Relapsing and progressive complications of severe hypertriglyceridemia – Effective long-term treatment with double filtration plasmapheresis

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Keywords

double filtration plasmapheresis, hypertriglyceridemia, acute pancreatitis, atherosclerotic cardiovascular disease, lipodystrophy, pregnancy

Abbreviations

ASCVD: atherosclerotic cardiovascular disease
AP: acute pancreatitis
DFPP: double filtration plasmapheresis
FPLD: Familial partial lipodystrophy
GW: gestation week
HTG: hypertriglyceridemia
HTG-AP: hypertriglyceridemia induced acute pancreatitis
IQR: interquartile range
LMNA Lamin A/C
LD: lipodystrophy
LDL-C: low-density lipoprotein-cholesterolLP: lipoprotein
Lp(a)-HLP: Lp(a)-hyperlipoproteinemia
PE: plasma exchange
SD: standard deviation
TG: triglyceride
VLDL: very low-densitiy lipoprotein

Abstract

Background Severe hypertriglyceridemia (HTG) is associated with major complications such as acute pancreatitis (AP) and atherosclerotic cardiovascular disease (ASCVD). Rapid elimination of triglyceride (TG)-rich lipoproteins with double filtration plasmapheresis (DFPP) without need for substitution has been found to be effective for the acute, short-term treatment of HTG-induced AP. Data on the long-term use of DFPP to prevent HTG-associated complications are scarce.

Objective To assess the clinical practice and to evaluate the efficacy of regular DFPP treatment for preventing recurrence of HTG-associated complications in therapy refractory patients.

Methods Retrospective multicenter study in patients with severe symptomatic drug and diet refractory HTG with regular DFPP treatment. Patients´ incidence of HTG-associated pancreatic or cardiovascular complications was compared before and with regular DFPP treatment.

Results Ten patients (3 female) were identified with baseline maximal TG concentrations of 2587 to 28090 mg/dl (median 5487 mg/dl; interquartile range (IQR) 4340-12636). The mean observation period was 3.9±3.4 years before and 3.8±3.0 years with DFPP. In five patients severe HTG was related to chylomicronemia, two patients had familial partial lipodystrophy Dunnigan, one patient had additional lipoprotein (a)-hyperlipoproteinemia. The main HTG-associated complication was recurrent AP in 8 patients, including 1 patient treated during pregnancy. Two patients presented with severe progressive ASCVD. With long-term DFPP treatment the annual rate of HTG-associated pancreatic or cardiovascular complications declined from median 1.4 (IQR 0.7-2.6) to 0 (IQR 0.0-0.4) ($p<0.005$). The absolute number of events was reduced by 77 %. In 6 patients (60%) neither episodes of AP occurred nor progression of ASCVD were detected clinically or by routine imaging techniques. DFPP was effective in elimination of TG-rich lipoproteins from plasma, safe and well-tolerated.

Conclusion Long-term regular DFPP treatment resulted in stabilization of patients with severe HTG and related recurrent AP or progression of ASCVD, who were refractory to conventional dietary and drug therapy.

Introduction

The complex causes of hypertriglyceridemia (HTG), which are associated with a highly variable clinical appearance, make diagnosis and therapy an interdisciplinary challenge. Increased synthesis or defective clearance can lead to increase of triglyceride (TG)-rich lipoproteins (LP) in plasma. According to a recent simplified classification HTG is defined mild-to-moderate with a plasma TG concentration of 2-10 mmol/l (175-885 mg/dl) with more likely polygenic or secondary basis, and the rare form of severe HTG with a TG concentration of >10 mmol/l, (>885 mg/dl) more likely related to monogenic causes affecting only 0.1 % of adults (Hegele 2014; Nordestgaard 2016). Severe HTG primarily results from genetic disorders leading to decrease in LP lipase activity. Uncontrolled diabetes mellitus, drugs, nephrotic syndrome, obesity and alcohol intake are secondary causes. Severe HTG is associated with serious complications such as acute pancreatitis (AP) with high-risk of frequent relapse, and atherosclerotic cardiovascular disease (ASCVD). Elevated TG-rich LP represent an independent causal risk factor for ASCVD (Nordestgaard, 2007, 2016). Results of a large Mendelian randomization study suggested that all ApoB containing LP particles, including TG-rich very-low-density lipoprotein (VLDL) particles and their metabolic remnants as well as LDL particles, have approximately the same effect on the risk of cardiovascular disease per particle (Ference 2019).

Severe HTG accounts for up to 10 % of all AP cases in non-pregnant individuals and is the third most common cause of AP after gallstones and alcohol. AP requires urgent therapy to prevent pancreatic necrosis, which has a mortality up to 30% (Adiamah 2018). In pregnancy up to 50% of all AP cases are related to HTG (Basar 2013). In the context of risk for HTG induced AP (HTG-AP) mostly the threshold of TG levels >1000 mg/dl is mentioned which should be regarded as equivalent to severe HTG (Adiamah 2018).

Standard treatment of HTG consists of dietary restrictions and lipid lowering medication, such as fibrates and omega-3 fatty acids. In medical emergencies, effective and rapid lowering of excessively elevated TG with therapeutic apheresis to control HTG-AP is recommended by national and international apheresis guidelines (Schettler 2019; Padmanabhan 2019). In general 1 to 3 daily treatments of plasma exchange or double filtration plasmapheresis (DFPP) are performed. DFPP is a selective method of LP apheresis based on membrane plasma separation with subsequent plasma filtration of high-molecular weight atherogenic LP from plasma. Clinical data on the long-term use of therapeutic apheresis to prevent recurrence of HTG-associated complications are scarce and limited to plasma exchange (Constantini 2016; Saleh 2017, Stefannutti 2009; Piolot 1996, Shaap-Fogler 2009). For this indication a selective procedure such as DFPP might be preferred to avoid the need of substituting human plasma products with their potential adverse effects.

The aim of our nationwide retrospective study was to assess the clinical practice and to evaluate the efficacy of long-term DFPP for patients with severe symptomatic HTG. For the first time a larger case series with regular DFPP treatment for this rare indication was analysed including 2 patients with familial partial lipodystrophy (FPLD) who are, to our knowledge, the first cases described in the literature.

Methods

Patents and outcome measures

An open retrospective multicenter non interventional study throughout Germany was performed. Criteria for patient enrolment were diagnosis of severe HTG, insufficient response to appropriate diet and lipid lowering drug therapy, including antidiabetic drugs, which was associated with relapsing or progressive HTG-related pancreatic or cardiovascular complications, and regular treatment with DFPP. The protocol was approved by the ethics committee Bayerische Landesärztekammer (No17090) and the ethics committee, University Duisburg Essen (No 18-8500-BO) and was reported to an open source online registry (No. DRKS00011704). The trial was performed in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent before enrolment. The clinical course before and with regular DFPP was recorded. Individual retrospective observation periods were determined by the date of commencing regular DFPP treatment and the date of data collection for the study. The analysed time before DFPP treatment varied depending on available clinical data of the individual patient.

The main outcome parameter for efficacy was the incidence of HTG-associated pancreatic or cardiovascular complications before and with DFPP treatment. HTG-associated complications were defined as follows: event of AP, episode of severe abdominal pain in patients with history of recurrent AP, event or progression of ASCVD as determined clinically or by routine imaging technique. An ASCVD event was accounted with occurrence of cardiovascular death, nonfatal myocardial infarction, coronary bypass surgery, percutaneous coronary intervention, or stent. Progression of ASCVD without clinical event was accounted as equivalent event with the following findings by routine imaging techniques: incidence of new or additional ASCVD at a new vascular location or region, or deterioration of existing ASCVD. Secondary endpoints were evaluation of safety and tolerability using treatment protocols in patient records. The validity of clinical findings was ascertained with careful review of medical records and based on standardized case report forms with source data verification.

DFPP treatments were performed using a membrane plasma separator (Plasmaflo OP-08W) in combination with a plasma component filter (Cascadeflo EC-50W or Rheofilter ER-4000 all Asahi Kasei Medical, Tokyo, Japan) and the tubing system MF-430 or CMF-430 (Effe Emme, Cigliano, Italy) together with the Octo Nova Technology, SW version 4.30.5 (DIAMED,

Cologne, Germany). In 2 patients with extreme chylomicronemia plasma separation was switched from membrane to centrifuge technique using the Spectra Optia (Terumo BCT Inc., Lakewood, CO, USA) in combination with a plasma filter (Rheofilter ER-4000). For vascular access in 2 patients peripheral veins were used, in 8 patients arteriovenous fistula were created for chronic treatment. The filtered plasma volume per treatment was 2000-5000 ml representing in mean 113% (±31) of patients' plasma volume. Anticoagulation was performed with unfractionated heparin, in two patients combined with citrate.

Statistics

The *t* test for paired samples was used to analyze changes in annual event rates; $p < 0.01$ was considered significant. Descriptive statistics were provided as mean with standard deviation (SD*)* or median with interquartile range (IQR).

Results

Characteristics of patients

Ten patients were identified (3 female) with a median age of 43 years (IQR 35-48) at commencing DFPP treatment. Patient characteristics are summarized in **table 1**. Ten treatment sites were involved in extracorporeal treatment of the patients. The patients presented with different forms of severe symptomatic HTG. In five patients (#2 - #5, #7) HTG was associated with chylomicronemia. In two patients the diagnosis of familial chylomicronemia syndrome (FCS) was considered as very likely. In patient #2 a heterozygous mutation of the *GPIHB* gene was detected. In patient #3 genetic testing was not available but FCS scoring according to Moulin et al. 2018 was 10, suggesting FCS, however, not completely excluding multifactorial CS. Two patients had familial partial lipodystrophy (FPLD) Dunnigan (#8, #9) confirmed by genetic testing. Patient #10 additional to severe HTG had lipoprotein(a)-hyperlipoproteinemia (Lp(a)-HLP) possibly representing a phenotype of familial combined hyperlipidemia. Diabetes mellitus has to be addressed as a major condition aggravating treatment of HTG in seven of the analyzed patients. In four patients diabetes type 2 was regarded as an underlying disease. In three patients diabetes mellitus was pancreoprive due to HTG-related pancreatitis episodes (patients #1,2,6) or additional pancreas tumour surgery (patient #6). Recurrent AP was the main HTG-associated complication in 8 patients (#1-#8), one woman (#7) was treated during pregnancy. Two patients (#9, #10) had a severe progressive ASCVD, #10 had in addition episodes of pancreatitis.

Appropriate diet and lipid lowering therapy, including antidiabetic drugs, were insufficient to prevent progression, or recurrence of HTG complications. In particular patients #9, and #10 received statins to reduce ASCVD risk. However, attainment of the LDL-C target for established ASCVD, which was reduced from 70 mg/dl to 55 mg/dl according to the 2019

guidelines of the European Society for Cardiology, could not be evaluated as LDL-C measurement must be regarded as invalid with TG levels of 800 mg/dl and above (Langlois 2018, Mach 2019). Due to unsatisfactory control of TG levels therapeutic apheresis was indicated, and DFPP was chosen as selective modality. At the time of current assessment in 2019 all but one patient (#7) were on regular treatment.

DFPP treatment

All patients received DFPP treatments, in general 1 to 2, maximal 3 treatments per week at discretion of the treating physician. One patient (#7) was treated with DFPP only during pregnancy for 2 months within the third trimester. The range of baseline TG concentrations was 2587–28090 mg/dl (median 5487 mg/dl; IQR 4340-12636). TG concentrations before/after DFPP treatment were in mean 2865 mg/dl (±1425), 1695 mg/dl (±1375). In two patients (#2, #8) with severe chylomicronemia plasma separation using a membrane technique was impaired by accumulation of chylomicrons. Elimination of these very large TG-rich lipoproteins was improved by using a centrifuge technique for plasma separation in combination with a plasma filter. The individual mean reduction rate of TG varied from 17-83% (for details see *online supplement Table 1*). Regular DFPP was well tolerated by all patients after analysis of 1085 treatment sessions. Eight patients had an arteriovenous fistula for vascular access. Recurrent fistula thrombosis occurred in one patient (#1), and could be resolved by thrombectomy and surgical revision. Minor adverse events typically associated with therapeutic apheresis methods e.g. transient hypotension, dizziness, hematoma at vascular access, or nausea were not reported in detail.

Clinical course

Patients´ clinical courses regarding HTG-associated complications before and with regular DFPP are depicted in **figure 1** (*for additional details of cases see online supplement*). Clinical course during DFPP treatment was analysed from the time of commencing DFPP until the time of data collection for each patient. The analysed time before DFPP treatment varied depending on available clinical data of the individual patient. The mean retrospective observation period was 3.9 years (±3.4) before and 3.8 years (±3.0) with DFPP. With long-term DFPP treatment the annual rate of HTG-associated pancreatic or cardiovascular complications declined significantly from median 1.4 (IQR 0.7-2.6) to 0 (IQR 0.0-0.4) ($p<0.005$) (Figure 2a). Additional subgroup analyses are provided in *online supplement*. In total 464 months were analysed before commencement and 457 months with DFPP treatment, evaluated absolute numbers of HTG-associated complications were 44 before and 10 with DFPP in 10 patients (**Figure 2b**). During regular DFPP treatment the absolute number of events declined by 77 %. In 6 cases (60%) including the pregnant patient neither episodes of AP occurred nor progression of ASCVD were detected clinically or by routine

imaging techniques. TG values during pregnancy and clinical course of patient #7 are depicted in **figure 3**.

Discussion

The role of long-term DFPP as final treatment escalation for relapsing or progressive complications of severe HTG was analysed in 10 patients. HTG in these patients had different etiologies and different pathogenic profiles. Diabetes mellitus was a comorbid condition of severe HTG in 7 patients. In 4 of these patients diabetes mellitus existed as underlying disease, in 3 patients developed due to deterioration of pancreas function (pancreoprive). Chylomicronemia was present in five patients. Two patients had the very rare form of FPLD Dunnigan. The indication for regular DFPP treatment to prevent HTG-associated complications, i.e. recurrent HTG-AP, or progression of ASCVD was established during routine care. The general approach for the long-term treatment of HTG aiming at preventing HTG-associated complications like HTG-AP includes dietary counselling, fat restriction, medium chain triglycerides, weight loss, avoiding alcohol intake, strict glycemic control in diabetes, and lipid lowering drugs (Rawla 2018). However, standard treatment strategies may be insufficient to prevent complications in some patients with severe HTG due to very rare monogenic forms, or individual comorbid conditions or predisposing factors.

HTG-AP

Recurrent HTG-AP was the main complication in 80% of patients in this study. HTG-AP is a rare and complex disorder with an underlying mechanism that is influenced by genetic, metabolic, environmental and patient specific factors. In guidelines for the treatment of acute or chronic pancreatitis prevention of HTG-AP relapse is not receiving appropriate attention. It has been recognized in current guidelines on the use of therapeutic apheresis (Padmanabhan 2019). Clinical studies are sparse. Plasma exchange and DFPP have been found to be safe and effective for the emergency treatment of severe HTG-AP also during pregnancy (Achard 1991, Basar 2013, Click 2015; Galan Carrillo 2015; Gavva 2016; Gubensek 2014, Huang 2016, Kadikoylu 2006; Kandemir 2018, 2019, Yeh 2003, Zeitler 2015). Shortening of hospitalization was reported as major benefit (Huang 2016; Chang 2016). The pathogenic model of HTG-AP includes disturbance of pancreatic microcirculation by very large TG-rich LP. The accumulation of chylomicrons reduces pancreatic capillary flow with resulting ischemia. Subsequent hydrolytic release of free fatty acids is toxic to the pancreatic endothelium and acinar cells. Activation and release of pancreatic enzymes induces autodigestion-related injury (Adiamah 2018). Rationale for the use of therapeutic apheresis is rapid extracorporeal elimination of TG-rich LP which is hypothesized to instantly stop further organ damage. DFPP treatment results in the improvement of blood flow and microcirculation (Klingel 2000). It is generally believed that TG levels >1000 mg/dl trigger AP

and its serious complications. However, this threshold for the risk of HTG-AP appears rather arbitrary (Huang 2018; Ewald 2013). Not all patients with severe HTG develop AP, and many patients with severe HTG never develop AP. In a recent retrospective study evaluating patients with very severe HTG and TG >2000 mg/dl, in 62 % no episodes of AP had been reported (Esparza 2019). Also the correlation of pancreatitis severity and TG level is different in individual patients (Click 2015). In one study more severe forms of pancreatitis were observed in patients with higher TG-levels compared to a group with lower-TG levels suggesting that high TG level may be associated with poor prognosis (Wang 2017). In our study clinical stabilization of patients was observed even though the TG concentration in plasma was not reduced to a level of in mean <1000 mg/dl in all patients, suggesting that additional individual predispositions for AP might be decisive. It can be hypothesized that the therapeutic effect of regular DFPP treatment is based on the pulsed immediate physical extracorporeal elimination of large LP with their load of oxidized lipid components from plasma thus subsequently reducing other pathophysiological processes of pancreatitis and atherosclerosis like inflammation, oxidative stress, and impaired rheology (Klingel 2000; Neumann 2013, Roeseler 2016). With long-term DFFP the incidence of AP was reduced in all affected patients in this study compared to the evaluated time period before commencing DFPP. A reduction of DFPP treatment frequency was followed by AP relapse in 2 cases (#4, #8). These results indicate a preventive effect of DFPP treatment.

Familial or Multifactorial Chylomicronemia Syndromes (FCS or MCS)

TG concentrations of >2000 mg/dl are indicative to large quantities of chylomicrons, which are composed of 86% TG. In the rare form of FCS arising from a genetic defect in intravascular lipolysis such as LP lipase, or glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein (GPIHBP1). Differentiation of FCS from MCS is difficult, since the clinical and biochemical phenotype can be very similar whereas the incidence of HTG-AP is more frequent in FCS. Methods of genetic assessment are not always available. Recently a pragmatic diagnostic scoring system for FSC was suggested (Moulin 2018). The scoring system revealed the diagnosis of FCS as very likely for 2 patients (#2, #3) in our cohort with history of multiple AP. In #2 a heterozygous mutation in GPIHBP1 was detected by gene analysis, #3 presented with eruptive xanthomas and chylomicrons were visible with a typical creamy layer on full blood samples. With long-term DFFP treatment the frequency of HTG-associated complications was markedly reduced in both patients. Antisense-mediated inhibition of hepatic APOC3 mRNA with volanesorsen represents a novel pharmacological option to lower TG levels for the very rare patients with confirmed monogenic FCS and failure of a strict low-fat diet, which has been investigated in phase 3 clinical trials, but long-term experience in routine care is not available (Witztum 2019). Thrombocytopenia and injection-site reactions were reported as common adverse events

(Witztum 2019). The expected annual treatment cost for volanesorsen will be far above that of DFPP.

HTG-AP during pregnancy

In pregnancy hormonal changes and a decrease in LP lipase activity predispose to increase of TG levels mostly in the third trimester. TG-lowering drugs are contraindicated. In particular in women with an existing dyslipidaemia, pregnancy may cause severe HTG with a high risk of HTG-AP. As in general, individual susceptibility differs, some pregnant women may tolerate higher TG levels without significant symptoms. AP is associated with life-threatening fetal complications and increased maternal mortality (Huang 2016). Our patient (#7) with known history of severe HTG and recurrent AP experienced intrauterine fetal death during her first pregnancy. Therapeutic apheresis was not considered at this time. With regular DFPP treatment during the third trimester the second pregnancy continued without complications until timely delivery of a healthy newborn. The prophylactic use of therapeutic apheresis to avoid HTG-AP in individual high-risk women during pregnancy seems to be rare in clinical practice, there are only few case reports including one describing the effective treatment with DFPP (Basar 2013, Michalova 2019, Sivakumaran 2009). An interdisciplinary cooperation of gynaecologists, lipidologists and nephrologists caring for women with previously known severe symptomatic HTG during pregnancy should become mandatory in these rare cases.

HTG- combined with Lp(a)-HLP

One patient (#10) presented with combined TG- and Lp(a)-HLP associated with severe progressive ASCVD involving coronary and peripheral arteries despite conventional dietary and drug therapy including effective lowering of LDL-C. Elevated apolipoproten B-containing TG-rich LP are an independent causal risk factor for ASCVD. High concentration of TG is strongly associated with increased risk for myocardial infarction, ischaemic stroke and early death (Nordestgaard 2016, Toth 2019, Hegele 2014). The atherogenic potential of elevated TG is underdiagnosed and undertreated due to diagnostic difficulties and occurrence in rare cases. Most forms of HTG are combined with other dyslipidaemias. The atherogenicity of TG is not only dependent on the concentration of TG but also on the composition of the TG-rich LP particles in the blood and their influence on lipid metabolism. With Mendelian randomization analyses a similar risk reduction of ASCVD was found in TG-lowering LP variants and LDL-cholesterol lowering LDL-receptor variants. (Ference 2019). However, there is not yet conclusive evidence whether lowering TG reduces the risk of ASCVD. Lp(a) is an independent causal cardiovascular risk factor, enhancing the risk of premature or progressive ASCVD. Regular LP apheresis can prevent cardiovascular events in patients with progressive ASCVD and Lp(a)-HLP (Roeseler 2016). Lp(a)-HLP along with progressive

ASCVD is approved as indication for regular LP apheresis in Germany. For patient #10 with combined HTG and Lp(a)-HLP (TG >3500 mg/dl; Lp(a) > 80mg/dl) regular DFPP treatment was of particular benefit as Lp(a) was eliminated with the same efficacy as LDL-C and even more effective than TG. With regular DFPP progression of ASCVD could be prevented.

HTG and lipodystrophy syndromes

Lipodystrophy (LD) syndromes are extremely rare disorders of deficient leptin action and body fat homoistasis, associated with serious metabolic complications, including diabetes, HTG, and steatohepatitis (Brown 2016). Dietary approaches and available pharmacological agents are often unsatisfactory in LD. Partial and generalized, congenital and acquired LD are distinguished. Diagnosis of LD is generally based on medical history, physical examination, body composition and metabolic status (Brown 2016). LD is a serious and complication-prone disease, data on the use of therapeutic apheresis as ultima ratio option to reduce TG are scarce. Intensive long-term plasma exchange therapy with dramatic clinical benefit has been described in one girl with acquired generalized LD (Bolan 2007). Two patients of our cohort were diagnosed with FPLD type Dunnigan with main complications of AP in #8 and ASCVD in #9. The majority of FPLD are autosomal dominant (Lightbourne 2017). Phenotypically, patients with FPLD lack extremity and gluteal subcutaneous fat becoming obvious around puberty in most cases. FPLD2, also known as the Dunnigan variety of FPLD, is caused by autosomal dominant mutations in the LMNA gene on chromosome 1q21-22 (Lightbourne 2017). LMNA encodes nuclear lamin A/C. Cardiovascular complications include CHD and hypertension, while metabolic complications include insulin resistance, diabetes mellitus, hypertriglyceridemia with resultant pancreatitis, and hepatic steatosis, which tend to increase with age. Women can also have preeclampsia, and miscarriages. Many complications of FPLD are secondary to deficient adipose tissue like insulin resistance leading to severe HTG and AP. Treatment with recombinant human methionyl leptin (metreleptin) may be considered for patients with FPLD and severe metabolic derangements (Hba1c>8%, and/or TG > 500 mg/dl). In #8, who was clinically stable with regular DFPP, metreleptin treatment was not effective to prevent AP. Simultaneous reduction of DFPP treatment frequency led to AP relapse. Our results correspond to the findings that in FPLD the response to metreleptin is less robust than in generalized LD (Brown 2016). There is evidence of an increased risk of early ASCVD in subjects with FPLD and LMNA mutations, notably in women (Brown 2016, Hegele 2001). Both female patients in our study with known mutations in the LMNA gene coding for Lamin A/C presented with two-vessel coronary artery disease, exhibiting a very progressive course in #9. After commencing regular DFPP treatment no progression of ASCVD was observed in both cases.

Therapeutic apheresis – methodological aspects

Data on the long-term use of therapeutic apheresis to prevent relapse or progression of HTG-associated complications are mostly limited to plasma exchange (Constantini 2016; Saleh 2017, Stefannutti 2009; Piolot 1996, Shaap-Fogler 2009). A selective method of LP apheresis capable of eliminating large TG-rich LP like DFPP might be preferred to avoid the regular need of substituting human plasma products. Protein replacement fluids bear the risk of allergic reactions, as well as a small but clinically relevant risk of virus transmission (Alvarez 2016, Hewitt 2014). With long-term DFFP treatment the frequency of HTG-associated complications was reduced by 77% in our cohort compared to the analysed time period before commencing DFPP. No relevant side effects were reported during DFPP. Sides effects related to vascular access are not specifically attributable to DFPP.

In rare cases with extreme large quantities of chylomicrons with a particle size of 75-1200 nm membrane plasma separation can be impaired. Treatment in a fasting state can be favourable to minimize a negative impact of postprandial increase of chylomicrons, remnants or large VLDL on the filtration process. The use of centrifugal plasma separation combined with plasma filtration represents a methodological alternative, however, the availability is mostly limited to in-hospital treatments. In 2 patients (#2, #8) of this case series reduction of very large TG-rich lipoproteins was improved with a change to this method. In-hospital care of acute HTG-associated complications is regulated in Germany, as DFPP and PE are equally implemented in the coding system for hospital reimbursement. However, for long-term outpatient DFPP treatment of high risk patients with severe HTG an individual approval for reimbursement by the statutory or private health insurance fund is necessary, representing a substantial hurdle for the actual clinical use.

Limitations

The retrospective nature of the analysis, variable observation periods, and lack of a control group of patients are limitations of the presented study results.

Conclusion

With long-term DFPP the incidence rate of HTG-AP and progression of ASCVD was substantially reduced in select high-risk patients with severe HTG and failure of previous standard therapies, including rare genetic forms like FPLD, and particular comorbid or clinical conditions like pancreoprive diabetes mellitus or pregnancy. Based on the limited data, only patients who already suffered severe complications can be candidates for secondary prevention by long-term apheresis treatment. A larger prospective trial would be required to ascertain conclusively the benefits of long-term DFPP as escalating treatment for severe refractory forms of HTG. However, it might be hardly feasible to enrol a sufficient number of patients with such rare morbid conditions in a controlled study design, which also from an

ethical point of view might be problematic in view of the putative benefit of therapeutic apheresis in these rare high risk patients.

10. References *(6 authors et al.; to be arranged in the order in which they are cited; tb done)*

1. **Achard** J, Westeel P, Moriniere Ph, Lalau J., de Cagny, Fournier A. Pancreatitis related to severe acute hypertriglyceridemia during pregnancy: treatment with lipoprotein apheresis. Intensive Care Med 1991; 187:236-237
2. **Adiamah** A, Psaltis E, Crook M, Lobo DN. A systematic review of the epidemiology, pathophysiology and current management of hyperlipidemic pancreatitis. Clin Nutr 2018; 37:1810-1822.
3. **Álvarez M**, Luis-Hidalgo M, Bracho MA, Blanquer A, Larrea L, Villalba J et al. Transmission of human immunodeficiency virus Type-1 by fresh-frozen plasma treated with methylene blue and light. Transfusion 2016; 56(4):831-836.
4. **Basar R,** Uzum AK, Canbaz B, Dogansen S, Kalayoglu-Besisik S, Altay-Dain S et al. Therapeutic apheresis for severe hypertriglyceridemia in pregnancy. Arch Gynecol Obstet 2013; 287:839-843
5. **Bolan C**.,Oral EA, Gorden P et al. Intensive, long-term plasma exchange therapy for severe hypertriglyceridemia in acquired generalized lipoatrophy.
6. **Brown RJ**, Araujo-Vilar D, Cheung PT et al. The diagnosis and management of lipodystrophy syndromes: a multi-society practice guideline. J Clin Endocrinol Metab 2016; 101(12):4500-4511
7. **Chang CT**, Tsai TY, Liao HY et al. Double filtration plasma apheresis shortens hospital admission duration of patients with severe hypertriglyceridemia-associated acute pancreatitis. Pancreas 2016; 45(4):606-612
8. **Click B**, Ketchum AM, Turner R, Whitcomb DC, Papachristou GI, Yadav D. The role of apheresis in hypertriglyceridemia-induced acute pancreatitis: a systematic review. Pancreatology 2015; 15: 313-320.
9. **Costantini** N, Mameli A, Marongiu F. Plasmapheresis for Preventing Complication of Hypertriglyceridemia: A Case Report and Review of Literature. Am J Ther 2016;23(1):e288-91.
10. **Esparza M,** Li X, Adams-Huet B, Vasandani C, Vora A, Das S et al. Very severe hypertriglyceridemia in a large US county health care system: associated conditions and management. J Endocrinol Soc 2019; 3(8):1595-1607
11. **Ewald N**, Kloer HU. Severe Hypertriglyceridemia: An indication for apheresis? AtherosclerSuppl (2009) 10: 49-52.
12. **Ference B**, Kastelein J, Ray K, Ginsberg H. Association of triglyceride-lowering LPL variants and LDl-C-lowering LDLR variants with the risk of coronary heart disease. JAMA 2019; 321(4): 364-373
13. **Galan Carrillo** I, Demelo-Rodriguez P, Rodríguez Ferrero ML, Anaya F. Double filtration plasmapheresis in the treatment of pancreatitis due to severe hypertriglyceridemia. J ClinLipidol (2015) 9: 698-702.
14. **Gavva C,** Sarode R, Agrawal D, Burner J. Therapeutic plasma exchange for hypertriglyceridemia induced pancreatitis: a rapid and practical approach. Transfus Apher Scie 2016; 54:99-102
15. **Gubensek** J, Buturovic-Ponikvar J, Romozi K, Ponikvar R. Factors affecting outcome in acute hypertriglyceridemic pancreatitis treated with plasma exchange: an observational cohort study. PLoS One. 2014;9(7):e102748.
16. **Hegele RA**. Premature atherosclerosis associated with monogenic insulin resistance. Circulation 2001; 103:2225-2229

17. **Hegele RA**, Ginsberg HN, Chapman MJ, Nordestgaard BG, Kuivenhoven JA, Averna M. The polygenetic nature of hypertriglyceridemia: implication for definition, diagnosis, and management. Lancet Diabetes Endocinol 2014; 2(8):655-666
18. **Hewitt P**, Ijaz S, Brailsford S, Brett R, Dicks S, Haywood B et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014; 384:1766-1773.
19. **Huang** C, Liu J, Lu Y, Fan J, Wang X, Liu J, Zhang W, Zeng Y. Clincal features and treatment of hypertriglyceridemia-induced acute pancreatitis during pregnancy: a retrospective study. J ClinApheresis 2016; 31: 571-578.
20. **Kadikoylu** G, Yavasoglu I, Bolaman Z. Plasma exchange in severe hypertriglyceridemia – a clinical study. Transfus Apher Scie 2006; 253-257
21. **Kandemir** A, Coşkun A, Yavaşoğlu I, Bolamani Z, Ünübol M, Yaşa M. Therapeutic plasma exchange for hypertriglyceridemia induced acut pancreatitis: the 33 cases experience from a tertiary reference center in Turkey. Turk J Gastroenterol 2018; 29(6):676-83
22. **Kandemir** A, Coskun A. Treatment of hypertriglyceridemia-induced acute pancreatitis with therapeutic plasma exchange in 2 pregnant patients. J Obstet Gynaecol 2019; 39:5, 702-704
23. **Klingel** R, Fassbender C, Fassbender T, Erdtracht B, Berrouschot J. Rheopheresis: Rheologic, functional and structural aspects. Ther Apher 2000; 4(5):348-357
24. **Langlois M, Chapman J, Cobbaert C, Mora S, Remaley AT, Ros E et al.** Quantifying atherogenic lipoproteins: current and future challenges in the era of personalized medicine and very low concentrations of LDL cholesterol. A consensus statement from EAS and EFLM. Clin Chem 2018;64:1006-1033
25. **Lightbourne** M, Brown RJ. Genetics of lipodystrophy. Endocrinol Metab Clin North Am 2017;46:539-554.
26. **Mach F**, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2019;doi.org/10.1093/eurheartj/ehz455
27. **Meehan C,** Cochran B, Kassai A, Brown R, Gorden P. Metreleptin for injection to treat the complications of leptin deficiency in patients with congenital or acquired generalized lipodystrophy. Expert Rev Clin Pharmacol 2016; 9(1):59-68
28. **Michalova** R, Mankova A, Nucak M, Mikulova S, Nehaj F, Raslova K et al. Therapeutic plasma exchange in secondary prevention of acute pancreatitis in pregnant patient with familial hyperchylomicronemia. Biomed Pap Med Fac Univ Palacky Olumouc Czech Repub 2019; 163(1):90-94
29. **Moulin P**, Dufour R, Averna M, Arca M et al. Indentification and diagnosis of patients with familial chylomicronemia syndrome (FCS): Expert panel recommendations and proposal of an "FCS score". Artherosclerosis 2018; 275:265-275
30. **Neumann C,** Schulz E, Haenah G, Platzer U, Wieland E, Schettler V. Lipoprotein apheresis-more than just cholesterol reduction? Atheroscler Suppl 2013; 14:29-32
31. **Nordestgaard BG**, Benn M, Schnohr P, Tybjaerg-Hansen A. Non-fasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA (2007) 298:299-308
32. **Nordestgaard BG.** Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease. Circ res 2016; 118:547-563
33. **Padmanabhan A**, Connelly-Smith L, Aqui N. Guidelines on the use of therapeutic apheresis in clinical practice – evidence-based approach from the writing committee of the American society for apheresis: the eight special issue. J Clin Apher 2019; 34:171-354
34. **Piolot** A, Nadler F, Cavallero E, Coquard J, Jacotot B. Prevention of recurrent acute pancreatitis in patients with severe hypertriglyceridemia: value of regular plasmapheresis. Pancreas 1996; 13:96-99

35. **Rawla P**, Sunkara T, Thandra KC, Gaduputi V. Hypertriglyceridemia-induced pankreatitis: updated review of current treatment and preventive strategies. Clin J Gastroenterology 2018; 11(6):441-448
36. **Roeseler E**, Julius U, Heigl F, Spitthoever R et al. Lipoprotein apheresis for liporprotein(a)-associated cardiovascular disease. Prospective 5 years of follow-up and apolipoprotein(a) characteriszation. Arterioscler Thromb Vasc Biol 2016; 36:2019-2027
37. **Saleh** MA, Mansoor E, Cooper GS. Case of familial hyperlipoproteinemia type III hypertriglyceridemia induced acute pancreatitis: Role for outpatient apheresis maintenance therapy. World J Gastroenterol 2017 October 28; 23(40): 7332-7336
38. **Schaap-Fogler M,** Schurr D, Schaap T, Leitersdorf E, Rund D. Long-term plasma exchange for severe refractory hypertriglyceridemia: a decade of experience demonstrates safety and efficacy. J Clin Apher 2009; 24:254-258
39. **Schettler V,** De Grot K, Fassbender C, Grützmacher P, Heigl F, Julius U et al. Standard der Therapeutischen Apherese 2019 der Deutschen Gesellschaft für Nephrologie e.V. in Zusammenarbeit mit der Gesellschaft für pädiatrische Nephrologie. [Standard of therapeutic apheresis 2019 of the German Societies for Nephrology and paediatric Nephrology] [https://www.dgfn.eu/apheres-standard.html]
40. **Sivakumaran** P, Tabak S, Gregory K, Pepkwitz S & Klapper E. Management of familial hypertriglyceridemia during pregnancy with plasma exchange. J Clin Apher 2009; 24:42-46
41. **Stefanutti** C, Di Giacomo S, Vivenzio A, Labbadia G, Mazza F, D'Alessandri G, Russi G, De Silvestro G, Marson P. Therapeutic plasma exchange in patients with severe hypertriglyceridemia: a multicenter study. Artif Organs. 2009 Dec;33(12):1096-102.
42. **Toth P**, Philip S, Hull M, Granowitz C. Hypertriglyceridemia is asscoicated with an increases reisk of peripheral arterial revascularization in high-risk statin-treated patients: A large administrative retrospective analysis. Clin Cardiol 2019; 1-6.
43. **Wang SH,** Chou YC, Shangkuan WC, Wei KY, Pan YH, Lin HC. Relationship between plasma triglyceride level and severity of hypertriglyceridemic pancreatitis. PloS ONE. **2016** Oct 11;11(10):e0163984.Witte PU, Schenk J, Schwarz JA, Kori-Lindner C:
44. **Yeh JH**, Chen JH, Hou-Chan C. Plasmapheresis for hyperlipidemic pancreatitis. J. Clin Apher 2003; 18:181-185
45. **Witztum J**, Gaudet D, Freddman S, Alexander V. Digenio A, Williams K et al. Volanesoren and triglyceride levels in familial chylomicronemia syndrome. N Engl J Med 2019; 381:531-542
46. **Zeitler H,** Balta Z, Klein B, Strassburg C. Extracorporeal treatment in severe hypertriglyceridemia-induced pancreatitis. Ther Apher Dial 2015; 19:405-410

Tables and Figures

Table 1 Baseline characteristics

Figure 1 Patients´ clinical courses regarding HTG-associated events. Clinical course during DFPP treatment was retrospectively analysed from the time of commencing DFPP until the time of data collection for each patient. The analysed time period before DFPP treatment varied depending on available clinical data of the individual patient. Triangles represent event of acute pancreatitis (red), TG-associated severe abdominal pain (orange), ASCVD event or progression (black) as detected by routine imaging techniques. #4 Missing of single DFPP treatments due to temporary non-adherence to apheresis treatment; in #8

DFPP treatment frequency was reduced during therapy trial with metreleptin. #7 was treated with DFPP during the last trimester of her 2nd pregnancy.

Figure 2a Annual rate of HTG-associated events including the composite of pancreatic and cardiovascular complications in patients with severe HTG (n=10). The mean retrospective observation period was 3.9 years (±3.4) before and 3.8 years (±3.0) with DFPP. Decrease of annual event rate from median 1.4 (IQR 0.7-2.6) before to 0 (IQR 0.0-0.4) with DFPP treatment. (*t* test for paired samples p<0.005, n=10).

Figure 2b Absolute numbers of events before and with DFPP treatment in patients with severe HTG (n=10). In total 464 months were analysed before commencement and 457 months with DFPP treatment.
HTG-associated events were defined as follows: event of AP, episode of severe abdominal pain in patients with history of recurrent AP, event or progression of ASCVD as determined clinically or by routine imaging technique.

Figure 3: DFPP treatment during pregnancy. Triglyceride levels and clinical course of #7. GW=gestation week.

Supplement

Tables and Figures

Supplement Table 1. Laboratory values and treatment characteristics.

Supplement Figure 1a. **Annual rate of HTG-associated events of acute pancreatitis in patients with severe HTG (n=9).** Decrease from median 2.0 (IQR 0.7-2.8) before to median 0.2 (IQR 0.0-0.4) with DFPP treatment. (*t* test for paired samples p<0.01). The mean retrospective observation period was 4.2 years (±3.4) before and 4.0 years (±3.1) with DFPP.

Supplement Figure 1b. Absolute numbers of AP events before and with DFPP treatment in patients with severe HTG (n=9). In total 452 months were analysed before commencement and 427 months with DFPP treatment. AP events were defined as event of acute pancreatitis (AP), episode of severe abdominal pain in patients with history of recurrent AP.

Supplemental information to the clinical courses of selected patients depicted in Figure 1

Patient #3: Substantial reduction of the AP event rate was achieved with regular DFPP, but AP could not completely prevented. Thirteen AP episodes occurred in the 2 years prior to chronic DFPP treatment. One relapse occurred 4 months after commencing DFPP treatment

followed by 2.5 years without recurrence. Then 3 episodes occurred despite chronic DFPP within 7 months. Diagnosis of FCS was very likely in this patient according to FCS scoring (Moulin 2018), a plasma sample gave the typical creamy chylomicron layer, but genetic testing was not available. Comorbid conditions of this patient putatively contributing to this instable course were diabetes mellitus with maximal insulin resistance (HbA1c 12.5%), steroid-dependent minimal change glomerulonephritis, hypothyroidism, steatosis hepatis, adipositas per magna (BMI 42), limited compliance to diet. Membrane plasma separation was impaired by chylomicrons, resulting in TG reduction rates below average. Centrifugal plasma separation was not available in the patient's local area. During the last 10 months of documentation no AP was reported.

Patient #4: A single episode of AP with regular DFPP must be attributed to temporary non-adherence to apheresis treatment resulting in increase of TG concentration. After continuing chronic DFPP treatment the patient was stable for the last 16 months of the observation period.

Patient #6: Available clinical data before commencing DFPP were limited in this case. Three months after pancreatic surgery the patient experienced a very severe exsudative pancreatitis requiring intensive care. Due to known unsatisfactory control of TG levels with combined lipid lowering medication and dietary restrictions regular DFPP treatment was commenced to prevent further episodes of AP.

Severe HTG and pregnancy (#7)

Patient #7: A 29-year-old women, presented with a severe familial HTG with chylomicronemia and initial TG values up to 9.000 mg/dl. According to the FCS score FCS could not be confirmed. Lipoprotein electrophoresis revealed markedly elevation in very low density lipoprotein (VLDL) and chylomicron fractions. She had a history of HTG-AP, and most recently during her first pregnancy at the age of 27 years experienced a necrotizing AP. Presumably by fat embolism intrauterine fetal death occurred in the 37th gestation week (GW). During further follow-up including strict diet with one fasting day per week in combination with maximal TG lowering drug treatment (omega-3 fatty acids, fibrate) TG could be reduced to concentrations between 960 mg/dl and a maximum of 2.300 mg/dl the patient remained clinically stable. At the age of 29 years the patient became pregnant again requiring termination of contraindicated lipid lowering medication. The TG level increased despite diet up to >3.700 mg/dl. DFPP treatment was commenced to rapidly reduce the TG in plasma and prevent AP. The patient was treated 12 times over a period of 2 months (**Figure 3**). After initiation of DFPP TG values could be maximally reduced by 2339 mg/dl and 64%. Reduction rates of TG varied, presumably in some treatments membrane plasma separation was impaired by chylomicrons. Overall, the treatments were well tolerated,

pregnancy was without complications. In GW 37 the patient delivered a healthy newborn by cesarean section.

Severe HTG and FPLD Dunnigan (#8, #9)

Patient #8: HTG was diagnosed at the age of 13 years, a positive family history for HTG was documented (brother and mother). Comorbidities were diabetes mellitus which is typically associated with FPLD, and two-vessel coronary artery disease. The first event of AP was reported at the age of 26 years. Until the age of 37 years the patient had experienced in total six AP, 3 episodes within the last 13 months before commencing regular DFPP treatment with a frequency of twice a week. Initial TG concentration was 14805 mg/dl. At that time the patient showed FPLD typical physical appearance with marked loss of subcutaneous fat from the upper and lower extremities and prominent muscles. Gene analysis confirmed the diagnosis of FPLD Dunnigan due to heterozygous mutation in the *LMNA* gene. With regular DFPP treatment the patient had no AP event within the next 3.5 years. After that period she had a trial with oral metreleptin (5 mg daily) therapy, and frequency of DFPP was reduced to once a week. Within *few weeks thereafter* (*Rü details Dr. T*) the patient developed typical symptoms of TG-associated abdominal pain, thus DFPP treatment frequency was increased again to twice per week. A second trial with an increased dose of metreleptin (7.5 mg) and decreased DFPP frequency led to similar symptoms. Consequently metreleptin therapy was terminated and DFPP treatments continued twice a week. With this regimen the patient was stable within the last three months of the observation period of our study.

Patient #9: FPLD Dunnigan was diagnosed confirmed by gene analysis (*LMNA* gene) at the age of 50 years. She showed FPLD typical physical appearance and great similarity to her mother who suffered also from FPLD with associated ASCVD. Comorbidities of the patient were also type 2 diabetes, breast cancer (surgery, chemotherapy, radiotherapy), morbus Crohn, chronic musculoskeletal pain syndrome. The rationale for commencing regular DFPP treatment in this case was diagnosis of severe progressive two-vessel coronary artery disease at the age of 57 years, confirmed by two ASCVD events within one year. Initial TG level was 2587 mg/dl with maximal tolerable lipid-lowering drug treatment, LDL-C was <70 mg/dl. With weekly DFPP treatment the patient had no further progression of ASCVD since 2.5 years. There was no trial with metreleptin due to concerns related to a potential involvement of leptin in the pathogenesis of breast cancer (Meehan 2016).

Severe HTG with Lp(a)-HLP

Patient #10: Early and severe progressive ASCVD involving coronary and peripheral arteries were underlying a non-fatal myocardial infarction at the age of 38 years. Appropriate diet, antidiabetic drugs (diabetes mellitus diagnosed at the age of 44 years) and maximal lipid-lowering therapy were insufficient to prevent progression of ASCVD and episodes of HTG-

AP. Mean concentration of TG during 2 years before commencing DFPP was 3.700 ±1.204 mg/dl. Baseline total cholesterol was 373 mg/dl, Lp(a) 89 mg/dl. The patient received DFPP treatment once to twice a week. Extracorporeal elimination of lipoproteins was useful in rapidly lowering elevated serum TG from in mean 1686±1492 mg/dl before to 953±847 mg/dl after DFPP. The mean reduction rate for TG was 40%, for LDL-C 67 % and for Lp(a) 68%. DFPP was safe and well-tolerated. The patient was clinically stable without any event since 12 months with regular DFPP.

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Statement of Ethics

Ethical approval for the study had been obtained. All patients gave their written informed consent.

Disclosure Statement

Authors are required to disclose any possible **conflicts of interest**. All forms of support and financial involvement (e.g. employment, consultancies, honoraria, stock ownership and options, expert testimony, grants or patents received or pending, royalties) which took place in the previous three years should be listed, **regardless of their potential relevance to the paper**. Also the nonfinancial relationships (personal, political, or professional) that may potentially influence the writing of the manuscript should be declared. If there is no conflict of interest, please state: "The authors have no conflicts of interest to declare."

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