Disease activity was assessed using SLEDAI-2K <sup>17</sup>. Controls (n=69) were healthy age- and sex-matched volunteers of which none had a history of VD. All the patients fulfilled at least 4 American College of Rheumatology (ACR) classification criteria for SLE <sup>18</sup>. The following SLE treatments were used at the time of blood sampling: hydroxychloroquine (n=46), azathioprine (n=19), mycophenolatmofetil (n=6), rituximab (n=1 within last 12 months), methotrexate (n=6), cyclophosphamide (n=3), cyclosporine A (n=2), non-steroidal anti-inflammatory drugs (n=12), acetylsalicylic acid (n=13), warfarin (n=24). Complement proteins and autoantibodies were measured according to routine analyses at the Department of Clinical Immunology and Transfusion Medicine, LabMedicin Skåne, Lund, Sweden. For further patient characteristics, see Table 1. The study was approved by the Regional Ethics Board in Lund, Sweden (LU 378-02). An informed consent was obtained from all participants.

### *In vivo complement deposition on platelets*

Blood was collected in sodium-citrate tubes (BD Biosciences Pharmingen, Franklin Lakes, NJ, USA) and used within 15 minutes. Platelet rich plasma (PRP) was obtained by centrifugation (280 g 10 minutes), and the plasma was immediately mixed with 10 mM

EDTA to prevent any complement activation during the isolation process. The PRP was centrifuged at 1125 g for 10 minutes and resuspended in 500 µl HEPES-buffer (145 mM NaCl, 5 mM KCl, 10 mM HEPES, pH 7.4). The platelets, 4 µl, were incubated with anti-C1q-FITC (Dako, Glostrup, Denmark) or antibodies against C3d, C4d, C3a and C4d neo (Quidel, San Diego, CA, USA) in HEPES-buffer at a total volume of 50 µl for 40 minutes at room temperature. For detection of C3 and C4 fragments, the platelets were washed once and incubated with FITC-conjugated rabbit anti-mouse IgG antibodies (Dako) for an additional 30 minutes at 4°C. The incubation ended with the addition of 500 µl 0.2% paraformaldehyde. The platelets were diluted 1/5 in PBS before analyzed by flow cytometry (Epics XL-MCL, Beckman-Coulter, Fullerton, CA, USA). An antibody isotype control was used as a negative control with a cut-off value of 2% positive platelets.

#### *In vitro complement deposition on platelets*

Platelets were isolated as described above and fixed with 2% paraformaldehyde for 10 minutes at room temperature. No attempts were made to inhibit platelet activation during platelet isolation. Experiments were also performed with non-fixed platelets with similar results, but due to extensive clotting in those samples, fixed platelets were used for all *in vitro* experiments. The platelets were washed and resuspended in Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl<sub>2</sub>, 0.36 mM NaH<sub>2</sub>PO<sub>4</sub>, 12 mM NaHCO<sub>3</sub>, 2 mM CaCl<sub>2</sub> and 5.5 mM glucose, pH 6.5) and incubated with serum (1/10) in Tyrode's buffer for 1 hr at 37°C. The platelets were washed in PBS and incubated with an antibody against C4d for 30 minutes at 4°C, washed once and then incubated with a FITC-conjugated rabbit anti-mouse IgG antibody for another 30 minutes at 4°C. In some experiments antibodies directed against C1q, C3a, C3d and C4d neo were used. The samples were analyzed by flow cytometry (Accuri C6, Accuri Cytometers, St Ives, United Kingdom).

### *Measurement of immune complexes*

ICs were measured as described previously<sup>6</sup>. Briefly, microtiter plates were coated with human C1q (10 µg/ml) and incubated at 4°C overnight. The plates were washed in PBS and blocked for 2 hrs at room temperature with 1% (wt/vol) gelatin in PBS and incubated with serum at 37°C for 1 hr and then at 4°C for 20 hrs. After the wash step, an alkaline phosphatase-conjugated goat anti-human IgG antibody (Sigma-Aldrich St. Louis, MO, USA) was added and incubated for 1 hr at 4°C. The phosphate substrate (Sigma-Aldrich) was added after a wash step, and the absorbance was read at 405 nm in a Wallac 1420 Multilabel Counter (PerkinElmer, Waltham, MA, USA)). Heat-aggregated IgG was used as a positive control.

### *Statistics*

Correlations were determined by Spearman's correlation test and the Mann-Whitney U-test was used for group comparisons. All *p*-values were considered significant at *p*<0.05.

## **Results**

### *Increased complement deposition on platelets in SLE*

One aim of this study was to evaluate whether components of the classical pathway could be detected on platelets from SLE patients *in vivo*. In our SLE cohort, the patients had statistically significant increased amount of C1q, C3d and C4d on their platelets as compared to healthy age- and sex-matched controls (*p*<0.0001 for all analyses, Figure 1). The simultaneous presence of C1q, C4 and C3 on platelets indicates activation of the classical complement pathway. Furthermore, there was a strong correlation between C3d and C4d



deposition ( $r=0.63$ ,  $p<0.0001$ ) and between complement activation fragment C3dg in serum and platelet C3d deposition ( $r=0.40$ ,  $p=0.001$ ), suggesting that the classical pathway of the complement system was indeed activated.

In our cohort, 48% of the patients were regarded as positive for C4d on their platelets and only 4% of the healthy controls were positive. The cut-off for a positive C4d value was calculated as the mean + 2 standard deviations of the healthy controls. The complement deposition was not associated with any treatments or disease activity measured as SLEDAI. Thus, complement deposition of C1q, C4d and C3d was increased in SLE and almost half of the SLE patients had increased complement deposition on platelets as compared to healthy controls.

#### *Activated complement components on platelets from SLE patients*

Naïve complement components have been described to bind directly to activated platelets<sup>9</sup>, but there are also studies supporting the idea of complement activation on activated platelets<sup>8</sup>. To investigate if the complement deposition on platelets seen in SLE was due to complement activation, antibodies directed against neo epitopes were used. The antibody directed against activated C4d (C4d neo) gave high fluorescence on platelets from an SLE patient (Figure 2C). Furthermore, staining of C3d-containing C3 molecules was seen, but the C3a epitope, the first to be cleaved once activated, was not detected indicating that the C3 molecule was also activated (Figure 2D and E). Thus, the complement components deposited on platelets in SLE patients had been proteolytically cleaved and activated through the classical pathway.

#### *Complement deposition is associated with increased platelet activation*

Complement components can be deposited on platelets upon platelet activation *in vitro* by shear stress, thrombin receptor activating peptide or ICs<sup>8, 12, 14</sup>. Increased platelet activation has been demonstrated in SLE by us and others<sup>6, 7, 19</sup>, but the possible association between platelet activation and *in vivo* complement deposition has not been investigated. In our patient cohort the platelet activation marker CD69 was correlated with platelet C3d and C4d deposition ( $r=0.41$ ,  $p=0.001$  and  $r=0.34$ ,  $p=0.004$ , respectively), a finding compatible with involvement of platelet activation in complement deposition on platelets. Furthermore, complement deposition was inversely correlated to the amount of platelets in the circulation (C1q:  $r=-0.34$ ,  $p=0.005$ ; C3d:  $r=-0.32$ ,  $p=0.008$  and C4d:  $r=-0.25$ ,  $p=0.04$ ), indicating that complement activation lead to the destruction or removal of the platelets. Immune complexes (ICs) are increased in SLE patients and could activate the classical pathway of the complement system as well as platelets<sup>14</sup>. However, no correlation between the levels of circulating ICs and complement deposition on platelets was seen. In summary, platelet activation might precede complement deposition on platelets with subsequent platelet destruction or removal from the circulation. Furthermore, the increased platelet activation seen in SLE might partly explain the increased *in vivo* complement deposition seen on platelets in SLE patients.

#### *Increased complement deposition on platelets in SLE patients with venous thrombosis*

Platelet C4d deposition has been described in non-rheumatic patients with acute ischemic stroke<sup>10</sup>. To investigate whether complement deposition on platelets is associated with VD in SLE patients, the patient cohort was divided into subgroups of patients with MI, arterial or venous thrombosis and no VD. SLE patients with a history of venous thrombosis had increased platelet C1q, C3d and C4d deposition ( $p<0.05$ ) whereas patients with arterial thrombosis (12/13 with stroke) or myocardial infarction did not (Figure 3). Only a trend of an

increased platelet C1q, C3d and C4d deposition was seen for patients with APS ( $p=0.16$ ,  $p=0.07$ , and  $p=0.07$ , respectively) probably due to the limited number of patients included in this study. However, patients with lupus anticoagulant (LAC) had increased C4 deposition ( $p=0.04$ ), which did not reach statistical significance for C1q and C3d (data not shown). Altogether, we found that SLE patients with a history of venous thrombosis, especially if combined with LAC, had increased complement deposition on platelets, suggesting that complement deposition on platelets is associated with development of VD and might be a potential biomarker of venous thrombosis in SLE.

#### *In vitro complement deposition on platelets is not increased in SLE patients*

Complement activation on platelets are thought to depend on the binding of C1q to chondroitin sulphate or phosphatidylserine on activated platelets. This is in concordance with our data showing a correlation between the platelet activation marker CD69 and complement deposition on platelets. To investigate whether serum from SLE patients had an increased ability to support complement activation on activated platelets as compared to serum from healthy individuals, the ability of serum to support *in vitro* complement deposition on fixed activated heterologous platelets was analyzed. Serum from a healthy individual supported platelet deposition of both C1q and C4, but not C3 deposition (Figure 4). Addition of EDTA, an efficient inhibitor of complement activation, inhibited the C4d deposition on the platelets but did not affect the binding of C1q. Furthermore, the C4d neo antibody only recognizing activated C4, bound to the platelets demonstrating that the complement system was activated on fixed platelets (Figure 4F). Since no platelet C3d deposition was seen *in vitro*, possibly due to the presence of complement regulators, only C4d deposition was analyzed on the platelet surface in the following experiments. Serum from SLE patients and healthy controls supported C4d deposition on heterologous platelet from a healthy donor and there was no

difference between these groups ( $p=0.46$ , Figure 5A). Similar results were seen when only using the C4-sufficient SLE patients demonstrating that even in the presence of normal complement levels SLE patients did not support increased complement deposition (data not shown). No correlation was seen between the *in vivo* and the *in vitro* C4d deposition ( $r=0.17$ ,  $p=0.15$ ). Thus, serum from SLE patients and healthy individuals had the same capacity to support complement activation on activated heterologous platelets.

*In vitro complement deposition on platelets is increased in SLE patients with a history of LAC, APS or venous thrombosis*

Even though our findings support the hypothesis of platelet activation-dependent complement activation, other factors, such as autoantibodies directed against platelets or phospholipids might amplify the platelet activation in SLE patients<sup>20-22</sup>, and potentially also lead to increased complement activation on the platelets. We observed that patients with a history of LAC supported increased *in vitro* complement deposition on platelets ( $p=0.03$ , Figure 5A). Furthermore, sera from patients with a history of APS or venous thrombosis (13/20 with APS) also supported increased C4d deposition on platelets as compared to patients without those manifestations ( $p<0.05$ , Figure 5A), as well as compared to healthy controls, even though not statistically significant ( $p=0.06$ ). In our patient cohort, a history of aCL antibodies was not sufficient to increase the complement activation ( $p=0.31$ , Figure 5A). Thus, we could conclude that LAC and aPL antibodies are associated with an increased ability to support C4d deposition on activated platelets, and suggests that this might be a mechanism operating in SLE leading to venous thrombosis and APS.

## Discussion

Lately, increasing attention has been given to the role of platelets in the development of vascular disease (VD) in SLE. We and others have demonstrated that platelets from patients with SLE are activated, which could contribute to the increased risk for VD<sup>6, 19</sup>. Platelet activation could lead to binding of C1q and C3 to the platelet surface<sup>8, 9</sup>. If the complement activation proceeds, the membrane attack complex will be formed and cause subsequent microparticle formation<sup>23</sup>. These particles are increased in SLE and are potent inducers of thrombin generation and could play an important role in the development of VD<sup>24</sup>. Platelet C4d deposition is increased in SLE patients as well as patients with stroke without any rheumatic disease<sup>10, 11</sup>. However, it is not known whether complement binding on platelets is associated with VD in SLE. The aim of this study was to investigate whether complement deposition on platelets could be a novel biomarker for MI, stroke or venous thrombosis in SLE, and to better understand the underlying mechanisms behind complement binding to platelets in SLE.

In this study, we could clearly demonstrate increased platelet C4d in SLE patients in accordance with the study by Navratil and co-workers<sup>11</sup>. However, our results show that a much higher percentage of SLE patients were positive for C4d deposition (48%) compared to the previous study (18%)<sup>11</sup>. Even though the patient cohorts were similar with regard to disease activity and age, our patient cohort was selected to have a high frequency of vascular disease, which might explain some of the differences.

Platelet C4d is increased in SLE patients<sup>11</sup> and in non-rheumatic patients with stroke<sup>10</sup>, and *in vitro* platelet C4d has been described to be increased in SLE patients with APS<sup>13</sup>.

However, no study has so far investigated the association between *in vivo* platelet C4d deposition and VD in SLE. In our SLE patient cohort, we could show that complement deposition on platelets was markedly increased in patients with VD and was primarily associated venous and not arterial thrombosis. Some patients with a history of an arterial thrombosis had increased complement deposition on platelets but most of those patients also had a history of a venous thrombosis. However, the patients included in this study are too few to draw any conclusions about the differences observed in arterial and venous thrombosis. Further studies are needed to clarify if SLE patients with arterial thrombosis have different patterns of complement deposition compared to patients with venous thrombosis.

Besides deposition of C4d on platelets in SLE patients, we could demonstrate increased C1q and C3d deposition which has, to our knowledge, not been reported before. The presence of C1q, C4d and C3d deposition on the platelet at the same time, as seen in SLE patients, suggests classical pathway activation. However, there are several mechanisms of how complement components could get attached to platelets. One initiator of the classical pathway is ICs which is important in many of the clinical manifestations seen in SLE. ICs could contribute to VD in SLE by activating platelets and allow complement activation on or in the proximity of platelets <sup>14, 25</sup>. However, in our patient material, we did not see a statistical correlation between the levels of circulating ICs and complement deposition on platelets. This is in accordance with previous reports where no deposition of IgM or IgG was detected on the surface of platelets from SLE patients even in the presence of high C4d deposition <sup>11</sup>. Besides the possible IC-mediated activation of platelets, several other mechanisms could be responsible for the platelet activation, including collagen exposure, inflammation and shear stress due to atherosclerosis, which is increased in SLE patients <sup>26</sup>. Furthermore, aPL

antibodies, seen in patients prone to develop VD, are able to bind to and amplify platelet activation<sup>20-22</sup>.

Upon platelet activation chondroitin sulphate deposit on the surface of the platelet and binds C1q and initiates classical pathway activation on the platelets<sup>8</sup>. In our study we found an association between platelet activation and complement deposition on platelets, which would favour the hypothesis of a platelet activation-mediated complement deposition in SLE. However, complement activation is not a prerequisite for the binding of complement components, but also native complement components could bind to activated platelets<sup>9</sup>. To address this hypothesis, we used epitope-specific antibodies and demonstrated that the complement components deposited on platelets in SLE patients were indeed activated. Furthermore, addition of EDTA, an efficient inhibitor of classical pathway activation, inhibited platelet C4d deposition *in vitro*. Thus, complement deposition on platelets in SLE seems to be due to complement activation.

Complement activation on platelets is highly regulated and is, in our experimental model, restricted to C1q and C4d deposition using platelets from a healthy donor. Besides the platelet surface complement regulators CD55, CD59 and the newly discovered C2 inhibitor CRIT, factor H and C4BP are able to bind to the surface of an activated platelet to regulate complement activation<sup>27, 28</sup>. However, in SLE patients, possibly due to improper complement regulation or impaired clearance of the platelets, complement activation might proceed to the formation of membrane attack complex and subsequent release of platelet microparticles<sup>23, 24</sup>. Such microparticles are increased in SLE and are important factors in the generation of thrombin, a key component in the initiation of the coagulation<sup>24</sup>. The inverse correlation between platelet count and complement deposition on platelets seen in our study might

indicate either impaired platelet clearance or microparticle formation. Further studies will address the association between complement deposition on platelets and generation of microparticles in SLE patients.

To further address the underlying mechanism of complement deposition on platelets, the ability of serum to support platelet C4d deposition on heterologous fixed platelet was measured by flow cytometry. Serum from SLE patients supported equal complement deposition on activated platelets as serum from healthy controls. Thus, increased platelet activation might explain some of the increased complement deposition seen on platelets in SLE patients. However, sera from patients with APS, LAC and venous thrombosis supported increased complement activation as compared to sera from patients without those manifestations. The increased *in vitro* platelet C4d deposition in sera from APS patients has previously been associated to the presence of aPL antibodies suggesting that autoantibodies directed against phospholipids also could be involved in the increased complement deposition seen on platelets in SLE patients<sup>11, 13</sup>. In both man and mice, development of aPL-mediated thrombosis seems to be dependent on activation of the complement system<sup>3-5</sup>. However, studies in hamsters suggest that the Fc-part of aPL-antibodies is not needed to induce LAC-mediated thrombosis<sup>29</sup>. Thus, even though classical pathway activation is necessary for aPL-mediated thrombosis, non-IgG mediated activation of the classical pathway might take place as well. It could be speculated that aPL antibodies partly mediate their prothrombotic effects through activating platelets<sup>20-22</sup> up-regulating chondroitin sulphate and phosphatidylserine, two ligands for C1q, and thus activating the classical pathway of the complement system. Further studies are needed to elucidate the exact role of aPL antibodies in the interaction with platelets and the subsequent complement activation. In conclusion, the *in vivo* complement deposition on platelets might be a marker of platelet activation, whereas the increased *in vitro*



complement deposition on platelets seen in SLE patients with LAC and APS could reflect a specific factor, perhaps aPL antibodies, able to increase the complement deposition on platelets further.

Altogether we have demonstrated that complement deposition on platelets is increased in SLE patients *in vivo* possibly due to increased platelet activation and the presence of aPL antibodies interacting with the platelets. Furthermore, complement deposition on platelets might be a valuable biomarker for platelet activation and venous thrombosis in SLE patients. Further studies, including prospective studies, are needed to elucidate the mechanism behind the complement deposition and the association to venous thrombosis and LAC in SLE.

### **Conflicting interests**

The authors declare that there is no conflict of interest.

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## Figures

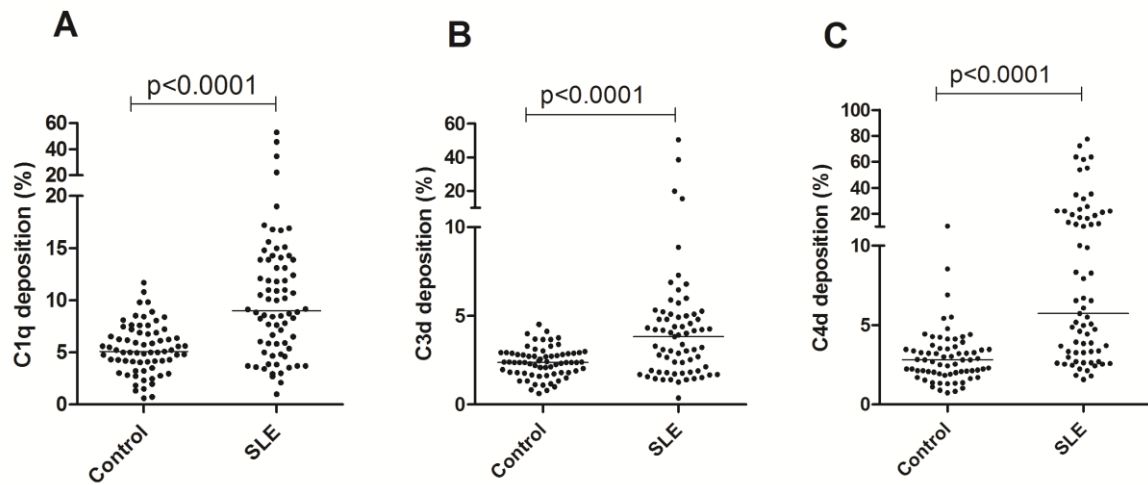


Figure 1. Increased complement deposition on platelets in SLE patients *in vivo*. Complement deposition on platelets was analyzed on isolated platelets from SLE patients and healthy controls by flow cytometry. A) C1q, B) C3d and C) C4d complement deposition on platelets in healthy controls and SLE patients. The values are expressed as a percentage of cells being positive as compared with a negative isotype antibody. The line represents the median value in each group.

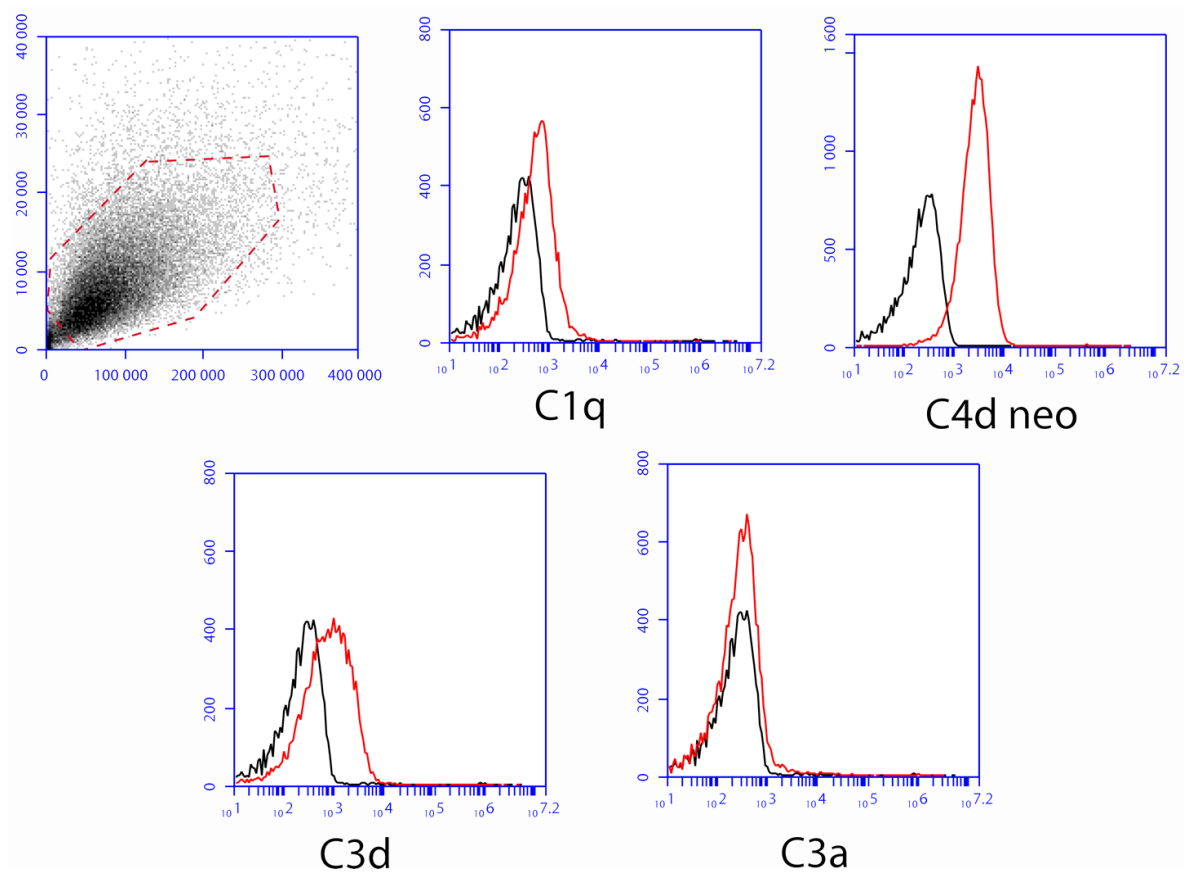


Figure 2. Representative flow cytometry plots of a SLE patient with increased complement deposition on platelets. A) Platelets were gated through forward and side scatter properties and initially confirmed to be platelets by the expression of CD42a. Platelet deposition of B) C1q, C) C4d neo, D) C3d and E) C3a was measured by flow cytometry. The black lines represent an isotype antibody and the red lines the complement deposition on the platelet.

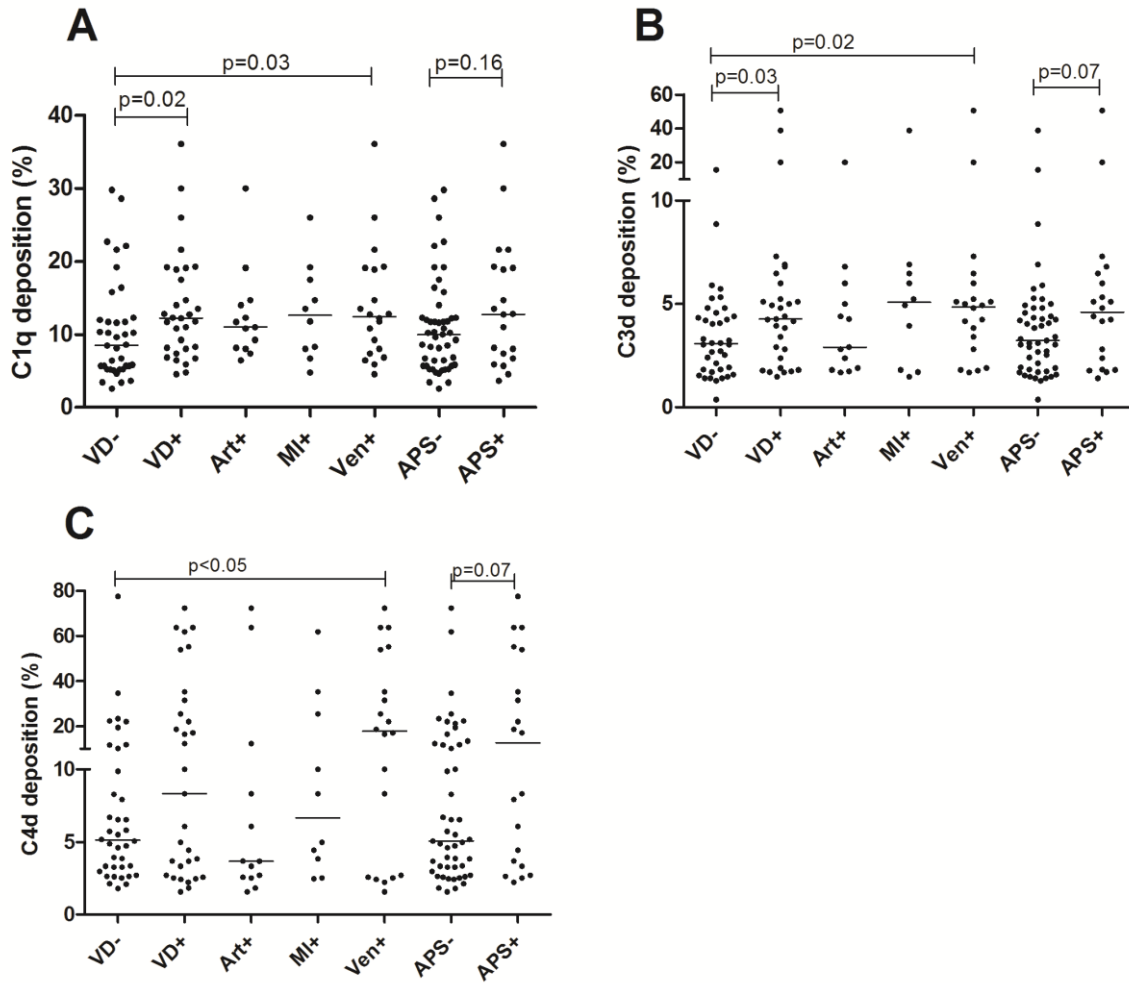


Figure 3. Increased complement deposition on platelets in SLE patients with vascular disease. The SLE patients were divided into subgroups (arterial thrombosis (Art+), myocardial infarction (MI+) and venous thrombosis (Ven+)) and analyzed for differences in platelet complement deposition levels. Platelet deposition of A) C1q B) C3d and C) C4d in subgroups of SLE patients. The SLE patients were also divided into patients with or without antiphospholipid antibody syndrome (APS). The values are expressed as a percentage of cells being positive as compared with a negative isotype antibody. The line represents the median value in each group.

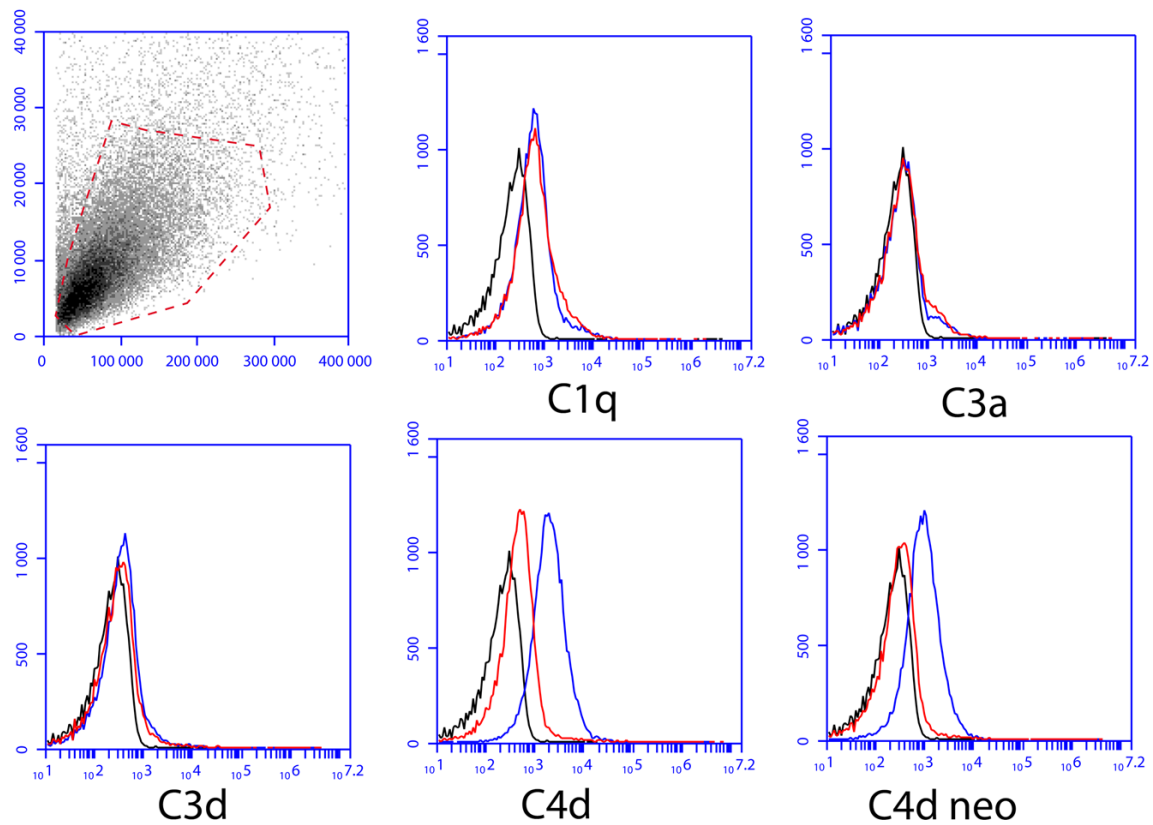


Figure 4. Representative flow cytometry plots of *in vitro* complement deposition on fixed heterologous platelets. A) Platelets were gated based on forward- and side scatter properties and analyzed for B) C1q, C) C3a, D) C3d, E) C4d and F) C4d neo deposition by flow cytometry. The black line represents an isotype antibody, the blue line serum from a SLE patient, and the red line the same SLE serum treated with 10 mM EDTA to inhibit complement activation.

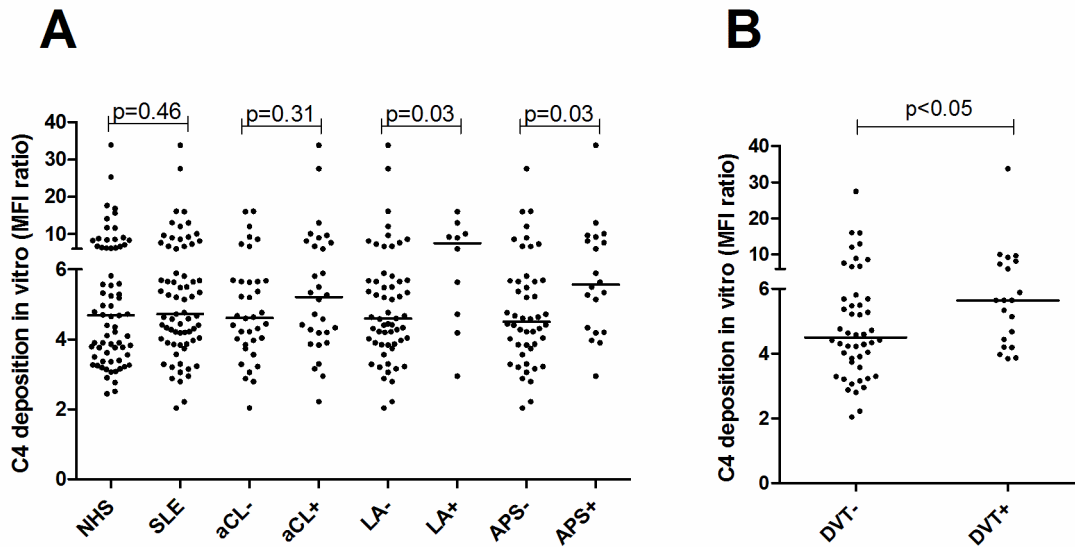


Figure 5. *In vitro* complement deposition on platelets. Heterologous paraformaldehyde-fixed platelets were incubated with serum and analyzed for the ability to support complement deposition on the platelets. A) SLE patients were divided into patients being positive or negative for anti-cardiolipin antibodies (aCL), lupus anticoagulant (LAC) or the antiphospholipid antibody syndrome (APS) and the ability of sera to support complement activation on platelets measured by flow cytometry. Serum from normal healthy individuals (NHS) served as controls. B) C4d deposition on platelets with serum from SLE patients with and without a deep venous thrombosis (DVT). The values are expressed as the MFI ratio for the target molecule as compared to an isotype antibody.



**Table I.** Clinical characteristics of the SLE patients according to the American College of Rheumatology (ACR) criteria and presence of vascular events at any time during disease.

	SLE n=69	Controls n=69
Age, median (range), years	51 (20-84)	53 (19-79)
Female %	87	87
Disease duration, median (range), years	13 (0-49)	-
SLEDAI score, median (range)	2 (0-14)	-
Malar rash %	61	-
Discoid rash %	30	-
Photosensitivity %	64	-
Oral ulcers %	30	-
Arthritis %	81	-
Serositis %	65	-
Renal disease %	42	-
Neurological disorder %	9	-
Hematological manifestations %	52	-
Leukopenia %	39	-
Lymphopenia %	28	-
Thrombocytopenia %	20	-
Immunology %	67	-
ANA %	100	-
Anti-DNA antibodies %	71	-
Anti-cardiolipin antibodies %	45	-
Lupus anticoagulant %	14	-
Anti-phospholipid antibody syndrome %	29	-
Vascular disease %	45	-
Median time since event, years (range)	8 (1-36)	-
Venous thrombosis %	29	-
Median time since event, years (range)	13 (3-36)	-
Arterial thrombosis %	19	-
Median time since event, years (range)	8 (1-18)	-
Myocardial infarction %	14	-
Median time since event, years (range)	5.5 (2-30)	-