Lipoprotein Apheresis for Lipoprotein(a)-Associated Cardiovascular Disease

Prospective 5 Years of Follow-Up and Apolipoprotein(a) Characterization

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Objective—Lipoprotein(a)-hyperlipoproteinemia (Lp(a)-HLP) along with progressive cardiovascular disease has been approved as indication for regular lipoprotein apheresis (LA) in Germany since 2008. We aimed to study the long-term preventive effect of LA and to assess hypothetical clinical correlations of apolipoprotein(a) (apo(a)) by analyzing genotypes and phenotypes.

Approach and Results—This prospective observational multicenter study included 170 patients with Lp(a)-HLP and progressive cardiovascular disease (48.9 years median age at diagnosis) despite other cardiovascular risk factors, including low-density lipoprotein cholesterol had maximally been treated (mean baseline low-density lipoprotein cholesterol: measured, 2.56 mmol/L [98.9 mg/dL] and corrected, 1.72 mmol/L [66.3 mg/dL]). Patients were prospectively investigated during a 5-year period about annual incidence rates of cardiovascular events. In addition, apo(a) isoforms and polymorphisms at the apo(a) gene (LPA) were characterized. One hundred fifty-four patients (90.6%) completed 5 years of follow-up. Mean Lp(a) concentration before commencing regular LA was 108.1 mg/dL. This was reduced by a single LA treatment by 68.1% on average. Significant decline of the mean annual cardiovascular event rate was observed from 0.58±0.53 2 years before regular LA to 0.11±0.15 thereafter (P<0.0001); 95.3% of patients expressed at least 1 small apo(a) isoform. Small apo(a) isoform (35.2%) carrying phenotypes were not tagged by single-nucleotide polymorphisms rs10455872 or rs3798220.

Conclusions—Results of 5 years of prospective follow-up confirm that LA has a lasting effect on prevention of cardiovascular events in patients with Lp(a)-HLP. Patients clinically selected by progressive cardiovascular disease were characterized by a highly frequent expression of small apo(a) isoforms. Only Lp(a) concentration seemed to comprehensively reflect Lp(a)-associated cardiovascular risk, however. (Arterioscler Thromb Vasc Biol. 2016;36:2019-2027. DOI: 10.1161/ATVBAHA.116.307983.)

Key Words: cardiovascular disease ■ coronary disease ■ lipoprotein(a) ■ lipoprotein apheresis ■ prevention ■ risk factors

Evidence from prospective epidemiological studies and Mendelian randomization studies has documented an independent and causal association of elevated lipoprotein(a) (Lp(a)) plasma concentrations with cardiovascular disease (CVD), including coronary artery disease, ischemic stroke, and peripheral arterial disease.1-4 Therefore, Lp(a) is regarded as a therapeutic target with the potential to lower cardiovascular risk and prevent clinical events.

Lp(a) is composed of a low-density lipoprotein (LDL)–like particle to which a single copy of apolipoprotein(a) (apo(a)) is covalently attached. Apo(a) is composed of a protease domain, and plasminogen-like kringle domains, namely, one kringle V and a variety of kringle IV (KIV) structures. Ten different KIV domains have evolved in the LPA gene with KIV type 1 and KIV types 3 to 10 being present in single copies only, whereas the KIV type 2 (KIV-2) domains show an extensive repeat

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*A list of all Pro(a)LiFe Study Group (Prospective Documentation of Isolated Lipoprotein(a)-Elevation With Progressive Cardiovascular Disease and Lipoprotein Apheresis for Effective Treatment of Hyperlipoproteinemia) Coauthors is given in the Appendix.

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copy number variation with 1 to >40 repeats. These are all translated and lead to a size polymorphism of apo(a), which is causally associated with Lp(a) concentrations in an inverse manner.\(^4\) Lp(a) isoforms have been categorized as small (≤22 KIV repeats) or large (>22 KIV repeats) with small isoforms, implying an ≈2-fold higher risk of CVD.\(^6\) Sequence variation in apo(a) other than the KIV-2 copy number variation is also associated with Lp(a) levels. Two common gene variants rs10455872 and rs3798220 have been found to be associated with CVD risk in whites.\(^7\)

Lipoprotein apheresis (LA) is an effective option for lowering blood LDL-cholesterol (LDL-C) concentrations in patients with severe hypercholesterolemia, in whom lipid-lowering medicines are insufficient or poorly tolerated.\(^8\) In 2008, the German Federal Joint Committee (GBA) decided to accept Lp(a)-hyperlipoproteinemia (Lp(a)-HLP) associated with progressive CVD as an indication for regular LA with reimbursement.\(^9\) To become eligible for treatment, the Lp(a) concentration should exceed 60 mg/dL, LDL-C concentration should be at treatment targets with maximally tolerated lipid-lowering medication, and CVD should be progressive despite optimal treatment of all other cardiovascular risk factors. The current reimbursement regulation in Germany has no equivalent in any other country and offered the unique opportunity to characterize this clinically selected high-risk patient group in a prospective observational study comparing the incidence rates of cardiovascular events in patients with Lp(a)-HLP and progressive CVD retrospectively before and prospectively after commencing regular LA.\(^10\) Here we report the follow-up of these patients after 5 years of regular ongoing LA to assess prospectively long-term sustainability of the preventive effect of LA. In addition, apo(a) was analyzed to assess hypothetical clinical correlations of genotypes and phenotypes in this clinically selected cohort.

### Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

### Results

#### Characteristics of Patients at the Time of the First LA and on y+5 of Follow-Up

A total of 170 patients all of white European ethnicity commenced regular LA at day 0, and 154 (90.6%) could be analyzed after completion of y+5 (Figure 1; Table 1). During a median period of 4.7 years of the pre-LA period, CVD was

![Figure 1](image_url)

**Figure 1.** Patient flow chart showing annual patient numbers during the entire 7 years of the study period. Reasons for terminating the study including deaths are listed in a table of the online Data Supplement.
progressive, which finally led to the initiation of LA. It should be noted that approval for LA because of Lp(a)-HLP was not based on the occurrence of a recent cardiovascular event because it requires careful consideration of the entire clinical course after diagnosis of CVD.

Between the time of the first LA and y+5, prevalence rates of coronary artery disease, cerebral atherosclerosis, and peripheral atherosclerosis did not change significantly (Table 1). The proportion of patients with renal artery stenosis decreased until y+5 because of patients who had terminated the trial. There was no obvious explanation for this coincidence. A table listing all 17 patients with their reason for terminating the study together with their renal artery status can be found in Table I in the online-only Data Supplement.10

**Safety of LA Treatment**

No serious adverse event related to LA treatment was observed during the entire prospective study period of 5 years. Also, no particular or sustaining clotting problems were reported. Minor adverse events typically associated with outpatient apheresis treatment, for example, transient hypotension, dizziness, hematoma at vascular access, or nausea, were not analyzed. Representative long-term safety analyses of 2 of the study sites have recently been published.11,12 Mean plasma concentrations of creatinine and fibrinogen remained stable throughout the entire study period. The patient group included 3 hemodialysis patients, 2 of whom died in y+2 or y+3 (see the analysis of events below) and 1 patient who successfully received a kidney transplant in y+4. Because iron deficiency can develop with chronic LA,13 the vast majority of patients received iron supplementation, mostly intravenously. Doses were determined individually according to monitoring of ferritin and transferrin saturation in intervals determined by local physicians. Hemoglobin levels of patients remained stable during regular ongoing LA treatment with a mean value of 13.7 g/dL at the time of the first LA and 13.3 g/dL in y+5.

<table>
<thead>
<tr>
<th>Table 1. Patients’ Timeline of CVD and Characteristics at the Time of the First LA Treatment, and in y+5 of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of the First LA (n=170)</td>
</tr>
<tr>
<td>Male/female*</td>
</tr>
<tr>
<td>Age, y†</td>
</tr>
<tr>
<td>Male, y†</td>
</tr>
<tr>
<td>Female, y†</td>
</tr>
<tr>
<td>Age at diagnosis of CVD, y†</td>
</tr>
<tr>
<td>Age at first CV event, y†</td>
</tr>
<tr>
<td>Age at second CV event, y†</td>
</tr>
<tr>
<td>Treatment intervals, 1.5× to twice per wk/weekly/biweekly/ every 3 wk*</td>
</tr>
<tr>
<td>Vascular access, peripheral veins/arteriovenous fistula</td>
</tr>
<tr>
<td>Coronary artery disease*</td>
</tr>
<tr>
<td>1-/2-/3-vessel coronary disease*</td>
</tr>
<tr>
<td>Cerebral atherosclerosis*</td>
</tr>
<tr>
<td>Peripheral atherosclerosis*</td>
</tr>
<tr>
<td>Renal artery stenosis*</td>
</tr>
<tr>
<td>Diagnosis of diabetes mellitus*</td>
</tr>
<tr>
<td>Antihypertensive medication*</td>
</tr>
<tr>
<td>Vitamin-K antagonist*</td>
</tr>
<tr>
<td>Antiplatelet medication*</td>
</tr>
<tr>
<td>Creatinine, (\mu\text{mol/L} (\text{mg/dL}))§</td>
</tr>
<tr>
<td>Hemoglobin, mmol/L (g/dL)§</td>
</tr>
</tbody>
</table>

LA indicates lipoprotein apheresis; CV cardiovascular; and CVD, cardiovascular disease.

*Numbers (percentages).
†Median (interquartile range).
‡Mean±SD (conventional units).
Laboratory Parameters

Laboratory investigations are summarized in Table 2. Mean Lp(a) concentration before regular LA was 108.1 mg/dL and was reduced by a single LA treatment on average by 68.1% during 5 years of chronic LA. Mean Lp(a) concentration before LA treatments averaged during 5 years of follow-up was 91.1 mg/dL, that is, 16% lower compared with the mean baseline concentration before the first LA treatment (P<0.05).

The mean LDL-C concentration before LA was 2.56 mmol/L (98.9 mg/dL). Mean LDL-C concentrations at baseline and before LA treatments averaged during 5 years of follow-up remained unchanged (Table 2). The mean LDL-C reduction was 66.3% per LA session. LDL-C as directly measured or calculated by the Friedewald formula includes the contribution of Lp(a) cholesterol, which is estimated as 30% to 45% of the total measured Lp(a) mass of a patient; thus, only corrected LDL-C reflects actually treatable LDL-C under the used lipid-lowering medication (Table 2).2,14,15

Medication

More than 90% of patients received lipid-lowering drugs throughout the entire study, in the vast majority consisting of a statin or a combination of statins with ezetimibe. The number of patients taking lipid-lowering medication with statins as one component decreased from 90.5% at first LA to 86.2% in year +5. The subgroup of patients taking only a statin without other lipid-lowering drugs increased from 24.1% at first LA to 39.2% in y+5. Both changes reflect that a statin without other lipid-lowering drugs increased from 91.1 mg/dL, that is, 16% lower compared with the mean plasma concentration before the first LA treatment (<0.05).

Analysis of Events

Absolute numbers and mean annual rates of major adverse cardiac event (MACE) and adverse cardiac or vascular event (ACVE) in selected study periods of all 7 study years are depicted in Figure 2. The commencement of regular ongoing LA was associated with a rapid stabilization of progressive CVD that had developed in the median interval of 4.7 years since the second cardiovascular event. Mean annual rates of MACE in periods of y+1 and y+2 versus y+3 to y+5 revealed a significant decrease, indicating the sustaining effect of LA. Annual incidence rates for MACE and ACVE were 85% and 81% lower during chronic LA, respectively, in comparison to the progressive phase of CVD before commencing LA. Annual MACE or ACVE rates in patients with the diagnosis of diabetes mellitus (n=37, ie, 21.8% in y+1; n=32, ie, 20.8% in y+5) were statistically not different as compared with patients without diabetes mellitus. Mean annual rates for y+3 to y+5 for MACE and ACVE seemed similar to all LA methods used. Because of the sample size, only for the largest subgroup treated by temperature-optimized double filtration plasmapheresis (n=101 [61%] in y+2 and n=89 [58%] in y+5),10 a separate statistical analysis could be performed, and no difference to the entire cohort was found for MACE (ie, y+3: 0.06, y+4: 0.03, y+5: 0.07) and ACVE (ie, y+3: 0.14, y+4: 0.05, y+5: 0.14).

In total, 12 deaths were recorded until y+5. Five cases were accounted for as death because of cardiovascular causes (Table I). In 7 cases, death had nonvascular causes (Table II). Two of 3 dialysis patients were among deaths, 1 patient with cardiovascular cause in y+2, and 1 patient with noncardiovascular cause in y+3. Accounting all deaths as cardiovascular deaths lead to mean annual rates for years y+3 to y+5 of MACE in y+3: 0.07, y+4: 0.03, and y+5: 0.08 and of ACVE in y+3: 0.16, y+4: 0.07, and y+5: 0.16. This did not change the significance levels of the comparative analysis of selected study periods (Figure 2A and 2B).

### Table 2. Mean Plasma Concentrations of Lipoproteins and Fibrinogen of the Pre-LA Phase and in the 5-Year Lasting Phase of Regular LA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-LA Phase y-2, y-1, and Before First LA</th>
<th>y+1–y+5, Before LA</th>
<th>LA Phase y+1–y+5, After LA</th>
<th>Reduction Rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a), mg/dL</td>
<td>108.1±46.1</td>
<td>91.1±36.5</td>
<td>28.5±13.5</td>
<td>68.1±9.7</td>
</tr>
<tr>
<td>LDL-C, measured, mmol/L/(mg/dL)</td>
<td>2.56±0.99/(98.9±38.4)</td>
<td>2.65±0.96/(102.2±37.2)</td>
<td>0.90±0.47/(34.7±18.3)</td>
<td>66.3±11.4</td>
</tr>
<tr>
<td>Corrected,* mmol/L/(mg/dL)</td>
<td>1.72±0.66/(66.3±25.4)</td>
<td>1.94±0.81/(75.0±31.2)</td>
<td>0.68±0.31/(26.1±11.8)</td>
<td></td>
</tr>
<tr>
<td>HDL-C,† mmol/L/(mg/dL)</td>
<td>1.35±0.56/(52.3±21.8)</td>
<td>1.29±0.37/(49.8±14.2)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol,† mmol/L/(mg/dL)</td>
<td>4.58±1.30/(176.8±50.2)</td>
<td>4.68±1.18/(180.8±45.6)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Triglycerides,† mmol/L/(mg/dL)</td>
<td>1.92±1.31/(169.8±115.6)</td>
<td>2.25±1.60/(199.1±141.2)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen,† mmol/L/(mg/dL)</td>
<td>10.33±3.99/(351.2±135.6)</td>
<td>9.37±3.03/(318.7±103.2)</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Values indicate mean±SD (conventional units). On average, 4 measurements were available in the pre-LA phase, during the LA phase measurements were done every 6 months. HDL-C indicates high-density lipoprotein cholesterol; LA, lipoprotein apheresis; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); and ND, not done.

*Correction of LDL-C for Lp(a)-derived cholesterol was done with the following formula: corrected LDL-C=measured LDL-C−0.3×(numeric value of Lp(a)).

†Concentrations were measured only immediately before LA treatments.
Genotype and Phenotype of Apo(a) Isoforms

For the analysis of apo(a) isoforms in genotypes and phenotypes, blood was collected at different times. Sample numbers differ from those of the clinical study because not all patients gave their consent for all genetic analyses.

One hundred thirty-six samples were available for analysis of genomic KIV domain copy numbers. The sum of KIV-2 repeats of both LPA alleles were determined by quantitative polymerase chain reaction. In comparison, 2550 participants of the Copenhagen General Population Study (CGPS) with a mean age of 59.5 years served as normal controls. Controls were all free of coronary artery disease or ischemic cerebrovascular disease according to the Danish patient registry. The distribution of Lp(a) concentrations differed significantly between both the cohorts: median of Pro(a)LiFe patients was 109.0 mg/dL (IQR, 77.0 mg/dL–132.0 mg/dL) and median of CGPS patients was 10.1 mg/dL (IQR, 5.2 mg/dL–32.4 mg/dL), \( P<0.0001 \). Pro(a)LiFe patients showed a significantly lower number of KIV-2 repeats in their genome (\( P<0.0001 \), Figure 3).

For 134 patients, apo(a) isoform sizes for both of the 2 LPA alleles were assessed by pulsed-field gel electrophoresis (PFGE), and the apo(a) isoform expression pattern was available from immunoblots. Encoded isoforms ranged in size from 14 to 37 KIV domain copies (Figure 4A). On the DNA level, 59.0% of all alleles were small; 95.3% of the analyzed patients expressed at least 1 small isoform in plasma (Table 3). For 6 patients, it could not be determined whether they expressed only one or both of their alleles in plasma because they carried 2 alleles of the same or closely neighboring isoform sizes, resulting in a single band on the immunoblot. There were 5 homozygote patients (3.9%) detected by PFGE. For the sake

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Seven-year course of the Pro(a)LiFe study with absolute numbers of events (A, major adverse cardiac event [MACE] or [B] adverse cardiac or vascular events [ACVEs] in all vascular beds) and comparative analysis of mean annual event rates in the select study periods.
of simplicity, their 10 alleles were accounted as 5 alleles expressing the total Lp(a) of patients and 5 null alleles. Thus, combined with the 59 heterozygotes with single-band phenotypes, we accounted 64 null alleles. Null alleles were in 90.6% encoding large isoforms (Figure 4A). Isoform-associated Lp(a) concentrations clearly showed that small isoforms carried the bulk of the Lp(a) in the vast majority of patients (Figure 4B). There was a striking difference in the Lp(a) concentrations associated with small (mean, 90.9±46.1 mg/dL) or with large isoforms (mean, 9.1±22.5 mg/dL), \( P < 0.0001 \).

For 121 patients with apo(a) phenotype data, information on their carrier status of variant alleles for the single-nucleotide polymorphisms (SNPs) rs10455872 and rs3798220 was available from previous genotyping. All of these 121 patients expressed at least 1 small apo(a) isoform, with 64.8% of them carrying also at least 1 SNP variant allele. Although all variant alleles were found in patients with such a small apo(a) phenotype, 35.2% of small apo(a) isoform carrying phenotypes were not tagged by either of the variant alleles.

**Discussion**

In this study, incidence rates of cardiovascular events were investigated prospectively during a period of 5 years in 170 consecutive patients who started regular LA to treat
apo(a) alleles in whites, and it had been suggested that they
not designed to investigate whether Lp(a) of small apo(a) iso-
sequence variations or particle compositions. Our study was
apo(a) isoforms confer a particular risk by still unidentified
2 population-based studies in Germany. The abundance of
expressed at least 1 small apo(a) isoform, which is 4× higher
and rs10455872 was markedly increased in Pro(a)LiFe
follow-up with chronic LA, corresponding to a 5-year mor-
ality rate of 3.0%. Thus, only 5 fatal cardiovascular events
reverted an accelerated progressive course of CVD to a stable
course in terms of the incidence rates of cardiovascular events
and mortality.

The most prominent finding of our characterization of
apo(a) genotypes and phenotypes is the high frequency of
patients with small apo(a) isoforms, which have been asso-
related decrease of mean annual incidence rates for MACE or
ACVE was observed comparing 2 years before commencing
regular LA and 2 years during chronic LA. We now extend
these findings by showing that the incidence rates of MACE
or ACVE continued to be low during a total period of 5 years.
The number of patients receiving antihypertensive medication
significantly increased from the time of the first LA (73.5%)
to y=5 (91.6%). There is no reason to think that this change in
medication exerted a major therapeutic effect on the clinical
course during the 5 years of LA treatment. Five deaths
because of cardiovascular causes occurred during 5 years of
follow-up with chronic LA, corresponding to a 5-year mort-
ality rate of 3.0%. Thus, only 5 fatal cardiovascular events
occurred during 804 patient-years. Regular LA seems to have
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The immediate effect of regular LA is pulsed physical
evacuum of apoB-containing lipoproteins including Lp(a), the latter is loaded with oxidized phospholip-
ids. Association of oxidized phospholipids with small apo(a)
isoforms may be a key determinant of cardiovascular risk, High Lp(a) levels and small apo(a) sizes are associated with
endothelial dysfunction. A single LA treatment improves
endothelium-dependent vasodilation, and the elimination of oxidized Lp(a) might be more important to this effect than
oxidized LDL. In particular about corrected LDL-C, Pro(a)
LiFe patients achieved low levels at least 2 years before commencing chronic LA, suggesting that the cardiovascular ben-
efit of LA substantially derived from the additional elimination of elevated concentrations of Lp(a) particles. There was no indication to suppose different clinical efficacy of one of the
LA methods. For all patients included in this study, treatment
volumes according to German reimbursement guidelines were
adjusted for a 60% to 70% reduction of baseline Lp(a) con-
centration. Treatment frequency, treatment volume, or removed
mass of the targeted plasma component can be regarded as gen-
eral parameters of apheresis efficacy. A dose–response relation-
ship for these parameters could not be investigated in this study.

Ruptured plaques tend to have large lipid cores. Improving
plaque morphology could be one underlying mechanism of
action for preventing clinical events by LA. It was hypothe-
sized that LA quantitatively reduced the number of vulnerable plaques and qualitatively limited the propensity of plaques to
rupture and their thrombogenicity. The resulting clinical
benefit of all these mechanistic aspects of LA is the prevention of
cardiovascular events.

PCSK9 inhibitors may play an indirect role in the man-
agement of Lp(a)-HLP because LDL-C needs to be brought
to treatment targets before LA is considered. More than 90% of Pro(a)LiFe patients received LDL-C–lowering medication
throughout the entire study period. PCSK9 inhibitors reduce
Lp(a) levels; however, relative reductions decrease substan-
tially with higher Lp(a) concentrations. Use of PCSK9
inhibitors with their potential to achieve ultralow LDL-C
}
concentrations could facilitate the earlier clinical identification of patients with Lp(a)-HLP and associated progressive CVD.

Mortality data for patients with a risk profile identical to this study are not available. The cohort at baseline is necessarily biased by survival because it does not consider patients with the same characteristics who had already died because of CVD events. Only a randomized controlled trial could finally confirm the results of this 5-year follow-up. Although such a trial of LA has so far been considered unethical in Germany, it might become feasible with novel medicines specifically lowering Lp(a), for example, an antisense drug that has been successfully tested in a phase I clinical trial.27 Because LA eliminates LDL-C and Lp(a), it is not possible to disentangle whether the therapeutic effect derives from lowering Lp(a) or LDL-C or both or even from other compounds that could have an effect on CVD and are eliminated by LA, for example, fibrinogen.2 However, all patients received maximally tolerated LDL-C-lowering drug treatment before their progressive CVD was identified as associated with Lp(a)-HLP; thus, supporting the hypothesis that lowering Lp(a) levels further reduced cardiovascular risk. Finally, there are pronounced differences across ethnicities with regard to Lp(a) levels and pathophysiological relevance of Lp(a). Therefore, our conclusions are valid only for white Europeans.

In summary, results of the 5-year follow-up of the prospective Pro(a)LiFe study support that prevention of cardiovascular events is a rapid and lasting effect of LA in patients with progressive CVD associated with Lp(a)-HLP. Patients were characterized by abundant expression of small apo(a) isoforms, which have been associated with increased cardiovascular risk, although, besides elevated Lp(a) plasma concentration, selection of this patient cohort was based on clinical criteria. Measurement of Lp(a) concentration must be recommended to assess individual cardiovascular risk and to consider extracorporeal clearance of Lp(a) by chronic LA as treatment option for select high-risk patients.

Appendix

Pro(a)LiFe Study Group Coauthors

All coauthors are from Germany, unless otherwise specified. Writing Committee: Eberhard Roeseler, Hannover; Franz Heigl, Kempen; Ulrich Julius, Dresden; Konrad Schmidt and Florian Kronenberg, Innsbruck, Austria; Volker Schettler, Gottingen; and Andreas Heibges and Reinhard Klingel, Cologne. Principal Investigator: Reinhard Klingel, Cologne. Data Monitoring Committee: Thomas Benzing, Cologne and Hildegard Christ and Walter Lehmacher, Cologne. Clinical Investigators: Josef Leebmann, Passau; Eberhard Roeseler, Sabine Wehner, Hannover; Franz Heigl, Ines Schulz-Merkel, Kempen; Ulrich Julius, Dresden; Ralf Spitthoever, Essen; Ralf Kuehn, Dennis Heutiling, Tangermünde; Paul Breitenberger, Germering; Albrecht Wagner, Trier; Wilfried Dtschietzig, Claudia Ernst, Cottbus; Michael Koziol, Gottingen; Johannes Bunia, Isertlohn; Peter Kulzer, Marktheidenfeld; Klaus-Dieter Kraenzle, Memmingen; Markus Toelle, Berlin; Gerhard Riechers, Christine Kuehnel, Braunshweig; Tobias Marsen, Cologne; Christina Saehn, Krefeld; Jens Ringel, Potsdam; Harald Messer, Wuppertal; Andreas Oehring, Suhr; Carsten Schuerfeld, Saarbröss: Michael Wintergarten, Olpe; Volker Schettler, Gottingen; Falko Neu mann, Dresden; Harald Kaul, Deggendorf; Martin Haesner, Juergen Passfall, Berlin; and Andrea Benschneider, Berlin; Stefan Heidemreich, Aachen. Laboratory Investigators: Winfried März, Ruediger Klaas, and Priska Binner, Mannheim; Pia R. Kamstrup and Børge G. Nordestgaard, Copenhagen, Denmark; and Asma Noureen, Konrad Schmidt, Hans Dieplinger, Gertraud Erhart, and Florian Kronenberg, Innsbruck, Austria. Data Management and Statistical Analysis: Andreas Heibges, Cordula Fassbender, Cologne; Walter Lehmacher, Hildegard Christ, Cologne; and Konrad Schmidt, Innsbruck, Austria.

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Disclosures

Dr Julius received honoraria from Fresenius Medical Care, Diamed, and Kaneka (all Germany). Dr Maerz is an employee with ownership interest of Synlab Holding, Germany, and he reports grants and personal fees from Aegerion, Amgen, AstraZeneca, Genzyme, Siemens Diagnostics, Sanofi, Hoffmann-Laroche, Alexion, MSD, Abbott Diagnostics, all outside the submitted work. Dr Klingel received research grants from Asahi Kasei Medical, Japan and Diamed, Germany. Dr Lehmacher received a research grant from Apheresis Research Institute, Germany. All other study group members had none declared. The other authors report no conflicts.

References

from more than 36,000 treatments at one center in Germany. Atheroscler Suppl. 2015;18:154–162. doi: 10.1016/j.atherosclerosisup.2015.02.013.


Lipoprotein Apheresis for Lipoprotein(a)-Associated Cardiovascular Disease: Prospective 5 Years of Follow-Up and Apolipoprotein(a) Characterization

Eberhard Roeseler, Ulrich Julius, Franz Heigl, Ralf Spithoever, Dennis Heutling, Paul Breitenberger, Josef Leebmann, Walter Lehmacher, Pia R. Kamstrup, Børge G. Nordestgaard, Winfried Maerz, Asma Noureen, Konrad Schmidt, Florian Kronenberg, Andreas Heibges, Reinhard Klingel and for the Pro(a)LiFe-Study Group

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http://atvb.ahajournals.org/content/36/9/2019
Clinical course of patients with Lipoprotein(a)-hyperlipoproteinemia and progressive cardiovascular disease.
## Supplemental Material

### Supplemental Tables

Table I: List of patients who terminated the trial before the end of y+5 with causes for termination, and diagnosis of renal artery stenosis.

<table>
<thead>
<tr>
<th>Patient ID code</th>
<th>Year of termination</th>
<th>Cause for termination of LA</th>
<th>Diagnosis of renal artery stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>y+3</td>
<td>Critical limb ischemia, end-stage renal disease, subsequent multi-organ failure</td>
<td>yes</td>
</tr>
<tr>
<td>147</td>
<td>y+4</td>
<td>Heart failure due to CHD</td>
<td>yes</td>
</tr>
<tr>
<td>151</td>
<td>y+4</td>
<td>Myocardial infarction</td>
<td>yes</td>
</tr>
<tr>
<td>83</td>
<td>y+5</td>
<td>Heart failure due to CHD</td>
<td>yes</td>
</tr>
<tr>
<td>142</td>
<td>y+5</td>
<td>Died after heart transplantation, which was indicated by terminal heart failure due to CHD</td>
<td>no</td>
</tr>
<tr>
<td>163</td>
<td>y+1</td>
<td>Neoplastic (colon carcinoma)</td>
<td>no</td>
</tr>
<tr>
<td>52</td>
<td>y+2</td>
<td>Non-medical (traffic accident)</td>
<td>yes</td>
</tr>
<tr>
<td>60</td>
<td>y+3</td>
<td>Neoplastic (colon carcinoma)</td>
<td>yes</td>
</tr>
<tr>
<td>70</td>
<td>y+3</td>
<td>Other medical (sepsis, multi-organ failure)</td>
<td>yes</td>
</tr>
<tr>
<td>114</td>
<td>y+4</td>
<td>Neoplastic (rectal carcinoma)</td>
<td>no</td>
</tr>
<tr>
<td>38</td>
<td>y+5</td>
<td>Neoplastic (gastric carcinoma)</td>
<td>yes</td>
</tr>
<tr>
<td>108</td>
<td>y+5</td>
<td>Other medical (sepsis, multi-organ failure)</td>
<td>yes</td>
</tr>
<tr>
<td>55</td>
<td>y+2</td>
<td>Termination of LA due to patient's wish</td>
<td>no</td>
</tr>
<tr>
<td>154</td>
<td>y+2</td>
<td>Change of treatment center</td>
<td>yes</td>
</tr>
<tr>
<td>94</td>
<td>y+3</td>
<td>Change of treatment center</td>
<td>no</td>
</tr>
<tr>
<td>115</td>
<td>y+3</td>
<td>Termination of LA due to lack of compliance</td>
<td>yes</td>
</tr>
<tr>
<td>12</td>
<td>y+4</td>
<td>Termination of LA due to lack of compliance</td>
<td>yes</td>
</tr>
</tbody>
</table>

LA: lipoprotein apheresis.
Table II: Distribution of LA methods, and treated plasma or full blood volumes of y+2 and y+5, and mean reduction rates of LDL-C and Lp(a) for the entire study period.

<table>
<thead>
<tr>
<th>Lipoprotein apheresis method</th>
<th>Patients y+2 (n=166)</th>
<th>Mean treated volume in y+2 [l]</th>
<th>Patients y+5 (n=154)</th>
<th>Mean treated volume in y+5 [l]</th>
<th>Mean LDL-reduction [%]</th>
<th>Mean Lp(a)-reduction [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods with plasma treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFPP, temperature optimized</td>
<td>101 (60.8)</td>
<td>3.68±0.59</td>
<td>96 (62.3)</td>
<td>3.67±0.63</td>
<td>63.9±11.5</td>
<td>68.1±11.0</td>
</tr>
<tr>
<td>HELP-apheresis</td>
<td>16 (9.6)</td>
<td>3.32±0.50</td>
<td>14 (9.1)</td>
<td>3.22±0.52</td>
<td>60.8±8.0</td>
<td>63.0±11.3</td>
</tr>
<tr>
<td>DSA</td>
<td>6 (3.6)</td>
<td>4.08±0.63</td>
<td>5 (3.2)</td>
<td>3.98±0.86</td>
<td>66.9±9.2</td>
<td>67.1±10.7</td>
</tr>
<tr>
<td>DFPP, simple</td>
<td>4 (2.4)</td>
<td>3.00±0.58</td>
<td>4 (2.6)</td>
<td>3.27±0.64</td>
<td>60.8±7.1</td>
<td>66.4±8.2</td>
</tr>
<tr>
<td>ApoB100-immunoadsorption</td>
<td>4 (2.4)</td>
<td>5.63±2.29</td>
<td>2 (1.3)</td>
<td>4.29±0.48</td>
<td>67.2±10.4</td>
<td>68.3±7.3</td>
</tr>
<tr>
<td>Methods with full blood treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA</td>
<td>24 (14.6)</td>
<td>8.71±1.23</td>
<td>22 (14.3)</td>
<td>8.66±1.30</td>
<td>72.9±9.9</td>
<td>65.3±9.4</td>
</tr>
<tr>
<td>Polyacrylate adsorption</td>
<td>11 (6.6)</td>
<td>8.56±1.81</td>
<td>11 (7.2)</td>
<td>8.41±1.80</td>
<td>74.2±9.2</td>
<td>67.6±8.3</td>
</tr>
</tbody>
</table>

Values indicate absolute numbers (percentages) or mean ± SD. DFPP: double filtration plasmapheresis, HELP: heparin-induced lipoprotein precipitation, DSA: dextran-sulfate adsorption.
Table III: Details of lipid lowering medication of patients throughout the entire study period (y-2 to y+5) which is briefly summarized in the results section of the main text.

<table>
<thead>
<tr>
<th></th>
<th>y-2</th>
<th>y-1</th>
<th>Time of first LA</th>
<th>y+1</th>
<th>y+2</th>
<th>y+3</th>
<th>y+4</th>
<th>y+5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-lowering medication, any</td>
<td>94.1</td>
<td>97.1</td>
<td>95.3</td>
<td>95.3</td>
<td>92.8</td>
<td>92.6</td>
<td>90.4</td>
<td>91.4</td>
</tr>
<tr>
<td>Lipid-lowering medication with statins as one component, all combinations†</td>
<td>90.0</td>
<td>92.5</td>
<td>90.5</td>
<td>90.5</td>
<td>88.5</td>
<td>86.4</td>
<td>84.7</td>
<td>86.2</td>
</tr>
<tr>
<td>Statins, no other drug</td>
<td>25.9</td>
<td>22.4</td>
<td>24.1</td>
<td>27.2</td>
<td>26.1</td>
<td>31.1</td>
<td>34.4</td>
<td>39.2</td>
</tr>
<tr>
<td>Statins + ezetimibe, only</td>
<td>30.0</td>
<td>27.1</td>
<td>30.0</td>
<td>33.7</td>
<td>30.3</td>
<td>26.7</td>
<td>29.9</td>
<td>30.7</td>
</tr>
<tr>
<td>Statins + other lipid-lowering medication*</td>
<td>21.2</td>
<td>27.1</td>
<td>22.9</td>
<td>17.8</td>
<td>20.6</td>
<td>17.4</td>
<td>12.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Statins + ezetimibe + other lipid-lowering medication*</td>
<td>12.9</td>
<td>15.9</td>
<td>13.5</td>
<td>11.8</td>
<td>11.5</td>
<td>11.2</td>
<td>8.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Ezetimibe, no statins, and/or other lipid-lowering medication*†</td>
<td>4.1</td>
<td>4.6</td>
<td>4.8</td>
<td>4.8</td>
<td>4.3</td>
<td>6.2</td>
<td>5.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Nicotinic acid, all combinations</td>
<td>24.7</td>
<td>34.1</td>
<td>27.6</td>
<td>21.8</td>
<td>23.5</td>
<td>21.1</td>
<td>10.2</td>
<td>3.3</td>
</tr>
<tr>
<td>No lipid-lowering medication†</td>
<td>5.9</td>
<td>2.9</td>
<td>4.7</td>
<td>4.7</td>
<td>7.2</td>
<td>7.4</td>
<td>9.6</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Values indicate percentages of patients receiving medication.
*Nicotinic acid, fibrates, cholestyramine, or omega-3-acid ethyl esters. †Figures add up to 100%.
Supplemental Material

Materials and Methods

Study design and patient population
The design of this prospective observational multicenter study conducted at 28 treatment sites throughout Germany has been described before. Timelines included a chronology of the CVD diagnosis, first and second cardiovascular events, a two-year-retrospective period before commencing chronic LA (y-2 and y-1) and 5-year-prospective period with chronic LA (y+1 to y+5). The prospective observation period started on the day of first LA as day zero. The study was approved by the appropriate ethics committee (No. 011/1504, International Ethics Committee, Freiburg, Germany) and reported to an open source online registry (No. DRKS00003119, German Clinical Trials Register, Freiburg, Germany). Sole criterion for patient inclusion was approval by the apheresis committee of health care payers according to German reimbursement guidelines and subsequent initiation of chronic LA. No re-assessment of patients’ approval was performed prior to enrollment in the study group. The approval had to be extended annually by submitting applications for renewal. After 5 years of follow-up, approval of the indication for LA was re-evaluated at least 4 times for all patients. Each patient provided written informed consent for the entire documentation. Since informed consent was not provided by all patients for all genetic analyses of blood samples, sample sizes for the genetic analysis differ from the number of enrolled patients and are given in the respective results section.

Data management
Study sites received standardized case report forms for data collection based upon original patient records as described before. A data and safety monitoring board continued to oversee the study (see list of study group members). Ascertainment of events or procedures relied on careful review of original medical records. All authors vouch for the completeness and accuracy of the data and all analyses that belong into their responsibility.

Lipoprotein apheresis
Standard selective LA procedures used during this study have been described before and were also used in study years 3 to 5. In clinical practice LA represents a highly standardized treatment. Table S2 of the online supplemental material summarizes the use of LA methods, treatment volumes, and reduction rates for LDL-C and Lp(a). For all patients included in this study treatment volumes, according to German reimbursement guidelines, were adjusted for achieving a 60% to 70% reduction of baseline Lp(a) concentration. The LA method and mode of anticoagulation were chosen at the discretion of treatment sites. For whole blood treatments (dextran sulfate adsorption, polyacrylate adsorption) combined anticoagulation with unfractionated heparin (UFH) and citrate was used in general, for HELP-apheresis only UFH can be used, for DFPP and apoB100-immunoadsorption in the vast majority of patients UFH was used, and few patients received citrate or a combination of UFH with citrate.

Laboratory measurements
LDL-C, Lp(a), total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, fibrinogen, hemoglobin, creatinine, and HbA1C in patients with diabetes mellitus were measured in laboratories with long-standing relationships to study sites as previously described, with no change during the 5-years prospective study period. Results are expressed as means values and standard deviations (SD) or as median values and interquartile ranges (IQR). LDL-C mg/dl was converted to mmol/l by factor 0.0259 following recommendations by the American Medical Association Manual of Style, 10th ed.. Lp(a) was reported in mg/dl only, because all measurements in participating sites were done by assays standardized to Lp(a) mass with assay calibrators as supplied by commercial manufacturers. A straightforward conversion of measurements to molar concentrations by a generally agreed factor is not possible due to the polymorphic nature of the molecule. Reduction
rates of LDL-C and Lp(a) were based on measurements immediately before and after LA sessions, and were documented every six months starting with first LA.

**Clinical outcomes**

The primary clinical endpoint was the mean annual incidence rate of cardiovascular events per patient during the first 2 years on chronic LA as compared to the 2 years prior to commencing chronic LA. Upon completion of 5 years of follow-up, incidence rates were analyzed for all single years. Event rates were calculated for each patient including any event in y-2 or y-1 and any event in y+1 until y+5. As previously described two composite endpoints were used. MACE (major adverse cardiac event) was defined as cardiovascular death, non-fatal myocardial infarction (MI), coronary bypass surgery, percutaneous coronary intervention (PCI) or stent. ACVE (adverse cardiac or vascular events) were defined as the sum of all documented cardiac or vascular events in arterial as well as venous vascular beds, i.e. MACE (see above), or cerebrovascular event (non-hemorrhagic, cerebrovascular event = transient ischemic attack (TIA) or prolonged reversible ischemic neurologic deficit (PRIND) or ischemic stroke or carotid percutaneous transluminal angioplasty (PTA) or carotid surgery) or peripheral vascular event (peripheral vascular event of lower extremities or renal arteries = PTA, stent, bypass surgery, amputation) or venous thrombotic event = deep venous thrombosis or pulmonary embolism. Cardiovascular death was not handled with a weight >1 event for calculations. Patients who died from non-cardiovascular causes or who terminated chronic LA were excluded from the analysis of that year (Figure 1). A table listing all 17 patients with their reason of terminating the study can be found in the online supplement (Table S1).

**Polymerase chain reaction (PCR) analysis of KIV polymorphism in LPA**

The apo(a) KIV-2 size polymorphism (KIV-2 CNV) was genotyped by real-time polymerase chain reaction (PCR) analysis using the multiplex real-time PCR genotyping assay on BiRad platform as previously described. Genotyping resulted in an estimate of the total sum of KIV-2 repeats on both alleles. Parallel to genotyping Pro(a)LiFe patients a sample of 2,550 participants from the Copenhagen General Population Study was genotyped and used as a normal control for the distribution of KIV-2 genotypes. The total KIV domain copy number for both alleles was obtained by adding 18 to the KIV-2 repeat copy number.

**Pulsed-Field Gel Electrophoresis (PFGE) for determination of KIV domain copy number**

DNA containing agarose plugs were prepared from whole blood as previously described. Following the previously established protocol, DNA was then subjected to digestion with the endonuclease KpnI (MBI Fermentas), PFGE was run on a Chef mapper system (Biorad, USA), and after Southern blotting and hybridization with a DIG labeled KIV-2 specific probe, the positions of the KpnI fragments were detected. A KIV-2 size standard previously typed by fiber fluorescence in-situ hybridization, and Lambda Ladder PFG Marker (New England Biolabs, USA), were applied as size markers. The total KIV domain copy number for one allele results by adding 9 to the KIV-2 repeat copy number.

**Immunoblotting**

On the protein level, apo(a) phenotyping was conducted by sodium dodecyl sulphate (SDS) gel electrophoresis followed by immunoblotting as described elsewhere with slight modifications (150 ng protein; 1.46% agarose gel, blotting time 30 min). Plasma drawn before a regularly scheduled LA session was used for phenotyping. A mixture of human plasma samples with five apo(a) isoforms of known size was used as reference material.

**Isoform specific Lp(a) concentrations**

In subjects expressing two apo(a) isoforms, the relative expression of the two isoforms was estimated by densitometric evaluation of the apo(a) bands on immunoblots as described elsewhere. Information from genotyping of the KIV-2 CNV was used to discriminate between
homozygotes and individuals with only one expressed allele in case of single-band phenotypes. Mean Lp(a) concentrations before commencing chronic LA were used to calculate the specific isoform associated amount of Lp(a) proportionately to the expression. The whole Lp(a) concentration counted for the expressed isoform in case a subject showed only one apo(a) band.

**Statistical analysis**

Sample size calculation resulting in a target size of 170 patients was described before. Two-sided paired Wilcoxon tests were used to compare MACE and ACVE rates for single years or periods. Dichotomous and continuous variables were compared by unpaired t-test, paired Wilcoxon test, or Mann-Whitney U tests as appropriate. SPSS statistical software package (version 20) was used for analysis.

**References**


4. Kamstrup PR, Nordestgaard BG. Elevated lipoprotein(a) levels, LPA risk genotypes, and increased risk of heart failure in the general population. JACC Heart Fail 2016; 4: 78-87.


