Clinical and Molecular Characterization of Patients with Lipoprotein(a)-Hyperlipoproteinemia and Progressive Cardiovascular Disease Treated by Lipoprotein Apheresis

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Composition of Lp(a)

Lp(a) is composed of apoB-100 covalently bound to apo(a), which is derived from kringle IV (KIV) and KV, and the protease domain of plasminogen.

Plasminogen has 1 copy each of KI to KV and an active protease domain. Apo(a) contains 10 subtypes of KIV repeats, composed of 1 copy each of KIV1, multiple copies of KIV2, and 1 copy of KIV3-10, KV, and an inactive protease-like (P) domain.

In these examples, apo(a) isoforms of 4, 8, 24, and 40 KIV2 repeats are shown, representing 13, 17, 33, and 49 total KIV repeats.

Oxidized phospholipids (OxPL) are present covalently bound to apo(a), and also dissolved in the lipid phase of apoB-100.

Tsimikas J Am Coll Cardiol 2017: 69: 692-711
The atherogenicity of Lp(a) can be broadly classified in 3 categories: proatherogenic, proinflammatory, and potentially antifibrinolytic. The major individual mechanisms within each category are listed. EC=endothelial cell; IL=interleukin; MCP=monocyte chemoattractant protein; PAI=plasminogen activator inhibitor; SMC=smooth muscle cell; TFPI=tissue factor pathway inhibitor.
Distribution of plasma Lp(a) concentrations in the Danish general population

Copenhagen General Population Study
N=69,454

Fraction of population

Lipoprotein(a), mg/dL

max. 387 mg/dl

Prevalence of elevated Lp(a) mass levels in 532,359 patients in the United States

Frequency distribution of Lp(a) mass levels at a referral laboratory, N=531,144.

Observational associations between high plasma Lp(a) concentrations and risk of CVD in the Copenhagen City Heart Study and Copenhagen General Population Study combined

MI: HR 2.4fold higher with Lp(a) >100mg/dl vs < 5 mg/dl

N=58,340; 1897=myocardial infarction

> 30 mg/dl elevated
< 50 mg/dl desirable

Demonstration of increased risk of events in patients with elevated Lp(a) and on statin therapy in the AIM-HIGH, LIPID, and JUPITER trials

This forest plot shows a study-level analysis of baseline LDL-C levels and Lp(a) levels > 4th quartile and the associated hazard ratio compared with the lowest quartile.

AIM-HIGH=Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes; JUPITER=Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; LIPID=Long-Term Intervention with Pravastatin in Ischaemic Disease.

2008 addition of Lp(a)-hyperlipoproteinemia as indication for lipoprotein apheresis to the German reimbursement guideline existing since 1991

...patients.....with isolated* Lp(a)-elevation > 60 mg/dl (or > 120 nmol/l) and LDL-cholesterol in normal range **, and progressive cardiovascular disease (coronary artery disease, peripheral arterial disease, cerebrovascular disease) as revealed clinically and by imaging techniques.

(*isolated stands for optimized control, i.e. maximum treatment, of all other cardiovascular risk factors of a patient.)

(**normal range stands for close to target levels).

Federal Joint Committee additionally stipulated that prospective clinical study data are required to maintain this decision on reimbursement. Ethical approval was denied for a randomized controlled trial.

According to the latest official figures there are app. 3000 chronic lipoprotein apheresis patients in Germany including app. 45% with the indication of iLp(a)-HLP.
Sole criterion for patient enrollment was approval and subsequent commencement of chronic LA due to isolated Lp(a)-HLP with progressive CVD by the apheresis committee of the regional association of statutory health insurance physicians, or directly by the individual statutory or private health insurance fund according to German reimbursement guidelines.

Application for approval of chronic LA was solely in the responsibility of patients’ physicians. No additional assessment of patients’ applications was performed prior to enrollment by the study group. Date of actual start of chronic LA for a single patient was the result of the clinical course, including previous diagnostic examinations and treatment, and the variable timeline of the application process until approval, which can be weeks to months. Therefore the actual date of the first LA was not affected by enrollment in the observational study. All participating centers confirmed, that all consecutive patients commencing chronic LA due to Lp(a)-HLP were enrolled during the study period.
According to the guideline of German statutory health insurances cardiovascular events cover coronary, peripheral and cerebrovascular beds, due to pathogenic effects exerted by Lp(a) also events of venous thrombosis were documented:

**MACE (major adverse cardiac event)**
was the primary composite outcome parameter, i.e. cardiovascular death, non-fatal myocardial infarction, coronary bypass surgery, PTA, or stent.

**ACVE (adverse cardiac or vascular events)**
is the secondary composite outcome parameter, 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 acute arrhythmia, or pacemaker implantation, or cerebrovascular event [non-hemorrhagic, cerebrovascular event = TIA or PRIND or ischemic stroke or carotid 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 (DVT) or pulmonary embolism.
Patient flow

171 patients

Enrollment

1 withdrawal of approval

170 retrospective data years -2 and -1

170 allocated to apheresis

Treatment

y+1: 169 analyzed

1 terminated treatment

y+2: 166 analyzed incl. 1 cv death

3 terminated treatment

Analysis

y+3: 161 analyzed incl. 2 cv deaths

5 terminated treatment

y+4: 157 analyzed incl. 1 cv death

4 terminated treatment

y+5: 154 analyzed incl. 1 cv death

3 terminated treatment

Analysis

90.6%
Pro(a)LiFe
5 years results: treatment intervals

**treatment intervals year +1**

- **weekly**
  - 92%
  - n = 157

- every 2 weeks
  - 5%, n = 9

- every 3 weeks
  - 1%, n = 1

- 2 per week
  - 2%, n = 3

**treatment intervals year +5**

- every 2 weeks
  - 10%, n = 15

- every 3 weeks
  - 2%, n = 3

- >1 – 2 per week
  - 6%, n = 9

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**Peripheral vascular access**

- 80%

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- **Leebmann et al. Circulation 2013; 128: 2567-2576**
Mean plasma concentrations of lipoproteins and fibrinogen of the pre-LA phase and in the 5 years phase of chronic LA

<table>
<thead>
<tr>
<th></th>
<th>Pre-LA phase</th>
<th></th>
<th>LA phase</th>
<th>Reduction rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y-2, y-1, and before first LA</td>
<td>y+1 - y+5, before LA</td>
<td>y+1 – y+5, after LA</td>
<td></td>
</tr>
<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>p</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C, measured, mmol/1/[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>p</td>
<td>0.140</td>
<td></td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>LDL-C, corrected*, mmol/1/[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>ND</td>
</tr>
<tr>
<td>HDL-C**, mmol/1/[mg/dl]</td>
<td>1.35±0.56 / [52.3±21.8]</td>
<td>1.29±0.37 / [49.8±14.2]</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Total cholesterol**, mmol/1/[mg/dl]</td>
<td>4.58±1.30 / [176.8±50.2]</td>
<td>4.68±1.18 / [180.8±45.6]</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Triglycerides**, mmol/1/[mg/dl]</td>
<td>1.92±1.31 / [169.8±115.6]</td>
<td>2.25±1.60 / [199.1±141.2]</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Fibrinogen**, μmol/1/[mg/dl]</td>
<td>10.33±3.99 / [351.2±135.6]</td>
<td>9.37±3.03 / [318.7±103.2]</td>
<td></td>
<td>ND</td>
</tr>
</tbody>
</table>

Values indicate mean ± SD [conventional units]. ND indicates not done; HDL-C, high-density lipoprotein cholesterol; LA, lipoprotein apheresis; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); and SD, standard deviation. On average 4 measurements were available in the pre-LA phase, during the LA phase measurement were done every 6 months. *Correction of LDL-C for Lp(a) derived cholesterol was done with the following formula: corrected LDL-C = measured LDL-C − 0.3x[numerical value of Lp(a)]. **Concentrations were measured only immediately before LA treatments.

Leebmann et al. Circulation 2013; 128: 2567-2576
### Pro(a)LiFe – lipid lowering medication

<table>
<thead>
<tr>
<th></th>
<th>y-2</th>
<th>y-1</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>
</tr>
<tr>
<td>Lipid-lowering medication including statins, 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>
</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>
</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>
</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>
</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>
</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>
</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>
</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>
</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%.
Mean annual rates of MACE (major adverse cardiac events) for single years before and after commencing lipoprotein apheresis (LA) demonstrating sustained efficacy of LA

<table>
<thead>
<tr>
<th>Year</th>
<th>MACE events</th>
<th>Mean annual rates of MACE in selected study period</th>
</tr>
</thead>
<tbody>
<tr>
<td>y-2</td>
<td>51</td>
<td>0.30±0.58</td>
</tr>
<tr>
<td>y-1</td>
<td>91</td>
<td>0.54±0.70</td>
</tr>
<tr>
<td>y+1</td>
<td>23</td>
<td>0.14±0.34</td>
</tr>
<tr>
<td>y+2</td>
<td>8</td>
<td>0.05±0.21</td>
</tr>
<tr>
<td>y+3</td>
<td>11</td>
<td>0.07±0.25</td>
</tr>
<tr>
<td>y+4</td>
<td>6</td>
<td>0.04±0.19</td>
</tr>
<tr>
<td>y+5</td>
<td>8</td>
<td>0.05±0.22</td>
</tr>
</tbody>
</table>

-78%

Leebmann et al. Circulation 2013; 128: 2567-2576
Mean annual rates of ACVE (adverse cardiac or vascular events) for single years before and after commencing lipoprotein apheresis (LA) demonstrating sustained efficacy of LA

Leebmann et al. Circulation 2013; 128: 2567-2576

5-year cv mortality 3% (5/166) or 5 fatal cv events during 804 patient-years
Clinical course of patients with Lp(a)-HLP-associated progressive cardiovascular disease according to results of the Pro(a)LiFe study

<table>
<thead>
<tr>
<th>Diagnosis of cardiovascular disease</th>
<th>1st cardiovascular event</th>
<th>2nd cardiovascular event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median ages</td>
<td>48.9</td>
<td>49.5</td>
</tr>
<tr>
<td>Study years</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5 prospective study years with regular lipoprotein apheresis (LA), peripheral vascular access: 80% in year+1, 72% in year+5, weekly LA: 92% year+1, 82% year+5

4.7 years (median) of emerging progressive course of cardiovascular disease and identification of Lp(a)-HLP

MACE: major adverse cardiac event

Imaging: progression of CVD without clinical event was accounted as equivalent event with the following findings: incidence of new or additional CVD at a new vascular location or region, or deterioration of existing CVD, e.g. 2-vessel CAD progressed to 3-vessel CAD, new appearance of stenosis or plaques within an already affected vessel or vessel region, >20% deterioration of existing stenosis, appearance of in-stent stenosis, or stenosis in artery bypass.
Baseline Lp(a) plasma concentration in Pro(a)LiFe patients compared to the Copenhagen General Population Study (CGPS)

Lp(a) plasma concentration in 136 Pro(a)LiFe patients. For comparison a sample of 2550 participants of the Copenhagen General Population Study is depicted (free of cardiovascular disease in 2013, mean age 59.5yrs [min/max 20.2/96.2] and mean Lp(a) 23.7 mg/dl [min/max 0.5/209.4]) (Pia Kamstrup, personal communication).
Lp(a) kringle-IV-type-2 (KIV-2) repeat numbers (sum of both alleles) in Pro(a)LiFe patients compared to the Copenhagen General Population Study (CGPS)

Total number of KIV-2 repeats (sum of both alleles) according to PCR analysis in DNA of 136 Pro(a)LiFe patients. For comparison a sample of 2550 participants of the Copenhagen General Population Study is depicted (free of cardiovascular disease in 2013, mean age 59.5yrs [min/max 20.2/96.2] and mean Lp(a) 23.7 mg/dl [min/max 0.5/209.4]) (Pia Kamstrup, personal communication).
Western blot analysis of apo(a) isoform expression with quantification by densitometry

Calculation of isoform associated Lp(a):

- total Lp(a): 50 mg/dl
- KIV-CNV genotype: 24 KIV / 34 KIV
- Expression (density on WB): 10% / 90%

→ 24 KIV isoform: 5 mg/dl
→ 34 KIV isoform: 45 mg/dl

PFGE: DNA
Western blot: apo(a) isoforms

Schmidt, Kronenberg, Innsbruck
Pro(a)LiFe patients:
small (≤22 KIV domain copies) allele genotype
and high risk allele variants of SNPs rs41055872 or rs3798220

- Genotype with at least 1 small LPA allele, phenotype with expression of at least 1 small apo(a) isoform
- Genotype with 2 large LPA alleles
- Negative for SNPs rs41055872 or rs3798220
- Positive for SNPs rs41055872 or rs3798220

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Lp(a) levels of Pro(a)LiFe patients in mg/dl and in nmol/l after conversion with KIV domain copy number specific conversion factors according to percentage contribution of isoforms to total patients’ Lp(a)

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Therapy escalation for Lp(a)-hyperlipoproteinemia according to current German guidelines

- **Lipoprotein-Apheresis**
  - $\downarrow >60\%$-$70\%$
  - Final step (annual re-evaluation)
  - (apo(a) antisense under investigation in clinical trials [IONIS-APO(a)-LRx])
  - $\downarrow \approx 80\%$

- **Max. LDL-C lowering drug treatment (target levels)**
  - $\downarrow \approx 30\%$ (-50%) for Lp(a) hypercholesterolemia (correction of measured LDL-C)

- **ODYSSEY ESCAPE**:
  - $\downarrow 4.1\% \pm 10\%$

- **Therapy of all existing cardiovascular risk factors**
  - **Steel, physical activity**
  - **Therapy of Lp(a)**
    - $\downarrow \approx 30\%$ with PCSK9 inhibitor
    - $\approx 15\%$-$20\%$ with high Lp(a)
    - $\uparrow \approx 80\%$ with statins
    - $\uparrow \approx 10\%$ with statins

- **No change**