Increased lipoprotein (a) levels as an independent risk factor for venous thromboembolism

Mario von Depka, Ulrike Nowak-Göttl, Roswith Eisert, Christian Dieterich, Monika Barthels, Inge Scharrer, Arnold Ganser, and Silke Ehrenforth

Elevation of serum lipoprotein (a) (Lp(a)) is a known risk factor predisposing to cardiovascular and cerebrovascular disease. However, little is known about the role of increased Lp(a) in venous thromboembolism (VTE). This study evaluated the role of Lp(a) among a panel of established hereditary thrombogenic defects in patients with VTE. A total of 685 consecutive patients with at least one episode of VTE and 266 sex- and age-matched healthy controls were screened with regard to activated protein C resistance, protein C, protein S, and antithrombin deficiency, elevated serum levels of Lp(a), and the factor V G1691A, MTHFR C677T, and prothrombin G20210A mutations. Elevated Lp(a) levels above 30 mg/dL were found in 20% of all patients, as compared to 7% among healthy controls (P < .001, odds ratio [OR] 3.2, 95% confidence interval [CI], 1.9-5.3). The coexistence of FV G1691A and elevated Lp(a) was significantly more prevalent among patients with VTE than in the control group (7% versus 0.8%; P < .001, OR 9.8, 95% CI, 2.4-40.7). No other established prothrombotic risk factor was found to be significantly combined with increased Lp(a). These data suggest that Lp(a) concentrations greater than 30 mg/dL are a frequent and independent risk factor for VTE. Furthermore, elevated Lp(a) levels might contribute to the penetrance of thromboembolic disease in subjects being affected by other prothrombotic defects, such as FV G1691A mutation.

Introduction

The most common risk factor for hereditary thrombophilia is the factor V (FV) G1691A mutation, which is in the majority of cases responsible for resistance against activated protein C (APC), and which is present in 20% to 50% of patients with venous thrombosis, depending on criteria of cohort selection. Other known markers of hereditary thrombophilia are the G20210A variant of the prothrombin gene and deficiencies of protein C, protein S, and antithrombin. Furthermore, the thermolabile variant of the 5,10 methylenetetrahydrofolate reductase due to the 677 CT transition, which appears to facilitate hyperhomocysteinemia especially in malnutrition with folic acid, has been discussed as a genetic risk factor for vascular disease and venous thromboembolism (VTE).

High serum lipoprotein (a) (Lp[a]) concentrations are associated with coronary heart disease, ischemic cerebrovascular disease, and chronic thromboembolic pulmonary hypertension. The major protein component of Lp(a) is apolipoprotein (a), which is disulfide-linked to apolipoprotein B-100. Because of the close structural homology of apolipoprotein (a) to plasminogen and in vitro and in vivo data, it seems reasonable that Lp(a) competes with plasminogen for fibrin binding leading to impaired fibrinolysis. Lp(a) can be found abundantly in atheromatous plaques and is therefore regarded to be a direct link between thrombogenesis and atherosclerosis. Although strong evidence suggests that Lp(a) is an independent risk factor for atherosclerotic vascular disease, little is known about the role of Lp(a) in VTE. Recently, in 2 pediatric case-control studies elevated Lp(a) was demonstrated to be an independent risk factor for childhood VTE and spontaneous stroke. Because the role of increased Lp(a) is unclear in venous thrombosis of adults, we evaluated Lp(a) as a single defect and in combination with established prothrombotic abnormalities of blood coagulation factors in patients with VTE and assessed its impact on the clinical manifestation of VTE.

Patients, materials, and methods

The present multicenter study was approved by the medical ethics committee at the Hannover Medical School, Hannover, Germany, and conducted in accordance to the ethical standards of the 1995 Declaration of Helsinki. All patients had given informed consent prior to study enrollment.

Patients

A total of 685 consecutive subjects with a history of VTE recruited from 3 catchment areas of Germany (Hannover, Frankfurt, Münster) were studied, including 414 women (60.4%) and 271 men (39.6%). Patients with arterial thromboembolism as the first thrombotic manifestation or with arterial thromboembolism only as well as patients with malignancies or laboratory evidence of antiphospholipid antibodies were excluded. In all patients exogenous risk factors were assessed by a standardized questionnaire comprising trauma, surgery, immobilization, use of oral contraceptives, pregnancy, and delivery. Diagnosis of VTE was confirmed by an independent radiologist who was unaware of the laboratory test results by duplex...
sonography/compression plethysmography, ascending phlebography, or computed tomography, respectively.

Controls were 266 healthy, age- and sex-matched white individuals (blood or potential bone marrow donors) from the same geographic areas as the patients. The criterion for recruitment of control subjects was the lack of any history of thromboembolic events.

**Blood sampling**

Blood samples were collected at least 3 months after the last acute thrombotic event and processed at each hospital according to standardized procedures. For coagulation assays, blood (9 parts) was obtained by fresh peripheral venipuncture and mixed with 0.109 mol/L trisodium citrate (1 part). Citrate plasma was prepared by immediate centrifugation for 15 minutes at 2000g, and the supernatant was immediately processed or stored at −20°C not longer than 3 weeks until analysis. For measurement of Lp(a), serum was obtained from venous blood following centrifugation at 1500g for 10 minutes and stored at −70°C until analysis. For gene analysis, venous blood was collected in EDTA-treated tubes. All laboratory tests were performed without knowledge of whether a sample was drawn from a control subject or a patient and without knowledge of the clinical outcomes.

**Lipoprotein (a) measurement**

In all subjects Lp(a) serum concentration was measured with a photometric “sandwich enzyme immunoassay” using mouse monoclonal antiapolipoprotein (a)-coated microtiter plates and peroxidase-coupled Fab fragments of mouse monoclonal antiapolipoprotein B-100 antibodies including human standards and controls (Roche Diagnostics, Mannheim, Germany). The lower detection limit of the assay was 5 mg/dL. A threshold value of Lp(a) more than 30 mg/dL was considered as the cut-off value, which is widely accepted as the cut-off in the assessment of increased risk for cerebrovascular and cardiovascular events.²⁹,³⁰

**Assays of hemostatic factors**

Determination of APC resistance was done using a commercially available kit (Chromogenix, Molndal, Sweden). Activities of protein C and antithrombin were measured by means of a chromogenic assay (Chromogenix). Total and free protein S antigen and protein C antigen were measured using a commercially available enzyme-linked immunosorbent assay kit (Asserachrom, Diagnostica Stago, Asnières, France). In patients treated with oral anticoagulants, no protein C and protein S were measured. Antithrombin and protein C deficiency were defined as reduction of the functional plasma activity below the lower limit alone or along with a reduced antigen concentration. Protein S deficiency was diagnosed when free protein S antigen levels were reduced below the lower limit combined with decreased or normal total protein S antigen levels, respectively.

**DNA-based assays**

The FV G1691A mutation, prothrombin (PT) G20210A variant, and the MTHFR C677T variant were determined. DNA was prepared from buffy coat using standard techniques, amplified by polymerase chain reaction, and detected by allele-specific hybridization.¹¹ The tests are based on a 3-step procedure consisting of DNA isolation, DNA amplification, and finally allele-specific hybridization and detection of amplified target DNA with 2 sequence-specific oligonucleotide reporter molecules in 2 separate cavities of a microwell plate. The labeling of the target DNA was done during amplification. Detection was done by binding of horseradish peroxidase-labeled specific antibodies against fluorescein. All analyses of FV G1691A mutation were consistent with analyses of APC resistance.

**Statistical analysis**

Data are expressed as means and standard deviations (SD) and compared by using the Student t test or as medians and ranges and compared by Mann-Whitney U test where appropriate. The significance of differences of the frequency of prothrombotic risk factors were tested using the χ² test. Odds ratios (OR), 95% confidence intervals (CI) and P values (χ² test, corrected for multiple testing according to Bonferroni) were calculated as a measure for relative risk. P values less than .05 were considered significant. In addition, the multifactorial role of prothrombotic risk factors was assessed using multivariate logistic regression analysis. All calculations were made with the SPSS for Windows Release 9.0.0 statistical package (SPSS, Chicago, IL).

**Results**

**Characteristics of thromboembolic manifestations**

At the first episode of VTE, patients’ ages ranged between 11 and 77 years, with a median age of 34 years (women 33 years, range 11-77; men 36 years, range 14-76). Of 685 patients, 476 (84%) suffered from juvenile thrombosis occurring before the age of 50 years. Of all patients, 333 (49%) suffered from one thromboembolic episode; the other 352 patients (51%) had recurrent VTE with a total of up to 6 thromboembolic events. Seventeen patients suffered from arterial thromboembolism as the second thromboembolic event (5%; myocardial infarction, n = 6; cerebral infarction, n = 5; and peripheral arterial thromboembolism, n = 6). Nine additional patients had arterial thromboembolism as their third thromboembolic event.

The first thrombotic onset consisted of lower extremity deep vein thrombosis in 427 patients (62%), thrombosis of the pelvic region in 77 (11%), thrombosis in the splanchic region in 37 (5%), deep vein thrombosis of the upper limb in 36 (5%), retinal venous thrombosis in 25 (4%), cerebral vein thrombosis in 16 (2%), and thrombosis of the superior or inferior caval vein in 4 patients (0.6%). Pulmonary embolism without detectable deep venous thrombosis was found in 63 cases (9%).

In 355 patients (52%) the first VTE developed spontaneously; in 147 patients (21%) VTE occurred immediately after trauma or surgery or while bedridden. Thirty-two patients (5%) suffered from obesity, 110 female patients (16%) used oral contraceptives, and 41 women (6%) developed VTE during pregnancy or immediately after delivery. Seventy-eight patients (11%) had more than one of the mentioned exogenous risk factors.

**Lipoprotein (a) levels**

In patients the mean Lp(a) level was 21.6 mg/dL (SD 40.6), whereas the mean level in controls was 11.9 mg/dL (SD 16.4; P < .01, t test). Elevated Lp(a) levels above 30 mg/dL, considered to be the atherosclerotic cut-off value as well as the threshold for venous thrombosis in the young,²⁸ were diagnosed in 135 of 685 patients (20%), whereas the prevalence of elevated Lp(a) levels was significantly lower in healthy controls (19 of 266, 7%, χ² test, P < .001). The OR of VTE associated with Lp(a) elevation was 3.2 (95% CI, 1.9-5.3, Table 1). If the cut-off level was set to more than 20 or more than 10 mg/dL, there was still a significantly higher proportion of Lp(a) elevation among patients compared to controls with a significantly raised OR (Table 2).

Patients with no additional exogenous risk factor and Lp(a) more than 30 mg/dL had a slightly lower OR compared to controls (OR 2.8, 95% CI 1.6-4.8, P < .001). A similar OR was calculated for patients who suffered from trauma or surgery or were immobilized (OR 2.9, 95% CI 1.6-5.5, P = .001). Women using oral contraceptives had the highest risk of all patient subgroups (OR 4.0, 95% CI 2.1-7.6, P < .001), which was comparable to patients with a combination of several exogenous risk factors versus controls (OR 3.9, 95% CI 1.9-7.9, P < .001). Patients with
recurrent VTE had a similar OR (2.9, 95% CI 1.6-5.5, \( P = .001 \)) compared to controls.

There were no differences of increased Lp(a) levels according to patients’ gender and age at first onset of VTE.

### Established prothrombotic risk factors

Analysis of the \( FV \) G1691A gene was performed in 683 patients; 205 were heterozygotes (29%) and 26 homozygotes (4%). Among the controls (\( n = 256 \)) we found no homozygotes and 19 (7%) heterozygotes, which was significantly lower compared to patients (Table 1). Because we found no homozygous \( FV \) G1691A mutations among our controls, the OR could not be calculated.

In 664 patients and 235 controls gene analysis of the PT G20210A genotype was performed. Forty-five of 664 patients (7%) were heterozygotes compared to 6 controls (2.6%). Thus, the prevalence was significantly higher in patients (\( \chi^2 \) test, \( P < .003 \), Table 1). The corresponding OR was 2.8 (95% CI 1.2-6.6).

The MTHFR 677TT genotype was found in 49 of 486 patients tested (10%) and in 22 of 161 controls (14%). Among the patients, 212 tested heterozygous for the MTHFR C677T variant (44%) as did 71 controls (44%); 225 (46%) of the patients and 68 (42%) of the controls were negative (none of the differences were statistically significant, \( \chi^2 \) test). No significant differences were found for the deficiencies of protein C (patients, \( n = 15 \), 2.5%; controls, \( n = 3 \), 1.3%), protein S (patients, \( n = 24 \), 3.9%; controls, \( n = 5 \), 2.2%), or antithrombin (patients, \( n = 17 \), 2.5%; controls, \( n = 1 \), 0.4%). The results of the univariate factorial analyses are shown in Table 1.

Coexistence of Lp(a) more than 30 mg/dL and \( FV \) G1691A was found in 49 of 683 patients (7%), but only in 2 of 256 (0.8%) of the control group (\( \chi^2 \) test, \( P < .001 \), corrected for multiple testing). Accordingly, the coincidence of elevated Lp(a) with \( FV \) G1691A mutation further increased the OR for thromboembolic events to 9.8 (95% CI 2.4-40.7, \( P < .001 \)). Coexistence of increased Lp(a) with the heterozygous PT G20210A mutation was found in 4 of 663 (0.6%) patients. Furthermore, neither deficiency of protein C or protein S nor antithrombin was found to be significantly combined with increased Lp(a).

### Multivariate analysis

To identify independent risk factors for VTE, multivariate logistic regression was performed including all parameters that proved to be statistically significant in univariate analysis. Lp(a) more than 30 mg/dL (OR 2.8, 95% CI 1.6-4.9, \( P < .001 \)), \( FV \) G1691A mutation (OR 5.4, 95% CI 3.2-9.0, \( P < .001 \)), as well as PT G20210A mutation (OR 3.1, 95% CI 1.3-7.6, \( P < .01 \)) could be identified as independent risk factors for VTE. In addition, Lp(a) levels more than 20 mg/dL still proved to be an independent risk factor with an OR of 2.0 (95% CI 1.3-3.1, \( P < .001 \)) as well as Lp(a) levels of more than 10 mg/dL (OR 1.5, 95% CI 1.0-2.0, \( P < .05 \)).

### Discussion

In this multicenter study we evaluated the prevalence of elevated Lp(a) levels and their association with VTE. Our data on consecutive white patients suggest that Lp(a) elevation is an independent risk factor for VTE.

Lipoprotein (a) inhibits the activation of plasminogen by streptokinase and tissue plasminogen activator in vitro and competes with plasminogen for binding to fibrin as well as for binding to annexin II and the plasminogen/tissue plasminogen activator receptor on endothelial cells and on platelets.\(^ {23,29,32-38} \) Recently, a French study provided the first quantitative evidence that binding of Lp(a) to lysine residues of fibrin and cell surfaces is directly related to circulating levels of both plasminogen and Lp(a) and that these glycoproteins may interact as competitive ligands for these biologic surfaces in vivo.\(^ {36} \) They observed an association of low plasminogen concentrations and high Lp(a) levels with an increased Lp(a) binding ratio onto fibrin and cells in nephrotic children during a flare-up of the disease.\(^ {36} \) Thus, because Lp(a) shares striking similarities with human plasminogen,\(^ {37} \) it is suggested that Lp(a) competitively interferes with plasminogen without exhibiting the proteolytic activity of plasminogen. Therefore, Lp(a) might interact with fibrin, platelets,\(^ {34} \) and cell surface receptors and might impair fibrinolysis and promote thrombosis.\(^ {35,38-41} \) In addition, Lp(a) is able to bind and inhibit tissue plasminogen activator.\(^ {33} \) This possible prothrombotic potential concerns on Lp(a) the direct link between thrombogenesis and atherosclerosis. High plasma concentrations of Lp(a) are associated with the development of atherosclerosis in coronary heart disease,\(^ {9,11-13} \) restenosis, and ischemic stroke.\(^ {16,18,27,42,43} \) However, there is only little information on the role of Lp(a) in venous thrombosis. In a small scale study Atsumi and coworkers\(^ {25} \) analyzed Lp(a) levels in patients with antiphospholipid antibody syndrome (APAS). They found significantly increased plasma Lp(a) levels in patients with APAS with arterial thrombosis as well

### Table 1. Frequency of Lp(a) elevation and hereditary risk factors of VTE and risk of VTE (univariate analysis)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Patients, n (%</th>
<th>Controls, n (%)</th>
<th>( \chi^2 ) test</th>
<th>( P ) values</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) &gt; 30 mg/dL</td>
<td>135/685 (20)</td>
<td>19/266 (7)</td>
<td>&lt;.001</td>
<td>3.2 (1.9-5.3)</td>
<td></td>
</tr>
<tr>
<td>( FV ) G1691A heterozygous</td>
<td>205/683 (29)</td>
<td>19/256 (7)</td>
<td>&lt;.001</td>
<td>5.7 (3.5-9.3)</td>
<td></td>
</tr>
<tr>
<td>( FV ) G1691A homozygous</td>
<td>26/683 (4)</td>
<td>0/256 (0)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>45/664 (7)</td>
<td>6/235 (2.6)</td>
<td>.03</td>
<td>2.8 (1.2-6.6)</td>
<td></td>
</tr>
<tr>
<td>MTHFR 677TT†</td>
<td>49/486 (10)</td>
<td>22/161 (14)</td>
<td>.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>15/611 (3)</td>
<td>3/228 (1.3)</td>
<td>.46</td>
<td>1.8 (0.5-6.6)</td>
<td></td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>24/612 (4)</td>
<td>5/219 (2.3)</td>
<td>.33</td>
<td>1.8 (0.7-4.7)</td>
<td></td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>17/685 (2.5)</td>
<td>1/226 (0.4)</td>
<td>.1</td>
<td>5.7 (0.8-43.3)</td>
<td></td>
</tr>
<tr>
<td>Lp(a) and ( FV ) G1691A‡</td>
<td>49/683 (7)</td>
<td>2/256 (0.8)</td>
<td>&lt;.001</td>
<td>9.8 (2.4-40.7)</td>
<td></td>
</tr>
</tbody>
</table>

*\( \chi^2 \) test, corrected for multiple testing.
†Only homozygotes.
‡Heterozygote and homozygote cases taken together.

### Table 2. Odds ratios for VTE according to different Lp(a) cut-offs (univariate analysis)

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Patients, n (%)</th>
<th>Controls, n (%)</th>
<th>( U ) test</th>
<th>( P ) values</th>
<th>OR (95% CI)</th>
<th>( \chi^2 ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) &gt; 30 mg/dL</td>
<td>135 (20)</td>
<td>19 (7)</td>
<td>&lt;.001</td>
<td>3.2</td>
<td>1.9-5.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lp(a) &gt; 20 mg/dL</td>
<td>172 (25)</td>
<td>35 (13)</td>
<td>&lt;.001</td>
<td>2.2</td>
<td>1.5-3.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lp(a) &gt; 10 mg/dL</td>
<td>272 (40)</td>
<td>78 (29)</td>
<td>&lt;.01</td>
<td>1.6</td>
<td>1.2-2.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Lp(a) &gt; 5 mg/dL</td>
<td>351 (51)</td>
<td>125 (47)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant.
as in patients with venous thrombosis. Mírza and colleagues, who investigated the apolipoprotein (a) levels of 203 patients with venous thrombosis before the age of 45 years, did not find a relevant association of apolipoprotein (a) levels and juvenile VTE. However, it should be taken into account that they measured apolipoprotein (a) levels using a sandwich radioimmunoassay. It is recommended to use a combination of antibodies against apolipoprotein (a) for capture with antiapolipoprotein B antibodies for detection to avoid cross-reactions to apolipoprotein (a), which may be present in free form in plasma as full-length and truncated apolipoprotein (a). On the other hand, it has recently been shown that increased Lp(a) is a risk factor for VTE in childhood. In the consecutively recruited patients investigated here we could identify elevated Lp(a) concentrations as a frequent and independent risk factor of VTE associated with an OR of 2.8 in multivariate logistic regression analysis. For recurrent disease the OR was similar. The OR is lower compared to other established risk factors of hereditary thrombophilia such as heterozygous FV G1691A mutation. On the other hand, it is higher than elevated prothrombin levels exceeding 1.15 U/mL with an OR of 2.1, which is similar to the OR of Lp(a) elevation if the cut-off level is set to 20 mg/dL. Lp(a) levels above 30 mg/dL are considered to be the atherosclerotic threshold. However, our study suggests that the thromboembolic risk associated with Lp(a) elevation in the cohort presented is concentration dependent, starting at Lp(a) levels well below the established cut-off (Table 2).

It is now widely accepted that thrombophilia is a multifactorial disorder. In our study, the combination of Lp(a) elevation and the FV G1691A mutation revealed a high prevalence among patients. In 7.2% of the patients but only in 0.8% of the control subjects, Lp(a) levels more than 30 mg/dL were coincident with the FV G1691A mutation, increasing the OR to about 10. Thus, it is likely that the simultaneous presence of Lp(a) elevation and FV G1691A mutation in a patient enhances the risk of VTE. Similar findings could be demonstrated in children with VTE. However, other than the coexistent FV G1691A mutation, no further combinations of genetic defects with elevated Lp(a) were found.

Additional exogenous risk factors are likely to modify the thrombogenic risk caused by Lp(a) elevation depending on the kind and number of the exogenous risk factors. Our data indicate that the strongest enhancing effect might be caused by intake of oral contraceptives. Although Lp(a) levels seem to be genetically determined, a reduction of Lp(a) has been achieved in postmenopausal women receiving hormone replacement therapy. However, little is known on the effect of oral contraceptives on lipoprotein metabolism. Thus, our results warrant further investigations.

In conclusion, our data indicate that the elevation of serum or plasma Lp(a) concentration significantly increases the risk of venous thromboembolic disease, especially with the coexistence of the FV G1691A mutation or further exogenous risks, thereby underlining the multifactorial genesis of thromboembolism. The exact pathogenic mechanism of elevated Lp(a) leading to VTE remains to be clarified.

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