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**ORIGINAL ARTICLE**

# Lupus anticoagulants and the risk of a first episode of deep venous thrombosis

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**Summary.** We have determined lupus anticoagulants, anti-$\beta_2$ glycoprotein I ($\beta_2$GPI) and antiprothrombin antibodies in the Leiden Thrombophilia Study, a population-based case–control study designed to determine risk factors for deep venous thrombosis (DVT). Lupus anticoagulant (LAC) was measured in 473 patients and 472 control subjects. Four control subjects (0.9%) and 14 patients (3.1%) had a positive LAC, resulting in a 3.6-fold increased risk [odds ratio (OR) 3.6, 95% CI: 1.2–10.9]. Of the total population, 49 were positive for anti-$\beta_2$GPI antibodies: 15 controls (3.4%) and 34 patients (7.5%), implying a 2.4-fold increased risk (95% CI: 1.3–4.2). Antiprothrombin antibodies were present in 114 subjects: 48 controls (11.0%) and 66 cases (14.6%) with an OR of 1.4 (95% CI: 1.0–2.1). When LAC was considered in the co-presence of antiprothrombin or anti-$\beta_2$GPI antibodies the OR increased to 10.1 (95% CI: 1.3–79.8). A LAC without a positive anti-$\beta_2$GPI or antiprothrombin test was not associated with a risk for DVT (OR 1.3, 95% CI: 0.3–6.0). This study demonstrates that the presence of LAC, anti-$\beta_2$GPI antibodies and antiprothrombin antibodies are risk factors for DVT in a general population. The strongest association holds for the combination LAC and the presence of anti-$\beta_2$GPI or antiprothrombin antibodies.

**Keywords**: anti-$\beta_2$ glycoprotein I antibodies, antiprothrombin antibodies, lupus anticoagulant, venous thrombosis.

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## Introduction

Deep venous thrombosis is a common disease in the Western society with an incidence of one to two per 1000 inhabitants per year [1]. The pathogenesis of venous thrombosis is complex, and it has become increasingly clear that deregulation of coagulation is a main cause of deep venous thrombosis. A large number of genetic risk factors related to unbalance of hemostasis have been recognized and in at least 60% of the patients one of these genetic risk factors can be found [2]. Besides genetic factors, acquired risk factors, such as immobilization, female hormone use and malignancies play a major role. The presence of antiphospholipid antibodies is considered as an important acquired risk factor that disturbs normal hemostasis [3]. The association between the presence of antiphospholipid antibodies and thrombosis has been firmly established for patients with autoimmune diseases such as Systemic Lupus Erythematosus [4].

The presence of anti-phospholipid antibodies ($\alpha$PL) in plasma can be detected by either a prolongation of phospholipid-dependent coagulation tests (lupus anticoagulants, LAC), or by solid-phase immunoassays [5]. Meta-analyses performed on all the published studies showed that the predictive value of assays that measure LAC activity correlate much better with a history of thrombosis than the presence of anticardiolipin antibodies. Different types of antibodies can cause LAC, both anti-$\beta_2$-glycoprotein I ($\beta_2$GPI)- and antiprothrombin antibodies have been shown to prolong coagulation *in vitro* [6,7]. The predictive value of ELISAs specific for autoantibodies directed against $\beta_2$GPI or prothrombin is unclear [8].

The incidence of LAC in the general population and its effect on the risk of thrombosis is not well established. The studies published included only small patient cohorts [9–11]. In the present study, we investigated the presence of LAC, anti-$\beta_2$GPI- and antiprothrombin antibodies in the Leiden Thrombophilia Study (LETS), a large population-based case–control study of unselected patients with a first venous thrombosis, designed to estimate the contribution of genetic and acquired risk factors to venous thrombosis.

## Patients and methods

### *Patients*

The LETS is a population based case–control study designed to determine risk factors for deep venous thrombosis [12]. The study includes 473 patients and 472 controls. The methods by which blood samples were obtained and interview data were collected have been described elsewhere [12]. The Leiden ethics committee approved the study protocol, and all participants gave their informed consent. Briefly, consecutively diagnosed patients younger than 70 years of age who had a first objectively confirmed episode of deep venous thrombosis between January 1988 and December 1993 and who had no known malignancy were selected through three anticoagulation clinics in the Netherlands. These clinics monitor anticoagulant treatment for all outpatients in well-defined geographic areas. All thrombi were proximal, 452 patients suffered from thrombosis in the leg and 21 patients suffered from thrombosis in the arm. Controls, who were either acquaintances or partners (225, 47%) of patients in the study, were frequency matched for age (within 5 years) and sex.

### *Samples*

Blood was collected in tubes containing 0.106 mol $L^{-1}$ trisodium citrate. Plasma was prepared by centrifugation at 2000 *g* for 10 min at room temperature and stored at −70°C in suitable aliquots. Samples were obtained at least 6 months after the thrombotic event and at least 3 months after discontinuation of oral anticoagulant treatment, however, 48 patients were still using oral anticoagulants at the time of drawing of blood because of early recurrences or arterial indications. Plasma samples used for the measurement of LAC activity had not been thawed before, whereas detection of anti-$\beta_2$GPI- and antiprothrombin antibodies was performed on samples that had been thawed and re-frozen once. As during the collection of the plasma samples the blood was centrifuged once while the recommendation for LAC testing is double centrifugation, we have tested with known LAC positive and negative samples whether this procedure was adequate to measure LAC. The results of these tests showed that the centrifugation procedure was sufficient to measure LAC.

### *Assays*

For all laboratory tests, the technician was unaware of whether the blood sample was from a patient or a control, and results were unblinded only after all results were entered into a database. LAC was measured with a dilute Russell's Viper Venom Time (dRVVT, Gradipore, Australia). Because of the limited amount of samples, the screening assay for LAC as recommended was bypassed and LAC testing was directly performed on samples diluted 1:1 with pooled normal plasma. A sample was considered positive when the clotting time was longer than the mean of 40 healthy controls + 3 SD (LAC screen). For those samples that showed a prolonged clotting time, a confirmation assay was performed with added phospholipids (LAC confirm). If addition of extra phospholipids resulted in a clotting time reduction of more than 20%, the sample was considered LAC positive [LAC ratio: LAC screen (1:1)/LAC confirm (1:1) $> 1.2$].

The presence of anti-$\beta_2$GPI IgG and IgM antibodies was measured as described before [13]. A positive patient sample was used for calibration and the results of this sample were arbitrarily set at 100 arbitrary units (AU). A sample was considered positive when the optical density (OD) for IgG or IgM measurement was higher than the OD of the mean of 40 controls + 3 SD (6.2 AU).

The presence of antiprothrombin IgG and IgM antibodies was measured as described before [13]. A positive patient sample was used for calibration and the results of this sample were arbitrarily set at 100 AU. A sample was considered positive when the OD for IgG or IgM measurement was higher than the OD of the mean of 40 controls + 3 SD (14.75 AU).

### *Other assays*

The activated protein C (APC) activity was measured as described previously in an activated partial thromboplastin time (APTT)-based assay [14]. APTT was measured using Cephotest (Nycomed, Oslo, Norway). C-reactive protein was measured with a sandwich-enzyme immunoassay as described previously [15].

### *Statistics*

As an estimator of the risk in those with abnormalities relative to those without, we calculated odds ratios (OR) with accompanying 95% CI, according to Woolf [16]. ORs were also calculated for various cut-off points for continuous variables, which were based on percentiles of the distribution in the control population. Adjustment for age, sex and other co-variates was performed by unconditional logistic regression.

## Results

The mean age of the patients and controls at the time of thrombosis was 45 years (range patients 15–69, controls 15–79). Fifty-nine percent of cases and controls were women (Table 1).

### *Lupus anticoagulants*

Of the total population, 18 persons were positive for LAC. Of these, 13 (72.2%) were women. The mean age of those with and without LAC was similar (45.0 and 43.0 years, respectively). Among the 472 control subjects, four persons were positive (0.9%), whereas 14 of the 473 patients with thrombosis were positive (3.1%). This implies a 3.6-fold increased risk for deep

**Table 1** Characterization of all patients and control subjects

| | LAC negative (ratio < 1.2) | LAC positive (ratio > 1.2) |
|---|---|---|
| Men (number) | 399 | 5 |
| Women (number) | 531 | 13 |
| Age (mean) | 44.9 | 43.4 |
| APTT (s, mean) | 27.8 | 34.9 |
| CRP (mg $L^{-1}$, mean) | 3.1 | 10.0 |
| Anti $\beta_2$-glycoprotein I antibody positive | 42 | 7 |
| Anti $\beta_2$-glycoprotein I antibody negative | 886 | 11 |
| Antiprothrombin antibodies positive | 105 | 9 |
| Antiprothrombin antibodies negative | 821 | 9 |

**Table 2** Odds ratios (OR) and 95% CI for thrombosis for antiphospholipid antibodies

| Assay | OR (95% CI) |
|---|---|
| Lupus anticoagulants | 3.6 (1.2–10.9) |
| Anti-$\beta_2$-Glycoprotein antibodies | 2.4 (1.3–4.2) |
| Antiprothrombin antibodies | 1.4 (1.0–2.1) |

**Table 3** Influence of LAC cut-off levels on the risk for thrombosis. OR (odds ratio), 95% CI

| Cut-off (LAC ratio) | No. of patients | No. of controls | OR (95% CI) |
|---|---|---|---|
| >1.1 | 38 | 23 | 1.9 (1.1–3.3) |
| >1.2 | 14 | 4 | 3.6 (1.2–10.9) |
| >1.3 | 7 | 3 | 2.4 (0.6–9.1) |
| >1.4 | 6 | 2 | 3.0 (0.6–15.1) |

venous thrombosis for individuals positive for LAC relative to those negative for LAC (OR 3.6, 95% CI: 1.2–10.9; Table 2). When patients ($n = 48$, of whom three were patients with positive LAC) on oral anticoagulants were excluded from the analysis, the OR decreased slightly (OR 3.1, 95% CI: 1.0–9.8).

We tested the influence of LAC on APC-sensitivity in an APTT-based assay. When restricted to those subjects that were not using anticoagulants, the APC-ratio was similar in patients with a positive LAC (APC ratio 0.85, 95% CI: 0.77–0.93) and patients without LAC (APC ratio 0.88, 95% CI: 0.86–0.90).

To determine whether the predictive value of the LAC assay would increase by varying the cut-off levels, the LAC ratios were set at 1.1, 1.2, 1.3 and 1.4. Elevated risks were found for all cut-off values, without a graded 'dose–response'. The a priori defined cut-off at a ratio of 1.2, which is used routinely in our center, gave the highest OR (Table 3).

### *Anti-$\beta_2$GPI-antibodies*

Of the total study population, 49 individuals had positive results for anti-$\beta_2$GPI antibodies: 15 controls (3.4%) and 34 cases (7.5%). This implies a 2.4-fold increased risk for deep venous thrombosis associated with having anti-$\beta_2$GPI antibodies (OR 2.4, 95% CI: 1.3–4.2; Table 2). This OR was determined for a cut-off level set a priori at the mean + 3 SD of 40 healthy controls (6.2 AU). When the cut-off level was set at the 70th (3.6 AU), 80th (4.1 AU), 90th (4.9 AU), or 95th percentile (5.8 AU) of the control population of our study, there was an increasing OR for increasing cut-off points, with a 1.9-fold increased risk (95% CI: 1.1–3.2) at antibody titers exceeding the 95th percentile (Table 4).

**Table 4** Influence of anti-$\beta_2$GPI antibody cut-off level on the risk for thrombosis

| Cut-off (percentile) | No. of patient | No. no controls | OR (95% CI) |
|---|---|---|---|
| P70 | 159 | 141 | 1.2 (0.9–1.6) |
| P80 | 104 | 94 | 1.1 (0.8–1.6) |
| P90 | 63 | 49 | 1.3 (0.9–2.0) |
| P95 | 42 | 23 | 1.9 (1.1–3.2) |
| Mean + 3 SD | 34 | 15 | 2.4 (1.3–4.2) |

**Table 5** Influence of antiprothrombin antibody cut-off level on the risk for thrombosis

| Cut-off (percentile) | No. of patient | No. of controls | OR (95% CI) |
|---|---|---|---|
| P70 | 175 | 141 | 1.4 (1.0–1.8) |
| P80 | 119 | 96 | 1.3 (1.0–1.8) |
| P90 | 64 | 47 | 1.4 (0.9–2.1) |
| P95 | 32 | 23 | 1.4 (0.8–2.4) |
| Mean + 3 SD | 66 | 48 | 1.4 (1.0–2.1) |

### *Antiprothrombin antibodies*

Of the total population, 114 were positive for antiprothrombin antibodies: 48 controls (11.0%) and 66 cases (14.6%), leading to a mildly elevated risk (OR 1.4, 95% CI: 1.0–2.1; Table 2). This OR was determined with a cut-off level of the mean + 3 SD (14.75 AU) of 40 controls, as routinely used. When the cut-off level was set at the 70th (10.4 AU), 80th (12.0 AU), 90th (14.9 AU) or 95th percentile (19.3 AU) of the control population in this study, the ORs were 1.3–1.4 for all cut-off values applied (Table 5).

### *Combinations of abnormal tests*

Of the 18 individuals with LAC, five were positive for antiprothrombin antibodies and anti-βGPI antibodies, two had only antiprothrombin antibodies, four only anti-βGPI antibodies, and seven had neither antiprothrombin nor anti-βGPI antibodies. Interestingly, three of the four control subjects with LAC had neither antiprothrombin nor anti-βGPI antibodies (as compared with three of 14 patients). When we only considered LAC in the co-presence of either antiprothrombin or anti-βGPI antibodies (10 of 473 patients and one of 472 controls), the risk of thrombosis associated with a LAC became much higher, with a 10-fold increased risk (OR = 10.1,

95% CI: 1.3–79.8). A LAC without antibodies to either prothrombin or βGPI, present in four patients and three controls, did not affected risk (OR 1.3, 95% CI: 0.3 to 6.0).

## Discussion

We have studied the risk of deep venous thrombosis associated with antiphospholipid antibodies in a population-based case–control study by measuring LAC, prothrombin antibodies and anti-$\beta_2$GPI-antibodies. LAC is present in 1% of the control population and 3.1% of the patients, and its presence increased the risk of thrombosis 3.6-fold. Anti-$\beta_2$GPI-antibodies were found in 3.4% of the population and 7.5% of the patients and increased the risk of thrombosis 2.4-fold, whereas antiprothrombin antibodies were detectable in 11% of the population and 14.6% of the patients, resulting in a relative risk of 1.4. When LAC was considered in combination with a positive anti-$\beta_2$GPI or antiprothrombin ELISA, the risk increased to 10.1. A LAC without positive ELISAs did not affect the risk. These findings demonstrate that antiphospholipid antibodies are present in a distinct incidence in the general population, and have a significant effect on the occurrence of thrombosis.

Several studies have demonstrated that LAC is a strong risk factor for thrombosis in patients with autoimmune disease [4]. We confirm this observation in a population who were otherwise apparently healthy. In an attempt to increase the predictive value for thrombotic complications, we analyzed the risk of combinations of assays. When besides LAC also anti-$\beta_2$GPI or antiprothrombin antibodies were present, the risk increased a further three times. This indicates that a combined screening for LAC and anti-$\beta_2$GPI and antiprothrombin antibodies will improve the diagnosis of the antiphospholipid syndrome. The percentage of patients positive for LAC was too low to analyze the contribution of anti-$\beta_2$GPI antibodies and antiprothrombin antibodies separately.

The incidence of LAC in patients with SLE and thrombosis is very high (40–60%) [17–19] and one of our aims was to assess the incidence of LAC in individuals without an underlying systemic autoimmune disease. In a study with 65 unselected patients, Ginsberg *et al.* [9] found nine patients positive for LAC (14%). In another study with 59 unselected patients with DVT, Simioni *et al.* [10] found five LAC positive patients (8.5%). Apparently, in unselected patients with thrombosis as in our study, the incidence of LAC is much lower than in patients with autoimmune disease.

We have found 4 positive LAC patients in a cohort of 436 controls (0.9%). Ginsberg *et al.* [9] found three positive samples in a total of 179 patients referred for suspected venous thrombosis who turned out not to have venous thrombosis (1.7%), while Simioni *et al.* [10] did not find any positive LAC in their control cohort of 117 persons (0%). In an older study Nencini *et al.* [10] studied 55 healthy volunteers and found only one LAC positive person (1.8%). These data seem to agree well on a incidence of LAC in the general population around 1%.

One of the major problems in diagnosing patients with the antiphospholipid syndrome is the decision whether a serological markers is positive or negative. It is not known above which level antiphospholipid antibodies should be considered as pathological. In this study, we have compared different cut-off levels for LAC, anti-$\beta_2$GPI and antiprothrombin antibodies. In our original publication [13], we used a ratio between dRVVT without extra phospholipids and with extra phospholipids of 1.2. Our current study demonstrates that this was a good choice, as this ratio had the highest association with DVT. It is not known if the same ratio is also the optimal cut-off level for arterial thrombotic complications. For anti-$\beta_2$GPI antibodies a cut-off level of the mean of 40 controls + 3 SD also appeared a satisfactory cut-off value. For antiprothrombin antibodies the choice of the cut-off level did not influence the association, which, although present, was much weaker than for the other so-called antiphospholipid antibodies.

It has been suggested that the presence of LAC leads to APC-resistance [20]. In the present study we did not find an effect of the presence of LAC on the APC-resistance, indicating that the increased risk for deep venous thrombosis found in patients with LAC was not because of an influence on protein C pathway.

Autoimmunity is more often seen in women than men. In accordance to this, about 80% of the patients with the antiphospholipid syndrome are women. In the cohort studied here, the majority of LAC-positive samples were from women (72%), and the sex distribution was not different between cases and controls.

Individuals with a positive LAC have higher levels of CRP in their blood compared with individuals without LAC. Blood was collected at least 6 months after the thrombotic event. It is difficult to imagine how infections would specifically raise LAC in patients who had suffered thrombosis so long ago. However, we could not exclude that a positive LAC because it is often related to autoimmune disease, leads to an inflammatory response and an increased CRP.

In conclusion, the presence of antiphospholipid antibodies is a risk factor for venous thrombosis of the deep leg veins in patients without a known underlying autoimmune disease. A combination of LAC with anti-$\beta_2$GPI or antiprothrombin antibodies confers the highest risk for deep venous thrombosis. Although the incidence of LAC in unselected patients with DVT is much lower than in patients with systemic autoimmune disease, around 1% of the population has a detectable abnormality increasing the risk of thrombosis.

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