

Dyslipoproteinemia therapy with lipoprotein-apheresis and/or human monoclonal antibodies

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Abstract

The prognosis of patients suffering from severe dyslipoproteinemia, sometimes combined with elevated lipoprotein (a) (Lp(a)), and coronary artery disease (CAD) refractory to diet and lipid-lowering drugs has been improved by the introduction of the lipoprotein-apheresis, and the human monoclonal antibodies (HMA) in different studies. All severe forms of dyslipoproteinemia can be treated successfully with these methods alone or in combination. Different lipoprotein-apheresis systems are available which reduce LDL cholesterol, Lp(a), triglycerides and others: cascade filtration or lipoproteinfiltration, immunoadsorption, heparin-induced LDL precipitation, dextran sulfate LDL adsorption, LDL hemoperfusion, and/or different HMA. There is a strong correlation between dyslipoproteinemia and atherosclerosis. Besides the elimination of other risk factors in severe dyslipoproteinemia therapeutic strategies focus on a drastic reduction of serum lipoproteins. In such patients in whom the maximum drug therapy failed, lipoprotein-apheresis (LA) is indicated. Technical and clinical aspects of these different lipoprotein-apheresis methods and results of the application of HMA are shown here. The published data clearly demonstrate that treatment with lipoprotein-apheresis in patients suffering from severe dyslipoproteinemia refractory to conservative therapy are effective and safe in long application. A disadvantage is the high costs and the expensive technologies of the different lipoprotein-apheresis methods. The costs of the therapy with HMA are lower than the costs of the lipoprotein-apheresis but larger studies are necessary to show what method could be preferred.

Introduction

In the last 35 to 40 years therapeutic plasma exchange (TPE) was used as an extracorporeal treatment option for patients with homozygous or severe heterozygous familial hypercholesterolemia (FH). Some years later more semi- or selective separation methods, the so-called lipoprotein-apheresis were successfully introduced in the therapy of dyslipoproteinemia.

Brown and Goldstein showed that circulating low density lipoprotein (LDL) is absorbed into the cell through receptor-bound endocytosis [1,2]. The adsorption of LDL into cell is specific and is mediated by a LDL receptor. This receptor is changed such that the LDL particles can no longer be recognized in patients with FH. The adsorption of LDL particles cannot be mediated and caused to an accumulation of LDL which resulted in the so-called hypercholesterolemia [3]. An excess supply of cholesterol also blocks the 3-hydrox-3 methylglutaryl-Co enzyme A (HMG CoA), reductase enzyme, which otherwise inhibits the cholesterol synthesis rate. The structure of the LDL receptor was determined by Brown and Goldstein. They found also structural defects in the receptor in many patients with FH [1-3]. The first metabolic disease which could be related to the mutation of a receptor gene was FH.

FH is an autosomal dominant disorder associated with well-characterized mutations of hepatocyte apolipoprotein-B (apo-B) receptors which are usually called LDL receptor. The result is a decreased LDL removal by the liver. Homozygote patients may have cholesterol in the range of 650–1,000 mg/dL, xanthoma by the age of 4 years, and death from coronary heart disease by the age of 20 and heterozygote patients may have cholesterol in the range of 250–550 mg/dL, xanthoma by the age of 20 years, and atherosclerosis by the

age of 30, both without therapy [4]. Lipoprotein-apheresis removes all cholesterol and lipoproteins very effectively and reduces the high inflammatory activity of the atherosclerotic vessel wall in patients with dyslipoproteinemia and overt atherosclerosis [5]. The standard therapy of patients with homozygous and severe heterozygous FH has been diet, lipid-lowering drugs, and in severe cases lipoprotein-apheresis.

A newer therapy of dyslipoproteinemia is the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Monoclonal antibody inhibition of the PCSK9 has demonstrated reductions in LDL blood concentrations in several studies, most of them have been of short duration with small numbers of patients, and relationship to dose, sex and background lipid therapy have not fully established [6,7]. But newer studies such as the ODYSSEY ESCAPE and the FOURIER trials with more patients and various doses of PCSK9 inhibitors showed a reduction of more than 60% of LDL cholesterol under this therapy [8,9]. PCSK9 inhibition significantly reduced cardiovascular risk in patients.

The authors will present the different therapeutic apheresis (TA) methods for cholesterol elimination and some results of the therapy with the HMA against PCSK9. All the techniques of lipoprotein-apheresis

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described here are effective and well tolerated [10]. A disadvantage is the high costs of the different artificial methods and of the application of HMA.

Pathophysiology of dyslipoproteinemia

There are more than 10,000,000 people with FH worldwide, mainly heterozygous; FH is one of the most common inherited disorders; Mutations along the entire gene that encode for LDL receptor protein are the most common FH cause. But mutations in apolipoprotein B and protein convertase subtilisin/kexin type 9 genes produce this phenotype have been also described [11]. A high concentration of circulating LDL is usually combined with a simultaneous increase in very low-density lipoprotein (VLDL) and a decrease in high density lipoprotein (HDL) especially for familial combined hypercholesterolemia. This situation leads to a development of atherosclerosis and, in particular, of CAD. Heterozygous FH has a frequency of 1: 500 may be closer to 1:250 and the homozygous form a frequency of 1: 1,000,000. Patients with FH nearly all die before the age of 30 without therapy [12]. In contrast to rare FH, in most patients dyslipoproteinemia have a complex genetic etiology consisting of multiple variants as established by genome wide association studies [13]. Genetic assessment can help to identify patients at risk for developing dyslipoproteinemia and for treatment decisions based on “risk allele” profiles.

Atherosclerosis with myocardial infarction, stroke, and peripheral vascular disease still maintains its position at the top of morbidity and mortality statistics in industrialized nations [14]. Risk factors which are widely accepted are besides familial disposition, smoking, arterial hypertension, diabetes mellitus, and central obesity stress, reduced HDL, increased Lp(a), and fibrinogen.

A strong correlation between dyslipoproteinemia and atherosclerosis was found [15,16]. The largest endocrine, paracrine, and autocrine participant in the regulation of numerous homeostatic vascular functions is the vascular endothelium. Changes in hemodynamic forces such as pressure and shear stress as well as circulating and locally formed vasoactive substances released by blood cells are sensed by endothelial cells. These endothelial cells synthesize and release biologically active substances such as nitride oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor, endothelin, prostaglandin H₂, thromboxane A₂, heparin sulfate, transforming growth factor, vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor, tissue plasminogen activator, plasminogen activator inhibitor-1, oxygen free radicals, and others [17]. All these substances together modulate vascular tone through their relaxing and contracting actions as well as vascular structure through production of growth promoting and growth-inhibiting factors.

In dyslipoproteinemia patients, intravenous reconstituted HDL infusion rapidly normalizes endothelium-dependent vasodilatation by increasing NO bioavailability. This can perhaps explain the protective effect of HDL on coronary heart disease and the potential therapeutic benefit of increasing HDL in patients at risk from atherosclerosis [18]. The endothelium also regulates hemostasis and thrombosis through its antiplatelet, anticoagulant, and fibrinolytic functions as well as inflammation through the expression of chemo tactic and adhesion molecules. The endothelium is in a strategic location between the blood and vascular smooth muscle and this is a primary target for injury from mechanical forces and processes related to cardiovascular risk factors [10]. The development of atherosclerotic plaques especially in

CAD results from high lipid concentration in the blood which leads to their accumulation in the intima. The changes in vessel tone and endothelium regulation seem to be accompanied by these alterations.

Hypertriglyceridemia is prevalent in 18.6 percent of men and 4.2 percent of women between the ages of 16 and 65 and a positive correlation between elevated triglyceride blood levels and heart attacks has been established [19]. Increased triglycerides (TG) are often accompanied by low-HDL cholesterol blood levels. High triglycerides represent a useful marker for risk of CAD, particularly when HDL levels are low [10]. The strong association between the ratio of TG/HDL and the risk of CAD suggests a metabolic interaction between the TG and cholesterol ester-rich lipoproteins in increasing risk of CAD [20]. A cumulative insult to the vasculature resulting in more severe disease which occurs at an earlier age in large and small vessels as well as capillaries is caused in dyslipoproteinemia in combination with diabetes mellitus. An independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemias on endothelial function, suggesting oxidative stress as common mediator of such effect was shown by Ceriello *et al.* [21].

The LDL/HDL ratio is a strong predictor of premature CAD events. In patients with a ratio of >5.0 with high triglyceride concentrations the risk of coronary events is to those with normal triglyceride concentrations four times higher [22]. The extent of postprandial-lipemia is an antiatherogenic factor and correlates inversely with HDL cholesterol. A high concentration of HDL is a sign that triglyceride-rich particles are quickly decomposed in the postprandial phase of lipemia. But often high HDL cholesterol concentrations are not protective against atherosclerosis. Excessively high triglyceride concentrations are accompanied by very low HDL counts. Acute pancreatitis is one of the most severe complications of severe hypertriglyceridemia [23]. Rapid lowering by TA of the excessively elevated triglyceride concentrations, activated enzymes, released cytokines and other inflammatory substances is therefore a primary goal in these patients.

Lp(a) which is very similar to LDL is a further important atherogenic substance. Lp(a) also contains Apo (a), which is very similar to plasminogen, enabling Lp(a) to bind to fibrin clots [24]. Into the walls of the arteries thrombi are integrated and become plaque components. Many studies show that high Lp(a) concentrations are associated with an early occurrence of CAD and apoplectic insult [10].

High plasma concentrations of Lp(a) are primarily genetically determined by variation in the Lp(a) gene coding for apolipoprotein(a) [25]. If CAD is mainly primarily associated with Lp(a) concentrations or with the six different phenotypes (S4, S3, S2, S1 Band F) has not been determined. High concentrations of Lp(a) are associated with CAD, and patients with premature CAD showed the highest Lp(a) concentrations. Lp(a) and Apo A enhance proliferation of human smooth muscle cells in culture by inhibiting the activation of plasminogen to plasmin, thus blocking the proteolysis activation of transforming growth factor- β , is an autocrine inhibitor of human vascular smooth muscle cells [26]. It was shown that Lp(a) is a major independent risk factor for atherosclerosis, increasing cardiovascular morbidity and mortality at a younger age [27,28].

Oxidized Lp(a) is more potent than native Lp(a) in stimulating vascular smooth cells and may play an important role in the pathogenesis of vascular disease [29]. Van der Valk found that Lp(a) induces monocyte to the arterial wall and mediates pro-inflammatory responses through its pro-inflammatory oxidized phospholipids (OxPL) content [30]. Elevated Lp(a) concentrations are correlated with the

extent of CAD and the mortality of these patients. These findings provide a novel mechanism by which Lp(a) mediates cardiovascular diseases [31]. As with lipoprotein-apheresis in homozygotes-specific Lp(a) is a life-saving therapy in severe cases with elevated Lp(a) as the sole risk factor.

An acquired Lp(a) excess in patients with renal disease is a marker for cardiovascular risk [32]. In renal disease high Lp(a) concentrations suggest an important role of the kidneys in the metabolism. In the United States, among older adults an elevated level of Lp(a) lipoprotein is an independent predictor of stroke, death from vascular disease, and death from any cause in men, but not in women [33]. Elevated Lp(a) blood concentrations and genotypes were associated with increased risk of aortic valve stenosis in the population, with concentration > 90 mg/dL predicting a threefold increased risk [34]. Future investigations and potential research projects must clarify the role of Lp(a) in stroke [35].

No sufficient drug therapy has been available to decrease high Lp(a) levels. N-acetylcysteine has been shown to induce a dose-dependent reduction in Lp(a) levels about seven percent by causing dissociation of the Apo A by cleavage of disulfide bonds [36]. Very high Lp(a) levels can only be normalized by lipoprotein-apheresis [37,38].

The newer therapy concept is the proprotein convertase subtilisin/kexin type 9 with Evolocumab, as a fully HMA directed against human PCSK9. Evolocumab up regulates LDL receptors causing increased catabolism of LDL and the reduction of LDL and is higher than of Lp(a) blood concentrations. Several studies using HMA inhibition of PCSK9, Evolocumab could reduce LDL of 53% to 75% and Lp(a) 24 to 39% in monotherapy or combination therapies, and is associated with minor adverse effects [6,39]. Further studies showed that the inhibition of PCSK9 with HMA is as effective as the regularly weekly or every two-week lipoprotein-apheresis. For both methods the lipoprotein-apheresis and HMA were comparable in the higher decreases of pre-treatment concentrations over the time. In the most severe cases both lipoprotein-apheresis and HMA can be used alone or combined.

Lipoprotein-apheresis

In the industrial nations, CAD remains one of the main causes of death in the mortality statistics despite considerable progress in diagnostics, development of new medications, such as HMG-CoA-reductase inhibitors as well as cardiosurgical measures. Cholesterol concentrations of over 200 mg/dL show an increased coronary risk. "This risk is double at cholesterol values between 200–250 mg/dL and fourfold at values of 250–300 mg/dL" [40].

A relative or absolute reduction of LDL receptors in the liver results in a decreased plasma clearance of lipids in severe forms of hypercholesterolemia. For all these patients, reduction intake of dietary fats is advised. Various medications are available, such as colestyramin, colestipol, β -fibrates, fenofibrate, β -pyridylcarbinol, probucol, and D-thyroxine depending on type of condition. With the introduction of HMGCoA-reductase inhibitors, which can also be combined with other lipid-lowering drugs, LDL reduction up to 50 percent of the original concentration can be achieved. In most cases, this appears to be sufficient. Studies with large numbers of patients have investigated the affectivity and safety of the various HMG-CoA-reductase inhibitors. Numerous side effects like diarrhea, obstipation, other gastrointestinal diseases, myositis, rhabdomyolysis, and others were observed [41]. But with the introduction of lipoprotein-apheresis all forms of previous therapy-resistant dyslipoproteinemia can now be effectively treated [42].

Severe heterozygous forms of FH or other forms of dyslipoproteinemia with cholesterol values between 250 and 600 mg/dL are indicated for lipoprotein-apheresis. These forms first require maximum dietetic and medication therapy, for example with, 24–32 g ion exchanger (e.g., colestyramin) in combination with 40–80 mg statins (HMGCoA-reductase inhibitors). If, despite this maximum therapy or due to therapy intolerance, LDL cholesterol cannot be constantly held below 150 mg/dL, then lipoprotein-apheresis is indicated. In exceptional circumstances should be patients over 60 years of age given lipoprotein-apheresis treatment. In patients with isolated Lp(a) higher than 60 mg/dL, normal LDL cholesterol and a progressed cardiovascular disease the lipoprotein apheresis is indicated, too. The patient should be a non-smoker. All patients should be placed under cardiologic observation with ECG under exercise, thallium scintigraphy, and, possibly, coronary angiography, to register reduced progression or the desired regression of the coronary heart condition.

The quotient relevant for cost-effective assessment: [cost of treatment—costs saved]: [improvement in life quality] cannot be exactly calculated at present. To calculate it, detailed information is required about the expenses saved through illnesses avoided (heart attack, angina pectoris, and premature coronary death) [10]. Besides diet and lipid lowering drugs the lipoprotein-apheresis can be the standard therapy. Up to now cascade filtration, immunoadsorption, heparin-induced LDL precipitation, LDL adsorption through dextran sulfate, the LDL hemoperfusion systems have been of clinical relevance. The acute reduction rates of LDL cholesterol and of Lp(a) depend on the treatment volume achieved with each LA method (Table 1) [43].

Cascade filtration (CF), membrane differential filtration (MDF), or lipid filtration seems to be superior to conventional plasmapheresis but less effective than adsorption or precipitation techniques (Table 2) [14,42,44]. The CF was developed by Agishi *et al.* 1980 in Japan and was the first semiselective technique used for treating dyslipoproteinemia [44]. The secondary membrane in cascade filtration has a cut off of approximately one million daltons. LDL particles with a molecular weight of approximately 2,300,000 daltons are thus retained by this membrane. All other molecules, which are larger than one million daltons are also retained, while plasma components smaller than one million daltons pass through the membrane and are returned to the patient. With a treatment of 1.0–1.2 total plasma volume (TPV), total cholesterol can be reduced by approximately 35–50 percent of the original value and LDL cholesterol by approximately 30–45 percent thereof (Table 1) [42]. Due to irregular pore distribution with different diameters in the secondary membrane, plasma components with smaller molecular weight can also be retained, such as fibrinogen (MW: Å 340,000), HDL (MW: Å 400,000), and IgM (MW: Å 1,000,000) [45]. To reduce fibrinogen, MDF is an effective method.

With synthetic secondary membranes such as the lipidfilter EC-50 (Asahi, Japan), and newer types of machines, better effectiveness and selectivity in the separation of the blood components can be reached in the treatment of dyslipoproteinemia. MDF is as safe and effective as the HELP-system with respect to the extracorporeal removal of LDL-cholesterol, Lp(a), fibrinogen, by treating identical plasma volumes. Fully automated apheresis machines have been developed so that continuous manual steering of flows and blood pressures is no longer required (Octo Nova/Diamed, Monet/Fresenius, both Germany) [45,46].

Immunoadsorption (IA) was first described by Stoffel *et al.* in 1981 as LDL-apheresis through sepharose columns coated with LDL antibodies, or other antibodies (Table 2) [47]. The LDL molecules in the

Table 1. Effectiveness of the various lipoprotein-apheresis, reduction of the original concentration (modified after 43)

| | Cascade filtration | Immunoadsorption | Heparin-induced LDL precipitation (HELP) | LDL-adsorption (dextran sulfate, Liposorber) | LDL hemo-perfusion (DALI) | LDL hemoperfusion (Liposorber D) |
|-------------------------------------|--------------------|------------------|--|--|---------------------------|----------------------------------|
| Treated total plasma volume | 1.0–1.2 | 1.6–2.0 | 1.0–1.2 | 1.0–1.2 | 1.6 blood volume | 1.6 blood volume |
| Reduction of original concentration | % | % | % | % | % | % |
| Cholesterol | 35 - 50 | 30 | 50 | 45 | 60 | 55 |
| LDL | 30 - 45 | 35 | 45 | 30 - 40 | 60 - 75 | 60 - 75 |
| HDL | 30 - 50 | 20 | 10 - 20 | - | 16 - 29 | 5 - 13 |
| Lp(a) | 60 - 70 | 60 | 46 | 60 | 60 - 75 | 60 - 75 |
| Triglycerides | 60 | 60 | 60 | 70 | ca 40 | ca 66 |
| Fibrinogen | 50 | 10 - 20 | 50 | 30 | 16 | 20 - 40 |
| IgM | 35 | 10 - 20 | - | - | 21 | 14 |
| IgA | 55 | 10 - 20 | - | - | - | - |
| Factor VIII | - | 10 - 20 | 10 - 20 | 20 | - | - |
| C 3 | - | - | 50 | - | - | - |
| C 4 | - | - | 50 | - | - | - |
| Plasminogen | - | - | 50 | - | - | - |

Table 2. Extracorporeal methods, human monoclonal antibodies and other drugs for elimination of LDL cholesterol

| Year | Authors | Method | Advantage | Disadvantage |
|------|-----------------------|--|--|-------------------------------------|
| 1980 | Agishi et al. [44] | Cascade filtration | Semi-selectivity | Expensive technology |
| 1981 | Stoffel et al. [47] | Immunoadsorption | Selectivity, effectiveness, regeneration, reusable | Expensive technology |
| 1982 | Seidel et al. [51] | Heparin-induced LDL precipitation (HELP) | Selectivity, effectiveness | Expensive technology |
| 1985 | Nosé et al. [52] | Thermo-filtration | Selectivity, effectiveness | Extensive technology, not available |
| 1987 | Mabuchi et al. [60] | Dextran sulfate LDL adsorption | Selectivity, effectiveness | Expensive technology |
| 1991 | Pokrovsky et al. [50] | Lp(a) immunoadsorption | Selectivity, effectiveness | Expensive technology |
| 1993 | Bosch et al. [67] | LDL hemoperfusion (DALI) | Selectivity, effectiveness, | Expensive technology |
| 1995 | Grossmann et al. [89] | Lomitapide, Mipomersen | Low effectiveness | Unknown, Not available |
| 2002 | Klingel et al. [68] | Lipid filtration | Semi-selectivity | Expensive technology |
| 2003 | Otto et al. [72] | LDL hemoperfusion (Liposorber D) | Selectivity, effectiveness, | Expensive technology |
| 2010 | Kreuzer et al. [46] | Lipoproteinfiltration (Monet) | Semi-selectivity | Expensive technology |
| 2014 | Raal et al. [6] | Evolocumab | Selectivity, effectiveness | Unknown |
| 2014 | Gaudet et al. [7] | Alirocumab | Selectivity, effectiveness | Unknown |

plasma after primary separation are adsorbed in the columns onto the antibodies. This is a reversible antigen-antibody bond accord based on the principle of affinity chromatography. Antibodies against the protein component of the LDL particles gained from sheep as heteroclonal sheep antibodies against apo protein B are covalently bound to sepharose particles. In one column, 3 grams of LDL cholesterol can be adsorbed. Both columns contain 300–320 ml or less volume of sepharose particles. Before one column is saturated with the adsorbed lipoproteins (600–800 mL plasma), the plasma flow is switched to the other column; while one column is used for adsorption, the off-line column is regenerated with neutral saline buffer solution, lysine buffer (pH 2.4), and neutral buffer again. The treated plasma is then mixed with the cellular components of the blood and returned to the patient. The whole procedure takes 2.5–3 hours with the computerized apheresis monitor [10]. After the treatment, the columns are rinsed, and filled with sterile solution.

Required volume of plasma of 3–10 L can be perfused in one treatment session. The advantage of this method is the high selectivity, effectiveness for all apo-B-containing lipoproteins, and regenerating

capacity of the columns. The disadvantage is the high expenditure required not only for the treatment itself, but also for the regeneration process [47]. A long-term basis that is to say, at least 20 times per patient is only viable for the high costs of the columns (LDL Therasorb system, Miltenyi Biotec, Germany) At a perfusion of 1.2 – 2.4 TPV per session, the LDL cholesterol is reduced to 30–40 percent of the original concentration. HDL, serum proteins, immunoglobulins, and fibrinogen, and others drop by approximately 15–20 percent and return to their normal level, after approximately 24 hours (Table 1). The system is safe and effective in clinical use, even in longterm treatment. The efficacy of the lipoprotein-apheresis IA columns did not decrease after 60 treatments sessions and the columns selectivity also remained unchanged [48,49].

Lipoprotein (a)-apheresis is the most effective therapeutic method in lowering Lp(a). Special immunoadsorption polyclonal antibody columns containing sepharose bound anti-Lp(a) have been available (Lipopak, Pocard, Moscow, Russia) (Table 2) [50]. It is an immunosorbent by immobilizing of antibodies to Sepharose CL-4B. Two personal columns are assigned to each patient for the treatment.

Each column is filled with 400 mL of sorbent tested for sterility and pyrogenicity. Anti-Lp(a) immunoabsorption columns are reusable. As with lipoprotein-apheresis in homozygotes-specific Lp(a) is a lifesaving therapy in severe cases with elevated Lp(a) as the sole risk factor.

Heparin-induced LDL precipitation (HELP) is available since 1982, in which after primary separation, the plasma is mixed in a ratio of 1:1 with an acetate-acetic acid buffer (pH 4.85), so that the pH of this mixture is 5.1. Then 100,000 U heparins per litre are added to the buffer [51]. The plasma has been mixed then thoroughly with the acetate-acetic acid buffer and heparin, LDL particles precipitates in the acid environment together with fibrinogen and heparin to form insoluble precipitates and these are then removed from the plasma by means of a polycarbonate membrane. The remaining free heparin is completely removed by a heparin absorber (DEAE cellulose). The acidulous plasma is returned to a physiological pH value using bicarbonate dialysis, and the plasma, free of LDL, is returned to the patient's blood. This method is technically complicated, but it is reliable and effective, but non-selective for in addition to the cholesterol, Lp(a), C3, C4, fibrinogen, plasminogen, and factor VIII, etc. are also eliminated. HDL reaches its original level after 24 hours, and the fibrinogen concentration only increases gradually; the amount of plasma should, therefore, be processed to 4 three litres [51].

A compact unit has been designed that somewhat reduces the cost of the equipment. The so-called Plasmator Futura (B. Braun, Germany) is easy to use and safe in handling. The priming rinsing and reinfusion are fully automated. The user is safely guided through all treatment steps and supported with message prompts and warnings [10]. Regular extracorporeal LDL-elimination studies indicate that the incidence of adverse cardiovascular clinical events can be reduced much earlier than by drug therapy alone. These immediate clinical benefits after the apheresis, cannot be due, however, to an improvement in coronary morphology (i.e., regression of atherosclerotic lesions), since such improvement can only be observed after several months of treatments [53]. An improvement in rheology as well as producing an immediate positive influence on endothelium function and an increase in the volume of vasodilators, anti aggressive nitrogen monoxide released or a reduction in the volume of available endothelin is likely due to the improved myocardial perfusion and clinical symptoms after lipoprotein-apheresis [54]. A more than 50 percent reduction of LDL at weekly intervals is clearly associated with an early regression of lipid-rich vascular lesions. Lipoprotein-apheresis also reduces the shear-stress of the flowing blood on vulnerable plaques either by its effect on plasma viscosity and/or on the vasomotor reserve, thus leading to a lower peripheral arterial resistance. A further point is lipoprotein-apheresis eliminates oxidized LDL, too, which might counteract plaque stabilization by its inflammatory effects [55].

In more than 120,000 treatments the safety and long-term applicability of the HELP system has been proved. Serious complications have never been observed, and the technology of the equipment has been improved over time [56]. There is clear clinical evidence that a drastic lowering of LDL concentrations by HELP reduces significantly the rate of total and coronary mortality as well as the incidence of cardiovascular events in high-risk dyslipoproteinemia patients which have been shown in many studies. Simultaneous reduction of proinflammatory and prothrombotic factors with atherogenic lipoproteins by HELP apheresis may contribute to improvement of endothelial dysfunction and thereby inhibit progression of atherosclerotic lesions and stabilize the plaque [57]. Lipoprotein-apheresis slightly, but significantly reduces CRP concentrations in patients with CAD on statin therapy, which may

contribute to the stabilization of atherosclerosis in dyslipoproteinemia patients treated with LDL apheresis [58]. These results are even more impressive when known age-related increase in CRP over treatment period is taken into account. Pentraxin 3 (PTX3) is the humeral arm of innate immunity possibly aiming at tuning arterial activation associated with damage vascular was acutely reduced by HELP apheresis [59]. The lowering of PTX3 concentrations in high risk patients is associated with disease regression of cardiovascular events.

The HELP apheresis has been demonstrated as a successful secondary prevention for patients with FH, CAD, cardiac bypass, heart transplantation, or acute cerebral infarction (stroke) [56]. The elimination of fibrinogen and other substances also has an influence on blood viscosity, rheology, and erythrocyte aggregation; thus, the microcirculatory situation as a whole can be significantly improved. Severe side effects are rare: so far, the only side effects reported have been transient shivering and hypotension.

Dextran sulfate low-density lipoprotein (Liposorber) was reported in 1987 from Mabuchi *et al.* (Liposorber LA 15, Kaneka, Japan). Low-molecular dextran sulfate (MW 4500) can selectively absorb all substances containing apolipoprotein B [60]. Dextran sulfate is covalently bound to cellulose particles. The dextran sulfate was selected as an affinity ligand of LDL adsorbent for its high affinity and low toxicity. The binding mechanism is the direct interaction between dextran sulfate and the positively charged surface of apolipoprotein B-containing lipoproteins (LDL, VLDL, and Lp(a)). The dextran sulfate has a structure similar to that of the LDL receptor and seems to act as a type of pseudo receptor [61]. Approximately 2.5 grams LDL can be bound per column. After primary separation, the plasma is perfused through the columns, where all material containing Apo-B such as cholesterol, LDL, VLDL, and triglycerides is absorbed. Low of cholesterol, the plasma and the blood cells are returned to the patient. After 500 mL of plasma, the columns are saturated and require regeneration with 4.1 percent NaCl solution. After rinsing with Ringer's solution, they are ready for use again. The effectiveness of this treatment is good, and cholesterol is eliminated selectively (Table 1). With a perfusion volume of more than four litres, a marked drop in the Quick level can occur, probably caused by the absorption of factor VIII.

The Liposorber system has also found widespread clinical use in recent years. Side effects are rare and of a minor nature such as hypotension, nausea, hypoglycemia, and light allergic reactions [62]. Dextran-induced allergic reactions have not been observed so far. Low-molecular dextran sulfate is much less allergenic than the forms of dextran which are normally implemented as a plasma expander with a higher molecular weight of 40,000 to 80,000. More than 60 percent reduction of the pre-treatment cholesterol values can be achieved by one treatment with a higher TPV than 1.2 with the Liposorber system. The effectiveness of therapy has also been observed over time in several long-term clinical studies. The observed side effects were between 0.5 and 4 percent [10]. The Liposorber system is safe and effective, even in high-risk dyslipoproteinemia patients. In children, the Liposorber system has proved to be safe and effective, too [63]. The advantage of the Liposorber system is the selectivity by elimination of all apo B-containing lipoproteins and the high effectiveness. A disadvantage is the labour-intensive technology.

With essentially all adsorbers complement activation takes place. The activated complement is removed by binding to specific proteins such as C4 binding protein. Bradykinin is formed in the presence of anticoagulating heparin. Generated bradykinin is immediately cleaved by kinase II/ACE in the pulmonary circulation, thereby not reaching

the arterial system to exert clinical symptoms. Angiotensin converting enzyme (ACE inhibitors) which is inhibited by different drugs can exacerbate the clinical effect of this amount of bradykinin to the point of serious adverse effect including anaphylaxis. This was reported on an anaphylactoid reaction in a patient during ACE inhibition therapy and lipoprotein-apheresis in 1992 [64]. The observed reactions were similar to those which frequently occur in ACE inhibition therapy in combination with AN69-high-flux dialysis as well as with reusable polysulphone membranes in intermittent hemodialysis. One of these anaphylactoid reactions is presumably induced by the increased release of bradykinin. Bradykinin is formed through activation of the contact activation system, which consists of the components such as high-molecular-weight kininogen, prekallikrein, Hageman's factor, and coagulating factor XI. The concentration of bradykinin in the plasma increases considerably, when plasma come into contact with very high negatively charged dextran sulfate. Bradykinin is quickly decomposed through the activity of kininase I and II. Thus, patients undergoing lipoprotein-apheresis are not normally affected.

Kininase II is identical to angiotensin-converting enzyme and is blocked by the application of ACE inhibitor resulting in an increase of bradykinin in the bloodstream and to an anaphylactoid reaction. These anaphylactoid reactions are not specific to a particular membrane or surface type but can always occur in ACE inhibition when blood or plasma comes into contact with contact-activating surfaces. These reactions are independent of the type of ACE inhibitor. The varying degrees of severity are presumably connected to the expression of the ACE-coding gene being subject to strong individual fluctuations [65]. ACE inhibitors in lipoprotein-apheresis using negatively charged surfaces, i.e., DALI and dextran sulfate columns/hemoperfusion cartridge are contraindicated [66].

The **low-density lipoprotein hemoperfusion** was described first by Bosch *et al.* in 1993 (**DALI**, Fresenius, Germany) (Table 2) [67]. The adsorber, which is compatible with human whole blood, uses a matrix of polyacrylate beads. In the DALI system whole blood is perfused through the adsorber, which contains 480 mL of polyacrylate coated polyacrylamide, without regeneration. The column has a capacity for hemoperfusion of more than 1.5–2.0 blood volumes for effective adsorption of cholesterol, LDL, Lp(a), and triglycerides and a regeneration is not necessary because the column is used for only one treatment [69]. There are 2 adsorber cartridges of different volumes/capacities available for the DALI system. The system is a simpler extracorporeal circuit, in which the blood is pumped through the LDL adsorber. The elimination of LDL, Lp(a) particles, and other lipoproteins from whole blood is performed by adsorption onto polyacrylate-coated polyacrylamide beads. The beads with a diameter of 150–200 µm have a porous structure which exploits the principle of size exclusion chromatography. The sponge-like structure of the beads offers a very large inner and outer surface for adsorption in which more than 99 percent of the overall surface of over 1,000 m² is located within the beads [69].

Like the LDL-receptor, polyacrylate, consists of polyanions, with negatively charged carboxylate groups [66]. The polyanions interacts selectively with the cationic groups in the apoprotein B moiety of LDL and Lp(a). By flowing, the whole blood passing the beads affects only a minor interaction between the blood cells and the similarly small outer surface of the beads [10]. HDL can also penetrate the beads, but because the apo AI-coated HDL is not attracted to the ligand, it is not affected by the adsorber and cannot be eliminated. Monitoring of this simple extracorporeal blood circulation system is carried out by measuring

the blood pressure in the afferent and efferent blood lines and at the adsorber inlet. Anticoagulation is carried out by first applying a heparin bolus, then by a continuous ACD-A solution infusion into the blood line.

The DALI system adsorbs besides lipoproteins all positively charged ions calcium and magnesium. Before start, the columns have to be pre-rinsed with 4–6 litres of a priming solution containing these electrolytes. The adsorber is thereby saturated with these cations, thus preventing hypocalcemia and hypomagnesia during the treatment. The advantages are good selectivity, high effectiveness, and a simpler technology. The potential for possible micro particle release from the columns as with all adsorbers can be avoided prevented by more and better rinsing of the columns and a careful handling [70]. In a five-year follow-up, long-term therapy with DALI was safe, effective, and selective as LDL and Lp(a) could be reduced by >60 percent per session in approximately 100 minutes treatment time, while decrease and the incidence of side effects were low [71]. The DALI system should be stated with caution, because of the bradykinin release and often observed symptoms of hypocalcemia.

A further whole blood lipoprotein apheresis system is the **Liposorber D** (Kaneka, Japan). The Liposorber D system is developed on the basis of the technology of the dextran sulfate and adsorbs all positively charged LDL, VLDL, and Lp(a) particles from whole blood using negatively charged polyanions. Liposorber D contains negatively charged dextran sulfate covalently bound to cellulose [72]. The negatively charged surfaces activate the intrinsic coagulation pathway. A prolongation of a PTT and shortening of PT have been observed in the lipoprotein-apheresis with the dextran sulfate column. Coagulation factors such as factors XI and XII were reduced by dextran sulfate adsorption, but those coagulation factors returned to normal range within one or two days after the treatment [73]. Adverse events, which were observed, were hypocalcemia during treatment caused by ACD-A solutions; the symptoms disappeared by administration of calcium, and slight hypotension.

The DALI system and the Liposorber D have clear advantages over the usual lipoprotein-apheresis systems that require plasma separation. These systems are simpler and easier to handle because no plasma separation procedure is necessary. The advantages are good selectivity, effectiveness, and a simple technology [71,72]. A treatment performed with the Liposorber D and the newly developed machine DX- 21 reduces the apoB lipoproteins without having great influence on HDL-C, other important plasma components or blood cells. The DALI system and the Liposorber D are comparable. The handling with the special machines is easy, safe, and the user is guided through all treatment steps. The whole blood perfusion lipoprotein-apheresis systems are simpler apheresis systems and thus a useful modality to remove LDL and Lp(a) from whole blood in dyslipoproteinemia patients.

Human monoclonal antibody

Proprotein convertase subtilisin/kexin type 9 is a serine protease involved in cholesterol metabolism that is enzymatically inactive following secretion. PCSK9 is a proprotein convertase belonging to the subtilase subfamily [74]. In healthy humans, plasma PCSK9 concentration decrease with fasting and increase following meals [75]. After discovery of PCSK9, it was shown that gain-of-function mutation PCSK9 is associated with FH [74]. Loss-of-function mutations in PCSK9 are associated with reduced LDL concentrations and that these lifetime reductions confer substantial protection against CAD [76]. PCSK9 gene expression is regulated by nuclear transcription factor

sterol regulatory element-binding protein-2. Concentrations of sterol regulatory element-binding protein-2 are increased by statin therapy, which thus also increases PCSK9 concentration. PCSK9 inhibition can be an useful therapeutic strategy in hypercholesterolemia. PCSK9 is expressed predominantly in the liver, and to a lesser extent in the intestine and kidney in adults. The only known physiologically relevant function of circulating PCSK9 is to regulate receptor in the liver [77]. In the management of many inflammatory disorders and cancers therapeutic HMA play important roles because of their ability to bind to a selected target highly specifically, but they have not been widely used in the treatment of cardiovascular disease as yet. HMA are target-specific antibodies created through recombinant DNA technology. These proteins have the characteristic Y-shaped protein structure of B-cell-derived antibodies and are designed to bind to a single therapeutic target with high specificity [78]. HMA exert their therapeutic action through a variety of mechanisms, including direct effects associated with the binding of the antibody to the target and indirect effects involving depletion of cells targeted by the monoclonal antibody [77]. HMA are administered intravenously, intramuscularly, or subcutaneously [79]. Elimination occurs not through the liver or kidneys, but primarily through antigen-specific target-mediated disposition and nonspecific pathways of the reticuloendothelial system [77].

The potential for development of neutralizing antibodies that can reduce the therapeutic efficacy of monoclonal antibodies is of particular concern [80]. Further development initially resulted in chimeric antibodies, which consisted of human antibody with murine variable regions. Fully HMA were developed using novel platforms [81,82]. Evolocumab and Alirocumab are fully human anti-PCSK9 monoclonal antibodies [7,39].

Several clinical trials with anti-PCSK9 antibodies have been shown that these agents lead to substantial reductions in LDL when administered as monotherapy or in combination with statins and/or ezetimibe to patients with dyslipoproteinemia. In patients with hypercholesterolemia, Evolocumab led to mean reductions in LDL of 48 to 76 percent compared with placebo and of 38 to 47 percent compared with ezetimibe after 12 or 52 weeks of treatment [77,81,82]. Alirocumab led to mean reductions of 46 to 62 percent compared with placebo and 24 to 32 percent compared with ezetimibe after 24 weeks of treatment [77]. Other clinical trials have shown that PCSK9 inhibitors yield an incremental 50–60% reduction in LDL cholesterol when added to statin therapy [83].

In the ODYSSEY ESCAPE study LA was discontinued in about 63% of patients on alirocumab who were undergoing regular LA. Alirocumab was well tolerated [8]. A meta-analysis of 16,721 patients from 33 randomized clinical trials with 10,532 patients who received PCSK9 inhibitors and 6,189 controls showed that PCSK9 inhibitors improve lipid profiles and lower all-cause mortality in patients with hypercholesterolemia [84]. The FOURIER study randomised controlled study of Evolocumab versus placebo included 27,564 patients with atherosclerotic diseases followed up for a median of 2.2 years. PCSK9 inhibition with Evolocumab significantly reduced cardiovascular risk in these patients [9].

Further larger studies are necessary to show that the inhibition of PCSK9 with HMA is as effective as the regularly weekly or two weekly lipoprotein-apheresis treatments. Comparable for both therapeutic methods as lipoprotein-apheresis and HMA were the higher decreases of pre-treatment concentration over the time in older patients with

initial higher Lp(a) concentration than in younger patients with lower Lp(a) and lower risk [6,85,86].

Inclisiran is a long-acting TNA interference that inhibits the synthesis of PCSK9. In a small study subcutaneous injection or placebo in a single-ascending-dose or a multiple-dose phase a reduction of the PCSK9 blood concentrations of up to more than 80% and of the LDL cholesterol concentrations of up to 60% was observed. In this only 1-month period no side effects were seen [87]. Here too, further studies are necessary.

The drugs lomitapide and mipomersen are no HMA [88]. But even today, the gene therapy is still no real alternative to regular lipoprotein-apheresis treatment [89]. The aim of gene therapy in FH is the expression on the LDL receptor by insertion of receptor-encoding transgene with the help of a suitable vector. So far, the adenovirus or adeno-associated virus vectors have been found particularly suitable. They infect both resting and dividing cells and remain episomal in the cytoplasm—not in the genome. They are easily to be manipulated at the molecular level so that they can have a high immunogenicity, i.e., a cellular and humeral immuno response against the foreign protein leads to the elimination of hepatocytes infected by the vector [88]. Only one trial for the treatment of homozygous FH in humans is available. “The hepatocytes were isolated, cultured, and then infected with one of the LDL receptor gene-encoding retroviruses. The liver cells were then reinfused into the portal vein” [89]. In some of the patients in this pilot study, this treatment lowered the LDL by 2–25 percent. These drugs are not available for the treatment of FH.

Conclusion of dyslipoproteinemia therapy

The lipoprotein-apheresis techniques described above are all effective and well tolerated [10]. With weekly or biweekly treatment, the average LDL cholesterol concentration can be reduced to approximately 30–60 percent and more of the original levels. LDL concentration increases again after each apheresis session. The increase after apheresis can be slowed down by lipid lowering drugs. The decrease of cholesterol from 400 mg/dL to 200 mg/dL treatment could almost double a patient's life expectancy. The lipoprotein-apheresis treatment must be repeated in homozygous and severe heterozygous FH life-long or until other therapy technologies such as HMA or gene therapy are available for everyone. Not only LDL mass decreases but also it improves the patient's life expectancy and performed with different techniques decreases the susceptibility of LDL to oxidation by lipoprotein-apheresis. This decrease may be related to a temporary mass imbalance between freshly produced and older LDL particles [10]. The baseline fatty acid pattern influences pre-treatment and post-treatment susceptibility to oxidation, too.

Despite drug therapy, lipoprotein-apheresis significantly stimulates the residual LDL-receptor expression in FH via the reduction of available extracellular cholesterol resulting in delayed reappearance of dyslipoproteinemia in between treatments [90]. “The acute effect of lipoprotein-apheresis on serum lipidome could be predominantly attributed to lipoprotein changes, while blood cell damages during this procedure caused additional, less-pronounced changes” [91].

All techniques vary somewhat in selectivity. Cascade filtration can reduce HDL concentration, which probably has an atherogenous effect in the long term. CF, HELP, and dextran sulfate adsorption to a lesser extent reduce the average fibrinogen concentration. This can be of advantage as the viscosity of the blood is reduced and the rheological characteristics improved.

Table 3. Indications for lipoprotein-apheresis (modified after 4,92,93)

| Year | Institutions | Indications |
|------------|--|--|
| 2002 | German Federal Committee and Health Insurance Funds (92) | <ul style="list-style-type: none"> - FH homozygotes - Patients with severe hypercholesterolemia in whom maximal dietary and drug therapy for >1 year have failed to lower cholesterol sufficiently - Patients with isolated Lp(a) higher than 60 mg/dL normal LDL cholesterol and progressed cardiovascular disease |
| 2013, 2016 | Apheresis Applications Committee of the ASFA (4, 93) | <ul style="list-style-type: none"> - Functional homozygote's with LDL >500 mg/dL - Functional heterozygote's with no known cardiovascular disease but a LDL >300 mg/dL - Functional heterozygote's with known cardiovascular disease and LDL >200 mg/dL - Patients without FH with high LDL or Lp(a) who cannot tolerate or whose conditions are unresponsive to conventional therapy |

The reducing of cholesterol concentration is to prevent the development and progression of atherosclerosis. The decrease and slower progression of atherosclerotic changes in coronary vessels and carotids after patients have been treated for one or more years with lipoprotein-apheresis is reported. Lipoprotein-apheresis represents a decisive breakthrough in the treatment of high-risk patients with dyslipoproteinemia, whose treatment has, up to now, been inadequate, despite strict diets and lipid-reducing medication. The indications of lipoprotein-apheresis for dyslipoproteinemia from the German Committee of Physicians and Health Funds and the Apheresis Applications Committee of the ASFA are summarized in Table 3 [92,93]. During pregnancy, LDL cholesterol levels in individuals affected by FH can rise to extreme levels that can compromise uteroplacental perfusion. The use of Lipoprotein-apheresis in these indications allows for successful completion of pregnancy [93].

The monoclonal antibody inhibition of PCSK9 have demonstrated reduction in LDL- and Lp(a) blood concentrations. Further larger studies are necessary to show if the inhibition of PCSK9 with HMA is as effective as the regularly weekly or two weekly lipoprotein-apheresis treatments and if with HMA besides cholesterol and lipoproteins other substances could be reduced such proinflammatory, prothrombotic factors and others. PCSK9 inhibition with HMA offers substantial LDL lowering in patients with dyslipoproteinemia and those with clinical atherosclerotic cardiovascular disease who are on maximally tolerated statins. Evolocumab and Alirocumab are generally well tolerated and the prolonged (Q2W and monthly) dosing schedules may offer the benefit of high patient adherence. Results from cardiovascular outcome have been published in studies in 2016 and 2017 (FOURIER; ODYSSEY ESCAPE studies) [8,9].

Moriarty *et al.* aim to evaluate the effect of Alirocumab on frequency of standard apheresis (weekly or every 2 weeks) in dyslipoproteinemia [8]. Guideline-recommended threshold values may be achievable in individual patients through treatment with Alirocumab alone or in combination with lipoprotein-apheresis. An unclarified point is the question if in long-term application with HMA an antibody expression against the HMA would be possible and what can be done than-lipoprotein-apheresis again? The antibody expression against HMA was observed in the cancer therapy by mutation of the oncogenes, therefore new HMA are necessary.

A reduction in costs is a valid demand in view of the scarce resources available in the healthcare system. Commissions, consisting of physicians, administration specialists and representatives of the health insurance funds and others, nowadays decide at a "round table" which will be granted medical facilities and who will be not, this is a clinical routine adopted in Germany [10]. Physicians are committed to helping all the patients entrusted to them to the best of their knowledge, and this means that medical treatment—and particularly the apheresis processes - must become affordable. This demand represents a great

challenge to physicians, politicians, health organisations, and, above all, to the manufacturers. Industry constantly justifies the high costs with the extensive research and development required. All those involved in the healthcare system must intensify their cooperation in this respect [10].

Medical progress is advancing and will not be stopped. Since the introduction of hollow fiber membranes, exceptional efforts in research and development have been undertaken in the apheresis sector alone, enabling, for example, the introduction of selective separation techniques into every day clinical practice—techniques which were un-thought of—at the beginning of the eighties. With lipoprotein-apheresis and the new human monoclonal immunoglobulin G2 (Alirocumab or Evolocumab) all severe hypercholesterolemia with cardiovascular disease are treatable. But the indication of lipoprotein-apheresis should be considered after a period of 12 months and refractory to diet and maximum lipid-lowering medications only as a secondary prevention if the patients suffering from progressive cardiovascular events with remaining LDL cholesterol concentrations >120-130 mg/dl and/or Lp(a) blood concentrations >60 mg/dl [94]. The application of HMA could be easier in future if no antibody expression is found. The costs of PCSK9 inhibitor therapy amount approximately 9,650 EUR per year against the LA therapy costs of approximately 50,000 EUR per year in Germany. But the number of patients with extremely elevated Lp(a) who need the extracorporeal therapy will increase. In future larger studies must be show which method the lipoprotein-apheresis or the PCSK9 inhibition therapy would be preferred or a combination of both.

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