Aus der medizinischen Klinik und Poliklinik

Gemeinschaftsklinikum Mittelrhein, Akademisches Lehrkrankenhaus der

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Glycosylphosphatidylinositol-verankertes Lipoprotein-Bindungsprotein 1 (GPIHBP1) Autoantikörper vermittelt ausgeprägte Chylomicronemie und ihre Behandlung

Glycosylphosphatidylinositol-Anchored High Density Lipoprotein Binding Protein 1 (GPIHBP1) Autoantibody Mediated Severe Chylomicronemia and its Treatment

Inauguraldissertation

zur Erlangung des Doktorgrades der

Humanmedizin

der Universitätsmedizin

der Johannes Gutenberg-Universität Mainz

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Mainz, 2022

Tag der Promotion: 06. Dezember 2022

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List of (non-)standard Abbreviations

AB	Antibodies
ANA	Antinuclear Antibodies
APO	Apolipoproteins
APS	Antiphospholipid Syndrome
Auto-Abs	Autoantibodies
BCT	Blood and Cell Technologies
BMI	Body Mass Index
CECR	Cat Eye Syndrome Chromosome Region
CENP	Centromere Protein
CHD	Coronary Heart Disease
CIN	Cervical Intraepithelial Neoplasia
CKD	Chronic Kidney disease
CRP	C-Reactive Protein
CTD	C-terminal Domain
CVD	Cardiovascular Diseases
DM	Diabetes Mellitus
DNA	Deoxyriboneucleic Acid

EDITA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
ENA	Extractable Nuclear Antigen
FA	Fatty Acid
FBG	Fasting Blood Glucose
FCS	Familial Chylomicronemia Syndrome
FFA	Free Fatty Acid
FCHL	Familial Combined Hyperlipidemia
FHTG	Familial Hypertriglyceridemia
GPIHBP1	Glycosylphosphatidylinositol-anchored high-density lipoproteinbinding protein 1
HCG	Human Chorionic Gonadotrophin
HLP	Hypolipoproteinemia
HRP	Horseradish Peroxidase
HSPGs	Heparin Sulfate Proteoglycans
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HTG	Hypertriglyceridemia

IA	Immunoadsorption
IBL	Immuno Biological Laboratories
IDL	Intermediate Density Lipoprotein
IFN	Interferon
IGF	Insulin-like growth factor
IVIG	Intravenous Immune Globulin
LDL	Low density Lipoprotein
LMF1	Lipase Maturation Factor 1
LPs	Lipoproteins
LPL	Lipoprotein Lipase
Ly6	Lymphocyte Antigen 6
MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid
MPO	Myeloperoxidase
MRT	Magnetic Resonance Tomography
NCEP-ATP	National Cholesterol Education Program Adult Treatment
NTD	N-terminal Domain
PAD	Peripheral Arterial Disease

PS	Plasma Separation
RNA	Ribonucleic Acid
RTX	Rituximab
SIADs	Systemic Inflammatory and Autoimmune Diseases
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-PyruvicTransaminase
SLE	Systemic Lupus Erythematosus
SS	Sjögren's Syndrome
TG	Triglycerides
TGAB	Thyreoglobulin Antibodies
TMB	Tetra Methyl Benzidine
TPE	Therapeutic Plasma Exchange
TPO	Thyriod Peroxidas
TRAB	TSH-receptor-AB
TSH	Thyriod Stimulating Hormone
VLDL	Very Low Density Lipoprotein
WHO	Wold Health Organisation

List of graphs, tables and figures

Introduction

Lipids are of central importance for homeostasis as they play an important role in many biological processes. They are an essential source for energy, form part of cell membranes and act as signal transducers (2). Based on their biosythesis, whether derived from ketoacyl thioesteres or isoprene units, lipids are grouped into 8 classes: fatty acyls (i.e. fatty acids), glycerolipids (i.e. triacylglycerides), glycerophospholipids (i.e. phosphatidylcholine), sphingolipids (i.e. sphongosin-1-phosphate), sterols (i.e. cholesterol, steroid hormones), saccharolipids and polyketides (i.e. macrolides, aflatoxins) (2). Cholesterol and triglycerides play a major role in the human lipid metabolism whose disturbance can have a substantial impact on morbidity and mortality as for example an increase of the cholesterol load is associated with an increased risk for cardiovascular diseases or an increase in triglyceride concentrations is related to an increased risk of acute pancreatitis (3-5).

Under physiological conditions triglycerides can be derived from the liver (endogenous form) mainly during fasting and are transported in very low density lipoproteins (VLDL) or they can be absorbed from the food by the intestine and are transported in chylomicrons (exogenous form). After reaching the adipose or muscle tissue triglycerides (TG) are hydrolysed into free fatty acids (FFA) by the enzyme lipoprotein lipase (LPL) (6). Apolipoproteins (apo) are very important in the metabolism of lipids. Apo-B is needed for the assembly of chylomicrons in the intestine and VLDL in the liver (7, 8). Apo E helps eliminating triglyceride-rich lipoprotein remnants. This process takes place in the liver, where apo E is secreted (9). Figure 1 shows a simplfied illustration of the pathway of dietary fat after being ingested under physiological conditions.

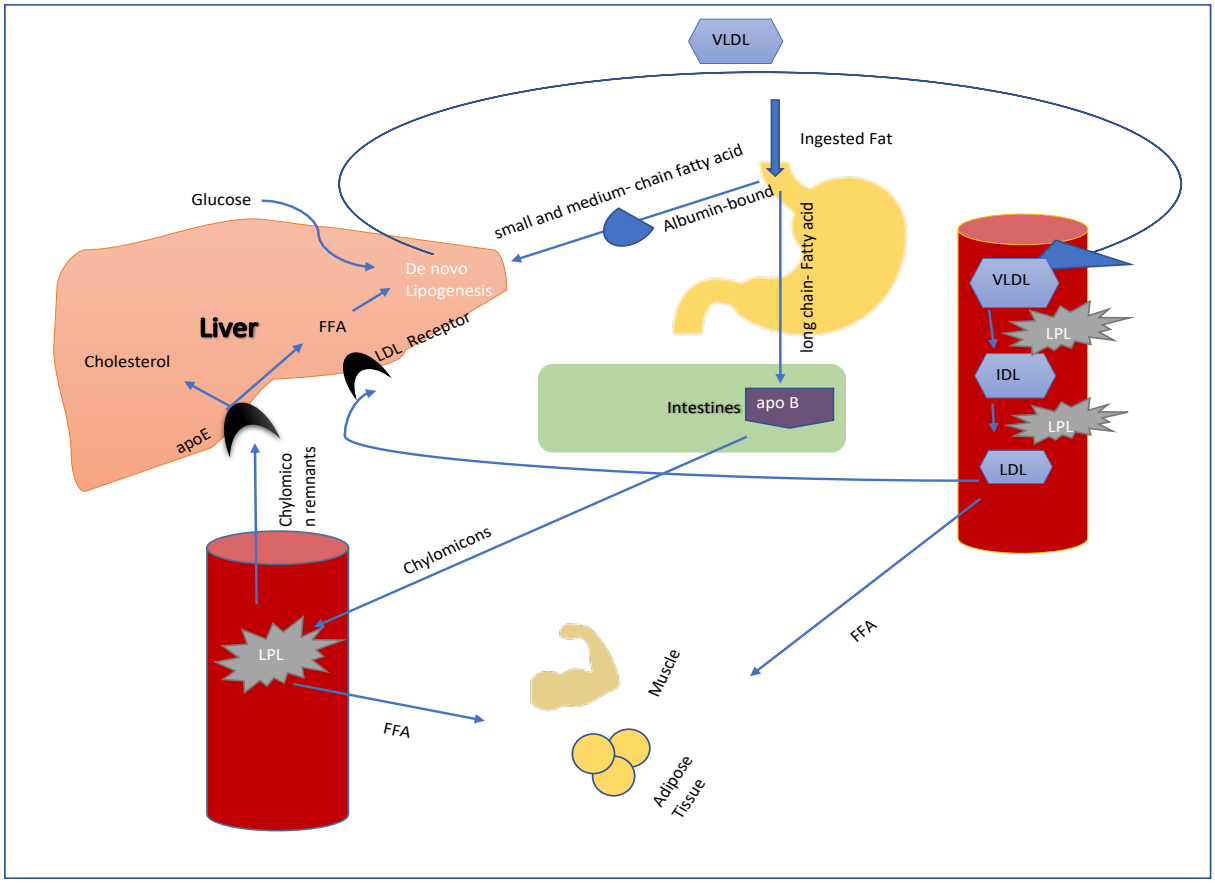


Figure 1: Physiological pathway of ingested fats. FFA (Free Fatty Acid), apo B-48 (Apolipoprotein B), apo E (Apolipoprotein E), LPL (Lipoproteinlipase), LDL (Low Density Lipoprotein), VLDL (Very Low Density Lipoprotein), IDL (Intermediate Density Lipoprotein).

Risk factors for the development of lipid disorders could be hereditary, or more commonly, acquired. These include for example nutritional habits, environmental factors, diseases such as: hypothyroidism, diabetes mellitus, central obesity, autoimmune disorders, metabolic syndrome, or chronic kidney disease (CKD) or drug-induced dyslipidaemia (10).

Lipid disorders, particularly those with an increased cholesrerol concentration, are associated with an increased cardiovascular risk through atherosclerosis and thrombosis, resulting in the development and progression of cardiovascular diseases (CVD) such as stroke, coronary heart disease (CHD), peripheral arterial disease (PAD), or sudden cardiac death. CVD represents a common cause of death and premature morbidity worldwide (10). However, it can be to a great extent prevented by lifestyle changes (for example avoiding fat and meat rich diet, smoking cessation, stress reduction, increased physical activity, etc.). If it is related to pathological conditions such as arterial hypertension, diabetes mellitus, or lipid disorders themselves, it

could be in addition to life style changes, the underlying disease could be pharmacologically controlled to reduce the risk of progressive CVD (10).

Dyslipidaemia together with central obesity, arterial hypertension and altered glucose metabolism collectively form a chronic debilitating and sometimes fatal syndrome called metabolic syndrome (11). This metabolic syndrome is very common nowadays with an incidence of up to 25% of the population in some developed countries. Sadly, it is also common among children and has a very serious impact on their health. As it contributes to many cardiovascular problems, hepatic steatosis, or diabetes mellitus type 2 (DM 2), etc. (11).

However, the differential diagnosis can be challenging in some patients especially with respect to hypertriglyceridemia/chylomicronemia (6, 10, 12). Since the rate of lipid disorders is increasing particularly in patients of younger age and in developed countries, the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) recommended that screening for lipid disorders regardless of the risk profile for coronary heart disease (CHD) should be started from an age of 20 years on (13). This shows how important it is to detect, manage, and keep lipid disorders under control (10).

Dyslipidaemia comprises a large group of lipid disorders, in which every disorder can be present alone or in combination with other lipid abnormalities. These include hypercholesterolemia (increased total cholesterol ≥240 mg/dL [6.20 mmol/L]), LDL-C (Low Density Lipoprotein- Cholesterol) (>160 mg/dL [4.13 mmol/L]), hypertriglyceridemia (triglyceride levels >200 mg/dL [2.25 mmol/L]) and decreased HDL-C (High Density Lipoprotein Cholesterol) (<40 mg/dL [1.03 mmol/L]). However, these levels are not fixed and can vary between regions (1) as well as race, individual risk, and age. Calculating the individual risk for CVD related to a certain cholesterol level by risk score calculation such as the Framingham Risk Score, the ACC/AHA Arteriosclerotic Cardiovascular Disease Risk Estimator, or the Reynolds Risk Score seems to result in a better risk estimation as compared to a sole analysis of particular laboratory parameters of the lipid metabolism (13).

Hypertriglycerdemia

Definition

HTG is a laboratory diagnosis, which is defined as increased TG levels above 150 mg/dl in a fasting state. Where levels between 150 - 999 mg/dl are classified as mild to moderate HTG and levels above 1000 mg/dl are classified as severe to very severe HTG (14). The aim of this

classification is to asses the risk of CVD, which is higher in mild to moderate HTG, and the risk of pancreatitis, which is higher in severe to very severe HTG (14).

Aetiology

The causes of HTG can be classified into primary and secondary (1, 6, 10). Primary HTG is considered when genetic causes are detected such as, LPL deficiency, apolipoprotein C-II (apoC2) deficiency, apolipoprotein AV deficiency, dysbetalipoproteinemia, GPIHBP1 deficiency (expressed in childhood und persists for life), familial hypertriglyceridemia (FHTG), familial combined hyperlipidaemia (FCHL) or primary metabolic susceptibility as in metabolic syndrome (10, 15). Secondary HTG is considered when one or more of the secondary causes (1) is/are present. These include (1, 10, 16): type 2 diabetes mellitus, hypothyroidism, HIV-infection, Cushing syndrome, acromegaly, growth hormone deficiency, nephrotic syndrome, renal disease, paraproteinemia, alcohol consumption, obesity, lipodystrophy, metabolic syndrome, pregnancy, chronic idiopathic urticaria, dietary causes as well as medications such as corticosteroids, estrogens (oral, not transdermal), non-selective β-blockers, thiazides, tamoxifen, raloxifene, protease inhibitors, retinoic acid, isotretinoin, sirolimus, L-asparaginase, bile acid resins, phenothiazines, antipsychotics (second generation) and immunosuppressants. Treating these causes could in some patients reduce hypertriglyceridemia.

The term “familial HTG” led to the misunderstanding of the aetiology of HTG, suggesting that HTG can be inherited through the classical Mendalian pattern of inheritance as it runs in families. However, it has been shown, that it is in most of the cases polygenic and that the genetic susceptibility carried on different chromosomes and do not always follow Mendalian pattern of inheritance (1). However, in some patients sever chylomicronemia is present without the mentioned underlying factors.

Chylomicronemia

Definition

Chylomicons are the largest lipoproteins, which transport most of the ingested and absorped triglycerides, cholesterol and phosphlipids to the liver (17). Chylomicronemia is defined as an abundance of chylomicrons in the blood, due to an impaired clearance, which results in the typically milky appearance of the plasma (18). Severe chylomicronemia is associated with a

severe elevation of TG concentrations above 1500 mg/dl that also persists after fasting (18, 19).

Pathophysiology

An average person would eat about 20-70 g of fat per meal, so that we spend most of the day in a variable lipemic state, that's why it is crucial to understand the pathophysiology of lipid metabolism before speaking about the abnormalities (8)

The metabolism of fat starts after its absorption in the intestines and conversion to lipoproteins (LPs). These are then processed by LPL (20). As shown above in figure 1 short- and medium-chain fatty acids bind to albumin and carried straightforward to the liver. Whereas long-chain fatty acids are directed to the intestine, only where apo B-48 is secreted in humans, to be converted to chylomicrons. After reaching the circulation, chylomicrons are then hydrolysed in the presence of LPL releasing free fatty acids (FFA) for storage or as a direct source of energy. Yet this process results also in the production of the so called 'chylomicron remnants', which are then taken by the liver in the presence of apo E for lysosomal degradation. On the other hand triglycerides are also produced from the liver (hepatic or endogenous source, mostly during fasting) as VLDL, which are also hydrolysed in the circulation through LPL and degraded to smaller particles (IDL and LDL) (8, 21).

Lipolysis is very important for our body in order to provide energy for the cells. As noted above, this process produces also particular remnants, which are highly atherogenic (e.g. LDL) (22). Apo B can be found in lipoprotein (a), LDL, IDL, VLDL as well as chylomicrons, which are all pro-atherogenic lipoproteins. Therefore, by measuring apo B one can directly detect how many atherogenic lipoproteins are circulating in the bloodstream and analyse the risk of developing CVD (8, 10). Usually, LPL clears with the help of cofactor apo c-II- chylomicrons from the plasma. Any disruption of this process leads to an accumulation of chylomicrons in the blood, which is called familial chylomicronaemia or hyperlipoproteinemia (HLP) type 1 according to the Fredrickson classification (6). The FCS is considered as one of the five HTG phenotypes according to the Fredrickson Classification/ WHO International Classification of Diseases based on patterns of lipoprotein fractions (1), in which the fat metabolism is impaired leading to an accumulation of chylomicrons and triglyceride-rich lipoproteins in the plasma. Figure 2 below shows the classification of hypertriglyceridemia. This results in various cardiovascular complications and comorbidities as well as in recurrent acute or chronic pancreatitis (23).

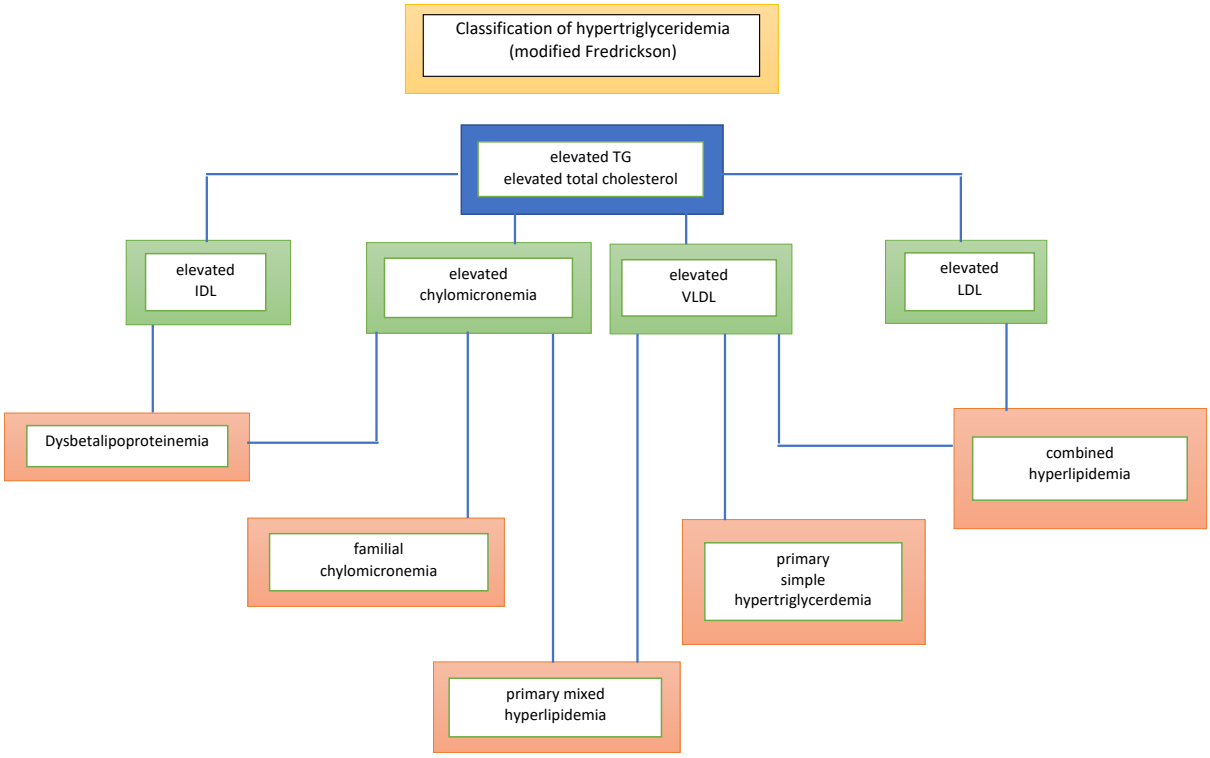


Figure 2 : simplified figure showing classification of HTG (modified Fredrikson) based on lipoproteins and their combinations (1). Familial chylomicronemia (HLP type 1), Combined hyperlipidemia (HLP type 2B), Dysbetalipoproteinemia (HLP type 3), Primary simple hypertriglyceridemia (HLP type 4), Primary mixed hyperlipidemia (HLP type 5) (TG: Triglycerides, IDL: intermediate Density Lipoprotein, VLDL: Very Low Density Lipoprotein, HDL: High Density Lipoprotein).

It took some effort to understand how LPL travels from its site of production in adipocytes and myocytes to its site of action, the capillary lumen. First scientists thought that positively-charged (heparin–binding domains e.g., apo-B, apo-AV, apo-E) LPL binds to negatively charged heparin sulfate proteoglycans (HSPGs) since LPL is released after the injection of heparin. The discovery of GPIHBP1 changed this view and revealed how important this protein is for the proceeding of lipolysis (15, 20, 22). As LPL binds to HSPG only in the absence of GPIHBP1, otherwise GPIHBP1 releases LPL from this binding in an on and off mode. Actually, LPL binding to HSPG is not essential for the process of lipolysis to proceed (15, 24, 25). Since both pre- and post-heparin plasma levels of LPL tend to be low in patients with GPIHBP1 deficiency, this demonestrates the inability of LPL to cross to the intravascular compartment without GPIHBP1 (19).

LPL has two domains an N-terminal domain (NTD) concerned with the catalysis and a C-terminal domain (CTD) binding to lipids (26).

GPIHBP1 1 is a protein on the interstitial surface of capillary endothelial cells, which binds to lipoprotein lipase and transfers it across the endothelium to the capillary lumen. GPIHBP1 is not only responsible for the transport of LPL, it also protects it from catabolism (15). This protein is found in nearly every capillary wall in our body with a different amount in every organ according to the demand of free fatty acids. For example, it is abundant in cardiomyocytes, adipose tissue, and pulmonary capillaries but not found in the cerebral capillaries, because our brain uses glucose as an energy supply (15, 22, 25). The exact function of GPIHBP1 in the lung is not yet understood. Although there is abundant GPIHBP1 in the lung capillaries, patients with GPIHBP1-deficiency do not present with pulmonary disorders (15).

GPIHBP1 is pivotal for the process of lipolysis as it is the only protein guiding LPL to the capillary endothelium, where lipolysis takes place (15, 27). Interestingly this protein, as well as it's antibodies, can cross the capillary endothelial cells bi-directionally (15, 28, 29).

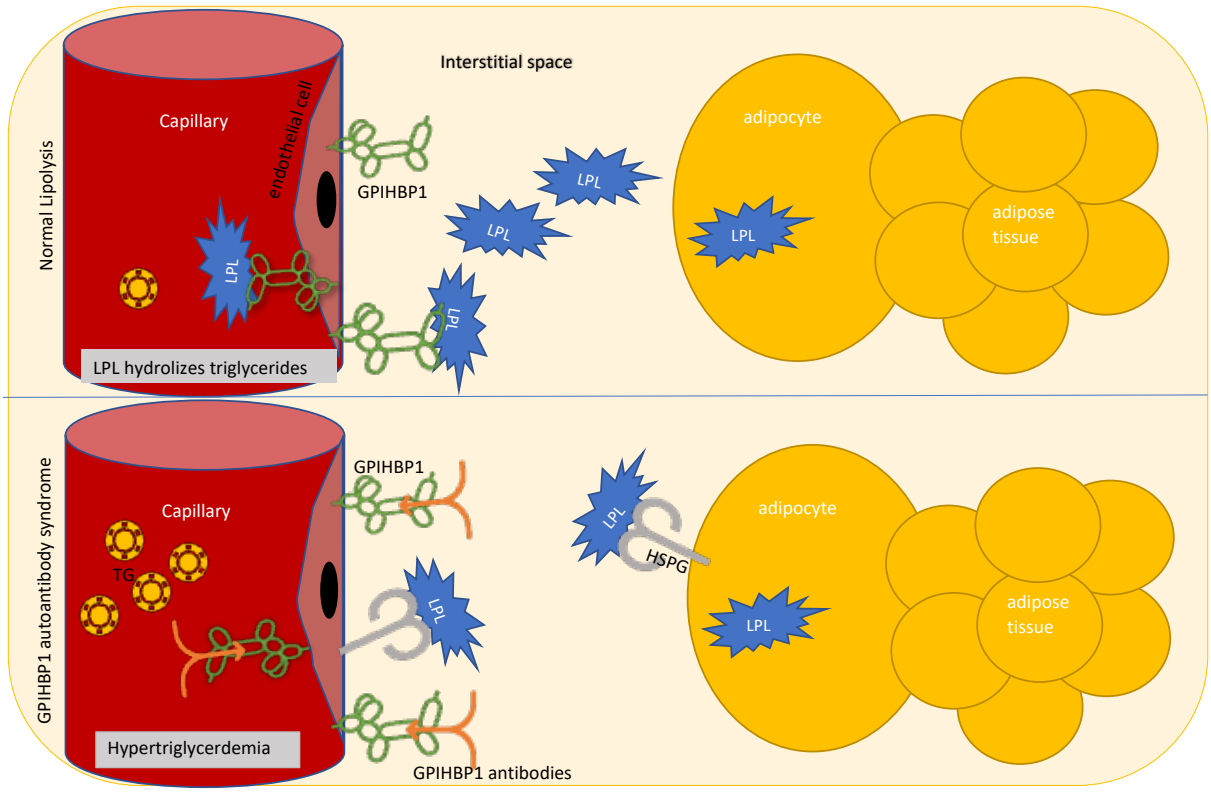


Figure 3: Difference between normal lipolysis and lipolysis in the presence of GPIHBP1 autoantibodies. (LPL: Lipoprotein lipase, GPIHBP1: Glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1, HSPG: Heparan Sulfate Proteoglycans, TG: Triglycerides).

The gene of GPIHBP1 encodes a protein called glycosylphosphatidylinositol-anchored protein. This protein, a part from lymphocyte antigen 6 (Ly6) family, has two important domains. One is a three-fingered LU-domain (which contains 10 disulfide bonded cysteines) and the other is an amino-terminal disordered acidic domain (which contains sulfated tyrosine in the middle of acidic domain and aspartates and glutamates). Both domains are very important for the binding with LPL and stabilizing this complex. But the acidic domain is explicitly needed for the binding with LPL, and its mutation disrupts this binding (15, 20, 24, 27, 28). GPIHBP1 acidic-domain also stabilizes and protects LPL by preventing its spontaneous catalytic-domain unfolding (15, 28).

Only patients with two mutant GPIHBP1 alleles suffer from chylomicronaemia, and the mutation is likely to be in the LU-Domain. However, heterozygotes have normal TG levels (15). For a normal lipolysis to take place, only half of the normally functioning GPHIBP1 is needed (28).

Autoantibodies against GPIHBP1 proved to be directed to the LU-domain and not to the whole protein (15). Whether GPIHBP1 autoantibodies cause tissue injury and inflammation is not clear, so far (19).

Thus, in absence of GPIHBP1, LPL remains in the interstitial space which results in severe hypertriglyceridemia i.e. chylomicronaemia, due to the absence of LPL in the capillary lumen (27, 29). It was proven that low plasma LPL is present in both genetic GPIHBP1 deficiency and GPIHBP1 autoantibody syndrome, which can be then as an evidence, that exclusively GPIHBP1 transfers LPL to the capillary lumen (30).

LPL associated molecules, other than GPIHBP1, for example Apo C-II, Apo A-V, and lipase maturation factor 1 are also crucial for lipolysis. Genetic defects in any of them also leads to severe hyperchylomicronaemia (31). In addition, anti-LPL undoubtedly affects the activity of LPL, either through increasing its turnover or through inhibiting its function (32).

Autoantibody mediated chylomicronemia

In 1970, autoimmune hyperlipidaemia was first described by Beaumont (33). The chylomicronaemia syndrome, in which the triglyceride level is severely elevated and exceeds 16.95 mmol/L [1500 mg/dL], was first described in 1981 (34).

Later in 1989 the first case of a patient with autoimmune hyperchylomicronaemia was reported, in which the investigators were able to confirm that anti- lipoprotein lipase (LPL) autoantibodies were responsible for a decreased LPL activity (31).

Recently autoantibodies interfering with glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) has been identified as a cause of chylomicronemia with severe hypertriglyceridemia (HTG) (29). The fact that inhibition of GPIHBP1 interferes with the degradation of triglycerides was first discovered in a study of mice, where mice lacking GPIHBP1 demonstrated severe HTG (28).

Epidemiology and prevalence of autoantibody mediated chylomicronemia

Until now only very few cases were diagnosed with autoimmune HTG as a result of LPL-, apo C-II- or GPIHBP1 antibodies (35). To the best of our knowledge, so far 22 cases have been diagnosed with the GPIHBP1 autoantibody syndrome worldwide (19, 29, 30, 36). The presented patient is the first one discovered in Germany so far.

It has been tried in many studies to analyse the prevalence of this syndrome. For example, in a study by Beigneux et al. in 2017 GPIHBP1 autoantibodies together with a low plasma lipoprotein lipase have been detected in 4.6 % (6/130) of patients with chylomicronaemia. Four of them had also a history of autoimmune diseases. Three patients had a history of systemic lupus erythematous (SLE) and one had Sjögren's syndrome. In another Dutch study of the same year, only one patient among 33 screened patients with unexplained HTG was diagnosed with this syndrome (29, 30).

So far, the prevalence of GPIHBP1 autoantibody mediated chylomicronaemia is not clear, but it could not be as rare as it seems. Most probably, it is only not well known and therefore it is rarely considered as a differential diagnosis. Moreover, diagnsotic tests for GPIHBP1 are not routinely available (19, 30, 36).

Diagnosis and differential diagnosis of chylomicronaemia

Diagnosing a patient with familial chylomicronaemia based on history and clinical examination is difficult. Starting with the history, patients with familial chylomicronaemia usually report recurrent epigastric pain with or without pancreatitis. They may also report neurological symptoms like irritability. Going through the clinical examination eruptive xanthomata and hepatosplenomegaly could be observed. Lipemia retinalis should also be excluded during the examination (6, 37).

Previously, familial chylomicronaemia was diagnosed by analysing the patients' plasma for a loss of LPL activity after an i.v. injection of heparin. This test is not widely performed nowadays (6). Patients with GPIHBP1 autoantibody syndrome usually have severe

chylomicronaemia that is irresponsive or rather poorly responsive to traditional therapy. It is also noteworthy that the chylomicronaemia in these patients is intermittent, so that patients come with episodes of chylomicronaemia that improves from time to time coinciding with episodes of autoantibody release (19).

It was observed that examining the plasma of patients with GPIHBP1 deficiency show macroscopically milky plasma regardless of a restricted fat diet (6) (28, 38)

The key for establishing the diagnosis of GPIHBP1 autoantibody syndrome consists of four key findings: very high triglyceride serum levels, very low serum GPIHBP1 levels, low LPL levels as well as the detection of GPIHBP1 autoantibodies. Yet GPIHBP1 levels are not necessary for the diagnosis and must not be tested (15, 19, 29). However, as GPIHBP1 autoantibody syndrome is not well recognized yet, some time may pass until the correct diagnosis is established (34). Thus, every patient with an acquired chylomicronaemia of otherwise unknown origin, even without autoimmune diseases, should be tested for autoantibodies against GPIHBP1 (29).

In 2018 a new method for detection of GPIHBP1 autoantibodies was developed using a solid-phase ELISA (Enzyme-linked Immunosorbent Assay) for quantifying GPIHBP1 autoantibodies, which can detect GPIHBP1 autoantibodies as low as 0.03 U/ml (39).

After establishing the diagnosis of Hypertriglyceridemia, one should search for a possible cause, which could be on one side one of the secondary causes of HTG (as mentioned above, page 4 and 5), or on the other side due to genetic disorder. As it is easier to exclude a secondary cause through simple investigations (examples are listed in figure 4 below) before doing a complex and expensive genetic work up, secondary causes should be first excluded. The genetic causes could be a mutation, whether homozygous or heterozygous, in the LPL gene itself or other genes encoding proteins responsible for its activity, such as APOC2 (LPL-co-activator), APOA5 (enables chylomicrons and VLDL interaction with LPL), LMF (important for LPL folding and intracellular trafficking) and GPIHBP1. Also autoantibodies against these proteins cause autoimmune chylomicronaemia (35, 37).

Moreover, drug induced GPIHBP1 autoantibody syndrome should be excluded if it occurs in a patient with autoimmune disease under therapy with an immune system modifying agent. This has been described in a patient with multiple sclerosis treated with interferon (IFN) β1. Here, a severe rise of plasma TG accompanied with a drop of LPL was observed under treatment with IFN. The plasma TG levels dropped back to normal levels and GPIHBP1 autoantibodies disappeared after discontinuation of the IFN therapy (36).

GPIHBP1 activity seems to be very low in patients with GIHBP1 autoantibody syndrome without real GPIHBP1 deficiency. This is only due to the inability of current immunoassays to detect GPIHBP1 in the presence of its autoantibodies (19, 40).

Algorithm for the Diagnosis of Chylomicrtonemia due to GPIHBP1 autoantibodies	
Initial Workup (History and Clinical Examiniation)	- recurrent epigastric pain with or without pancreatitis - associated autoimmune diseases (with or without positive ANA) - neurological symptoms as irritability, confusion, memory loss - eruptive xanthomata - Lipemia retinalis - hepatosplenomegaly
Diagnostic Workup (Laboratory Investigations)	- severe intermitent chylomicronemia, irresponsive or poorly responsive to traditional therapy - serum triglyceride (very high, usually >1500 mg/dL) - serum LPL (low)
Advanced Workup (Specific Investigations)	- very low serum GPIHBP1 levels (not essential for diagnosis) - GPIHBP1 autoantibodies
Aetiological Workup	- exclude secondary causes of HTG (e.g.: FBG, HBA1c, TSH, T3, T4, HIV-PCR , 24-hour urinary cortisol, low-dose dexamethasone suppression test, IGF-1, Growth hormone suppression test, creatinin, albumin, ß-HCG) plus genetic susceptability. - drug induced GPIHBP1 autoantibody syndrome (e.g. IFN) - genetic testing: homogynous or heterogynous mutations in LPL, APOC2, APOA5, LMF1 or GPIHB - Autonatibodies against LPL, APOC2, APOA5, LMF or GPIHBP1

Figure 4: Algorithm for the diagnosis of chylomicronemia due to GPIHBP1 autoantibodies. FBG: Fasting Blood Glucose, HBA1c: Haemoglobin A1c, TSH: thyroid stimulating hormone, T3: Triiodothyronine, T4: thyroxine, HIV: Human Immunodeficiency Virus, IGF-1: Insulin-like growth factor 1, ß-HCG: Human chorionic gonadotropin (6, 15, 19, 28, 29, 34, 37, 41)

Complications and prognosis

Any disruption in the process of lipolysis leads to formation of a lot of atherogenic particles that cannot be processed by vital tissues and leads to severe hypertriglyceridemia with its complications, first and foremost, pancreatitis as well as CVD including stroke and myocardial infarction (26). The incidence of developing pancreatitis in patients with HTG (TG above > 10 mmol/L) is about 2% (42). However, even if TG-level is below 10 mmol/L and in absence of other CVD risk factors, the risk of CVD is high. A study in Copenhagen showed that 80 % of the untreated patients with TG >3.0 mmol/L developed CVD (43). Having combined hypertriglycerdemia increases the risk of CVD up to 14% (44).

Patients with GPIHBP1 autoantibody syndrome suffer from severe hypertriglyceridemia and subsequently are at increased risk of acute and chronic pancreatitis and lower risk of cardiovascular diseases with its comorbidities (6, 23).

The risk of acute pancreatitis is directly proportional to the plasma TG-Lvels (6, 42, 45). Although the specific mechanism of how HTG causes acute pancreatitis is not clear, it is considered as the third most common cause of acute pancreatitis, where about 10% of the cases with acute pancreatitis are believed to be due to HTG (45, 46). Recent studies assume that FFA accumulation in the pancreas is responsible for the pancreatic injuries through activation of inflammatory cascades and a direct cytotoxic effect (42, 47)

Regardless of traditional risk factors of atherosclerosis, SLE patients have increased risk to premature atherosclerosis (8.3-fold compared to normal population) due to high TG levels, which is strongly related to LPL-antibodies. This makes the prognosis worse when it is combined with GPIHBP1 autoantibody syndrome (32).

So as a matter of principle the higher the plasma TG levels are, the poorer is the prognosis and the higher is the mortality rate (45).

In this study we describe a patient with GPIHBP1 antibodies, who developed severe hypertriglyceridemia without any history of familial lipid metabolism disorder or any other secondary causes of lipid metabolism disorder.

Study design and objectives

Study design

The present study reports to the best of our knowledge the first patient with a GPIHBP1 autoantibody syndrome in Germany. So far 22 cases have been documented worldwide. The patient was followed up for about 34 months in our clinic including the diagnosis and course of the disease, treatment, and complications starting from her initial presentation in March 2018 with a severe chylomicronaemia until now.

Questions

1.) What is the course of the GPIHBP1 autoantibody syndrome after initiation of a conventional triglyceride lowering therapy or fat reduced diet?
2.) Is the removal of GPIHBP1 autoantibodies with extracorporeal techniques such as plasma separation or immunoadsorption effective for lowering triglyceride concentrations in this patient?
3.) Is a B-cell depleting therapy with the anti-CD 20 antibody (Rituximab) effective for lowering triglyceride concentrations in this patient with respect to the pathological importance of GPIHBP1 autoantibodies ?
4.) Which alternative therapeutic options could exist for this patient?
5.) Is the GPIHBP1 autoantibody syndrome associated with other autoimmune diseases?

Case Presentation

Patient's Examination/Presentation

A 27 year old female patient presented to our clinic for the evaluation and further analysis of severe HTG with chylomicronaemia, which was accidentally discovered by her general practitioner. She had a TG serum concentration of 7911 mg/dl and a total cholesterol serum concentration of 1036 mg/dl. By the time of presentation, the patient was in a good general condition with normal weight and a calculated BMI (body mass index) of 20.3 (weight = 60 kg, height =172 cm). Apart from occasional mild abdominal pain, she reported no other symptoms. In addition, the physical examination revealed no pathological findings and in particular no signs of a pancreatitis.

Past Medical history

She already had a remarkable past medical history. An antiphospholipid syndrome (APS) with venous sinus thrombosis had been diagnosed in September 2015 followed by a continuous oral anticoagulation with the vitamin K antagonist phenprocoumon. There was no history of any other thrombotic events or miscarriages. She also had a thyroidectomy due to Graves' disease. A myocarditis with temporary extra systoles was diagnosed in 2016. Cardio-MRT did not show any significant pathologic findings. The coronary angiography revealed a small fistula from the marginal branch of coronary artery to the left pulmonary vein. In 2002 she had a trauma related splenic rupture. She had also two benign right sided ovarian cysts. In 2016 a conization was performed to treat a cervical intraepithelial neoplasia (CIN). Furthermore, the patient was on a balanced diet predominantly vegetables und some meat.

After excluding secondary causes of hypertriglyceridemia including type 2 diabetes mellitus, alcohol consumption, obesity, metabolic syndrome and dietary causes, we started to search for genetic and other pathogenic factors of primary HTG with severe chylomicronaemia.

Materials and Methods

LPL activity was analysed from patient's serum using LPL-Activity-Assay-Kit for a quantitative measuring of LPL activity. Samples could be plasma with an anticoagulant (for example heparin, citrate or EDTA) or serum without anticoagulant or even tissue sample. After mixing the sample with the reagents lipase hydrolyzes a specific substrate and this reaction leads to fluorescence, where fluorometric intensity is directly proportional to the amount of hydrolyzed substrate and hence LPL activity (48).

GPIHBP1 was measured from the patient's serum using sandwich ELISA, where diluted patients' samples are added to standards into the wells. After the first reaction occurs HRP-conjugated secondary antibody is added into the wells, where the second reaction takes place. Then after washing Tetra Methyl Benzidine (TMB) is added to the wells and color develops (49).

GPIHBP1 antbodies were analysed from patient's serum by indirect ELISA, which is now available in Japan (Immuno-Biological Laboratories, Fujioka) only for research purposes and not yet approved for diagnostics (19). This Kit (Human GPIHBP1 Autoantibody Assay Kit – IBL) acts as a primary antigen when added to samples from patients (samples could be human serum, EDTA-plasma, heparin plasma or post-heparin EDTA-plasma) the first reaction occurs. Then Horseradish peroxidase (HRP)-conjugated 2ry Anti-human IgG Goat IgG is added to the wells for the secondary reaction. Finally Tetra Methyl Benzidine (TMB) is added after washing to the wells and chromogenic reaction happens (19, 50).

Plasma exchange

Here we used Spectra Optia apheresis system (Terumo BCT, Lakewood, USA) for therapeutic plasma exchange (TPE) based on centrifugal therapeutic apheresis and continuous flow with centrifugal blood cell separator according to our routine protocol. In each session we exchanged approximately 2000 ml of plasma through a dual lumen dialysis catheter. The separated plasma volume was continuously replaced with an equal amount of a 5% Human-Albumin (1500 ml multiLac 2 mmol/l potassium + 500 ml Human albumin solution). Under prophylactic use of 10% calcium gluconate according to our stanard protocols no signs of hypocalcaemia were observed. The patient tolerated all plasma exchange sessions without significant problems.

Immunoadsorption

Furthermore immunoadsorption (IA) was performed using the same Spectra Optia apheresis system with Plasmaflo OP-05W(L) as plasma separator, Immusorba TR-350 column as an adsorber through a dual lumen dialysis catheter according to our routine protocol. The anticoagulation was initially performed using fractionated heparin (500 I.E as bolus followed by continuous flow 1500 I.E./h). Due to very low venous pressure as low as 20 mmHg during IA we switched the anticoagulation to citrate.

Rituximab

RTX, the anti-CD20 monoclonal antibody, was administrated with a total dose of 375 mg/kg/m^2 at incrementally increasing infusion rates over six hours with monitoring of heart rate and blood pressure. This was repeated after a week, and then after about 6 months in a dose of 375 mg/kg/m^2.

Results

Blood and urine analysis at her first presentation were done according to the standard routine procedures in the associated clinical chemistry laboratory of our institution.
The results of the initial laboratory analysis are presented in table 1.

Parameter	**Results**	**Reference**
Sodium (mmol/l)	134	132-146
Potassium (mmol/l)	3.8	3.5-5.5
Calcium (mmol/l)	2.08	2.08-2.65
Creatinine (mg/dl)	0.53	0.5-1.1
SGOT (U/l)	16	13-40
SGPT (U/l)	14	7-40
AP (U/l)	40	46-116
g-GT (U/l)	3	<38
Lipase (U/l)	50	12-53
Amylase (U/l)	45	30-118
Protein (g/l)	41	57-82
Albumin (g/l)	60.7	32-48
CRP (mg/l)	< 4	<10
Cholesterol (mg/dl)	693	<200
Triglycerides (mg/dl)	> 5500	<150
HDL (mg/dl)	9.7	>60
Lp(a) (mg/dl)	15.1	
Urine analysis	**Results**	**Reference**
Leucocytes	neg.	neg.
Protein	neg.	neg.
Glucose	neg.	neg.
Erythrocytes	neg.	neg.
Protein/creatinine ratio (g/gKrea)	0.09	<0.1
Albumin/creatine ratio (g/gKrea)	2,2	<0.02
Blood count		
Thrombocytes (/nl)	180	186-353

Parameter	Results	Reference
Hemoglobin (g/dl)	14.7	10.6-13.5
Erythrocytes (/pl)	2.2	3.7-4.87
Leukocytes (/nl)	6.85	4.37-9.68
Antibodies		
Anti-phosphatidyl serine IgG (U/ml)	< 6.3	
Cardiolipin antibodies	<7.5	
IgM (U/ml)	24.1	
ANA	0.263888889	neg.
ENA differentiation		
U1-nRNP	neg.	neg.
SmD	neg.	neg.
SS-A 60/52 kDa	neg.	neg.
SS-B	neg.	neg.
Scl-70	neg.	neg.
Jo-1	neg.	neg.
CENP-B	pos.	neg.
Thyreoglobulin-AB (TGAB) (IU/ml)	< 100	<100
Peroxidase-AB (TPO) (IU/ml)	< 33	<33
TSH-receptor-AB (TRAB) (U/ml)	<1.8	<1.0
ds DNA (IU/ml)	0.6	<10.0
pANCA/MPO U/ml	< 0.2	<3.5
cANCA/PR3 U/ml	< 0.2	<2.0
direct Coombs test	neg.	neg.

Table 1 Patient's initial laboratory findings at presentation.

(SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase, AP: alkaline phosphatase, g-GT: gamma-glutamyl transferase, CRP: c-reactive protein, HDL: high density lipoprotein, Lp (a): lipoprotein a, Ig: immunoglobin, ANA: antinuclear antibodies, U1-nRNP: Uridin Ribonucleoproteins, Sm D: Smith, SS-A: or Anti-Ro autoantibodies for systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS), kDA: kilodalton, SSB: or Anti-La Anti-Sjogren syndrome antigen B, Scl-70: scleroderma antibodies, Jo: John P. antibodies, which are associated with inflammatory myopathies, CENP-B: Centromere protein B, AB: antibodies, TSH: thyroid stimulating hormone, dsDNA: Anti-double stranded DNA antibodies, pANCA: perinuclear anti-neutrophil cytoplasmic

antibodies, MPO: myeloperoxidase, cANCA: cytoplasmic anti-neutrophil cytoplasmic antibodies, PR3: proteinase 3.

Laboratory parameters most strikingly outside the normal range were a profound hyperlipidemia with triglyceride concentrations > 5500mg/dl, high LDL Cholesterol >120 mg/dl and a low HDL concentration < 10 mg/dl. Her serum was further analyzed to identify the cause of the severe lipid disorder. In view of her multiple autoimmune disorders (thyreoditis, myocarditis, anti-phospholipid syndrome) and after exclusion of common secondary causes of hypertriglyceridemia such as diabetes mellitus, adipositas, excessive alcohol consumption or familial causes we searched for an autoimmune pathogenesis of her lipid disorder. So the key enzyme in the triglyceride metabolism pathway lipoprotein lipase (LPL) and its transporter protein GPIHBP1 together with the respective autoantibodies against this transporter protein were analyzed. This revealed extremely low levels of LPL (< 10 ng/ml) while GPIHBP1 was undetectable in her serum. In congruence with the low concentration of LPL and the absence of GPIHBP1 we detected high levels of anti-GPIHBP1 autoantibodies in her serum (table 2). She also had positive ANA and positive ENA screening for CENP-B. However, a diagnosis of a systemic lupus erythematodes (SLE) seems unlikely as the dsDNA antibodies were repeatedly negative and there were no further clinical signs of SLE present.

	Results	**Reference**
GPIHBP1 autoantibodies (U/ml)	5414	< 58.4
Serum TG range (mg/dl)	>5000	< 150
GPIHBP1 mass (pg/ml)	0	570.6 - 1625.6
LPL mass (ng/ml)	7	26.5 - 105.5

Table 2 GPIHBP1, - autoantibodies, Triglycerides range and LPL mass at diagnosis.

Patient's management including extracorporeal therapy

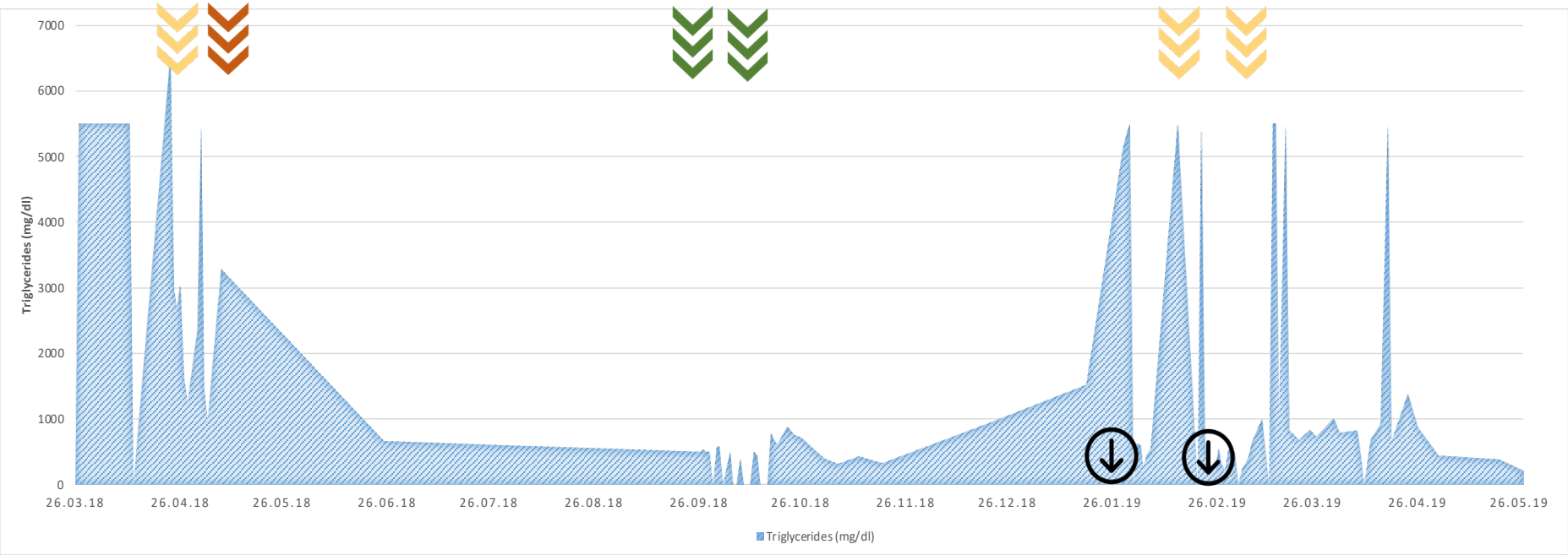
The patient was initially treated with fenofibrate (1 x 250 mg/day) and ezetimibe (10 mg/day) and a diet with a restricted amount of fat and glucose in order to lower the triglyceride levels. Additionally, as an underlying autoimmune disorder seemed to be present, she received a steroid bolus (3 x125 mg/d for three days) followed by tapering of the steroids to a maintenance dose of 7.5 mg/d. However, this did not result in a substantial decrease of the triglyceride concentrations. We then performed a course of four plasmaphereses in April 2018 to reduce the risk of an acute pancreatitis (Graph 1). Triglyceride concentrations were then reduced reaching 987 mg/dl but increased again shortly thereafter.

Despite the initial pharmacologic therapy, dietary restrictions and the exracorporeal therapies, triglyceride concentrations did not decrease constantly.

She developed a severe acute necrotizing pancreatitis together with an immune mediated thrombocytopenia as well as a haemolytic anaemia in September 2018. During the following months multiple abdominal and retroperitoneal pseudocysts developed, while some of them had to be continuously drained. Following the pancreatitis, she received 14 plasmaphereses, 10 immunoadsorptions, and 2 infusions of immunoglobulins. Triglyceride concentrations varied between 200 mg/dl and 11.000 mg/dl. She lost approximately 10 kg of her body weight and was distressed by severe episodes of recurrent abdominal pain as well as fever. Blood cultures and urine cultures were all without evidence of pathogens. Her condition did not improve substantially and the hypertriglyceridemia improved only transitionally after the extracorporeal treatments.

In view of the autoimmune pathogenesis with a detection of GPIHBP1 autoantibodies we planned a therapy with the B-cell (CD20) depleting antibody (rituximab) in order to interfere with the B-cell – T-cell interaction as well as the reduction of antibody production through an indirect reduction of plasma cells by the depletion of B-cells.

Thus, she received 3 infusions of rituximab (RTX dose of 375 mg/1.73 m2 body surface area) in 2019. Her triglyceride concentrations normalized with constant values around 60 mg/dl. Normalization of triglyceride concentrations was accompanied by disappearance of GPIHBP1 autoantibodies and increasing serum levels of both GPIHBP1 and LPL (table 2 and graph 2). She receives a regular check-up every 3-4 months in our clinic and her medical condition continues to be good.

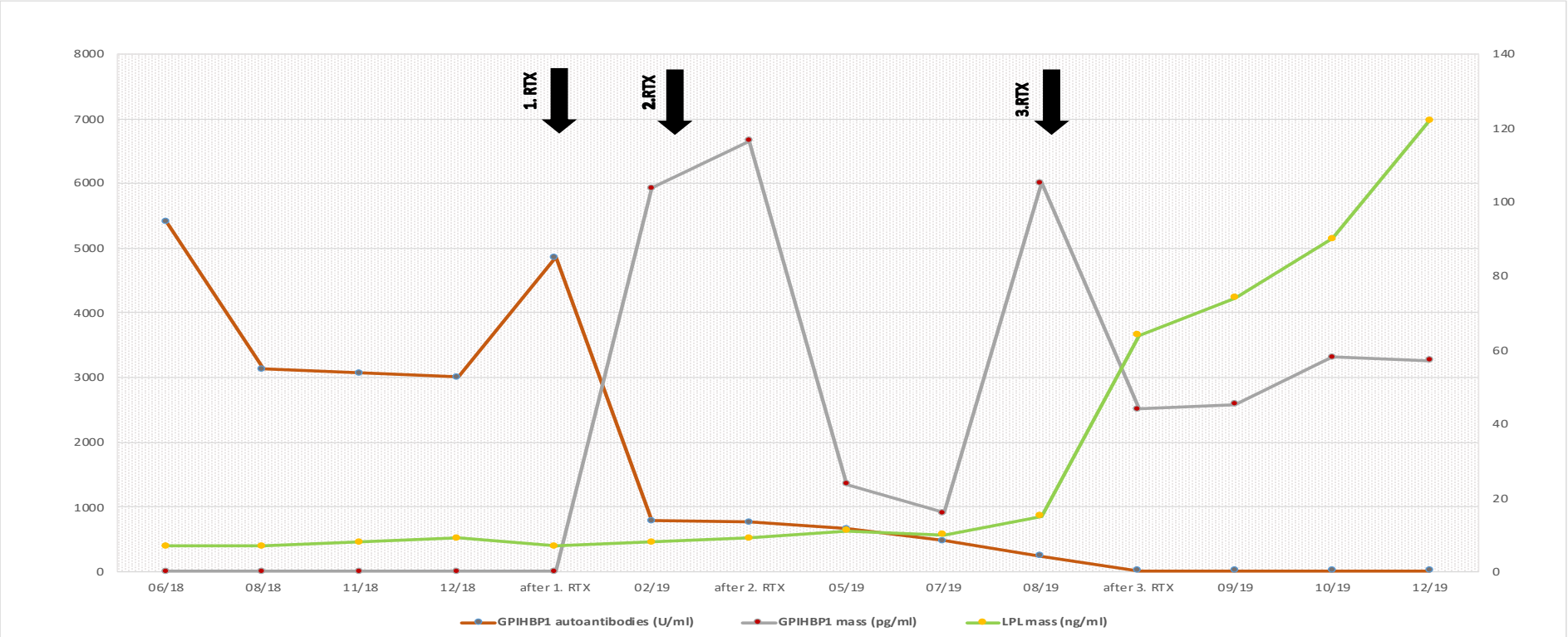


Graph 1: Serum triglyceride concentrations after first presentation with severe chylomicronemia, and after treatment; plasma separations: 4 sessions in April 2018, 7 sessions in February and 3 sessions in March 2019 (yellow arrows), LDL-apheresis: 3 sessions in Mai 2018 (brown arrows), immunoadsorptions 3 sessions in September and 5 sessions in October 2018 (green arrows), Rituximab: first and second infusions in January and February 2019 (black arrows). Our patient showed also no significant response to this therapy (see table 3).

	GPIHBP1 autoantibodies (U/ml)	Serum TG range (mg/dl)	GPIHBP1 mass (pg/ml)	LPL mass (ng/ml)
Refrences	<58.4 U/ml	<150 mg/dl	570.6-1625.6 pg/ml	26.5-105.5 ng/ml
Jun 18	5414	>650	0	7
Aug 18	3130	**>500**	0	7
Nov 18	3073	334-472	0	8
Dec 18	3011	530-935	0	9
Jan 19 (1. RTX)	**4862**	**614-5500**	**0**	**7**
Feb 19	787	271-5500	5924	8
Feb 19 (2. RTX)	**763**	**180-5500**	**6660**	**9**
May 19	662	215-1468	1347	11
Jul 19	474	51-52	914	10
Aug 19	239	58-78	6012	15
Aug 19 (3.RTX)	**11**	**52**	**2512**	**64**
Sep 19	11	55-67	2583	74
Oct 19	15	>60	3312	90
Dec 19	16	42-53	3260	122
Jan 20	20.2		732.5	64
May 20	29.1	52	2621.9	69.3

Table 3 Levels of GPIHBP1 antibodies (autoAbs), range of serum triglycerides (TG), and levels of GPIHBP1 and LPL mass after the patient's initial presentation with chylomicronemia till last check up.

Data for the first, second, and third (final) rituximab infusions (1/19, 2/19, 8/19) are highlighted in yellow.



Graph 2: GPIHBP1-, autoantibodies and LPL course under Treatment with RTX.

Plasma GPIHBP1 autoantibodies (autoAbs, left y-axis, orange curve), GPIHBP1 mass (left y-axis, grey curve), and LPL mass (right y-axis, green curve) before and after rituximab (RTX, black arrows) treatments. During calendar year 2020 and 2021, the plasma triglyceride levels remained normal.

Discussion

Despite increased awareness and lipid lowering drugs, disorders of the lipid metabolism, including hypertriglyceridemia und hypercholesterolemia, are not uncommon within the population with relatively high incidence and prevalence up to over 20% in some developed countries (1, 3, 51). FCS is a very rare autosomal recessive disorder with a prevalence of 1 - 2 persons per million population (52).

Here we report on the first patient with GPIHBP1 autoantibody syndrome in Germany. So far 22 patients have been reported worldwide. And this is the first patient who received a well-documented and successful therapy with TPE/immunoadsorption and the antiCD20 antibody rituximab.

Course of the GPIHBP1 autoantibody syndrome after initiation of a conventional triglyceride lowering therapy

A decrease of dietary intake of fat and glucose together with weight loss, regular physical exercise and restricted alcohol intake would be the first line of mangement of HTG (6, 10, 12). The second line of treatment consists of traditional pharmacological therapy, including: Fibrates (e.g: gemfibrozil, fenofibrate, and bezafibrate) and Omega 3 preparations, which mainly target TGs, while Statins, ezetimibe, bile acid sequestrants, and PCSK9 Inhibitors mainly target LDL and are less important in the treatment of HTG (53). Generally monotherapy should be tried first before combinations with other drugs are applied (6, 10, 16).

However, such treatment options seem not to be very effective in patients with familial chylomicronemia and in particular with the GPIHBP1 autoantibody syndrome. This is reported in different cases treated with restricted-fat diet, fibrates, and *n*-3 fatty acid supplements, where patients – if at all- showed slight response to conventional therapy (6, 19, 29, 54).

Our patient showed also no significant lasting response to dietary modulation and medical therapy with fenofibrate and ezetimibe (see table 2 and graph 1).

Removal of GPIHBP1 autoantibodies with extracorporeal techniques

Triglycerides and antibodies can be removed from plasma with unselective plasma separation, while Immunadsorption selectively removes antibodies from plasma. (55, 56). Table 3 below shows the difference between TPE, IA and lipoprotein-apheresis.

	Therapeutic plasma seperation	Immunadsorption	Lipoprotein apheresis
Procedure	Non-selective	Selective	Selective
Anticoagulation	Heparin and/or citrate, alternative: Argatroban or Hirudin	Heparin and/or citrate, alternative: Argatroban or Hirudin	Heparin and/or citrate, alternative: Argatroban or Hirudin
Technique	Plasma seperation by centrifugation or plasma filter	Plasma seperation by centrifugation or plasma filter + Adsorption of Immunglobuline, complement and Immuncomplexes	Plasma seperation by centrifugation or plasma filter + Filtration of LDL, TG and Lp (a)
Elimination of	Proteins, complements, coagulation factors, antibodies, TG, etc	Dependent on the adsorper used	Dependent on the filter used
Substitution	Albumin or fresh frozen plasma (FFP)	Not necessary	Not necessary
Advantage	Short tern reduction of cholesterol + TG	Short term reduction of antibodies	Short term reduction of TG, LDL

Table 4: Different extracorporeal techniques in GPIHBP1 autoantibody syndrome (57).

As a short term therapy plasmapharesis could be considered in acute attacks to lower plasma TG rapidly, as only a few sessions of plasmaphersis are able to reduce TG levels by about over 60% (37, 55, 58). Plasmaphersis is recommended in patients with severe hypertriglycerdemia (usually above 1000 mg/dl), who are suffering from or at increased risk of acute pancreatitis, till TG reach a level below 500 mg/dl (56). It was proven that, the earlier (within 1-3 days after symptoms' onset) the plasmaphersis is started, the better the results are (symptoms improvement including less abdominal pain, nausea and emesis, earlier hospital discharge, improved prognosis, etc) (47, 55, 59, 60).

In order to lower the plasma triglyceride and to eliminate the autoantibodies, we performed 14 courses of total plasma exchange (TPE). Although TPE removes circulating immune complexes and circulating disease mediators, it resulted in our patient in the reduction of the TG levels only for a short time. However, TPE was successful in reducing the acute pancreatitis bouts.

In order to reduce antibodies and TG more selectively, we performed IA and LDL-apheresis. Where in IA her plasma, after being separated from blood, was allowed to circulate in an

adsorber columen to remove Igs (Immunglobulins). However LDL-apharesis is considered as more selective apharesis, where lipoproteins could be removed without affecting other componenets (61). The results after IA and LDL-Apharesis were by our patient also unsatisfactory, where here TG-levels showed only a short term reduction (see graph 1). Currently, there is no strict indications for aphersis by hypertriglycerdemia and the indication should be individually assessed (61, 62). Some authers believe that PS (Plasma separation) is not necessary even in severe HTG, as it did not offer significant difference in the mortality compared to tradional medical therapy (58).

B-cell depleting therapy in GPIHBP1 autoantibody syndrome

Rituximab (RTX)

RTX, which was primarily used in treatment of neoplasia, is now emerging as an option in the treatment of autoantibody related inflammatory and/or autoimmune diseases (SIADs) especially if it is refractory to conventional therapy (63). This occurs through selective B-cell depletion, where rituximab binds to CD20 on the surface of B-cells causing its death (63-65). The effect of RTX is believed to be also direct, through interrupting the B- / T-Cell interaction. This would result in a relatively faster effect when compared to the indirect antibody level reduction via reduction of the number of plasma cells (65, 66).

That means that, the anti-CD20 monoclonal antibody could have two important therapeutic effects in our patient: 1. Interruption of B-cell-T-cell interaction (short-term). 2. Secandary reduction of antibodies producing plasma cells by reduction of B-cells (long term). Thus inflammatory/immune process as well as the generation of antibodies-producing plasma cells could be reduced or stopped.

Based on the fact that RTX is a successful and safe treatment of some autoimmune and lymphoproliferative diseases (67, 68) and as autoantibodies play a pivotal role in the pathogenesis of HTG in our pateint, we decided to start a therapy with RTX. The decision for RTX was made consciously by our young female patient as it is well tolerated and the intervals between the infusions in the maintenance phase is about 6 months.

After the first two RTX infusions her serum GPIHBP1 and triglyceride concentrations fluctuated. This was probably due to the delayed maximum therapeutic effect of Rituximab that is reached after some weeks/months. Moreover, RTX affects first memory and mature B cells and second line plasma cells with the antibody levels. It is important to note, that B cell

number start to rise after approximately 12 months, while the half life time of RTX is one week (69-71).

After about 6 months from the first RTX infusion, her serum TG-, LPL- and GPIHBP1-levels were back in the normal range and GPIHBP1 autoantibodies could not be detected anymore. In addition, this treatment resulted in a significant improvement of her general condition toghether with regained appetite. Since her last discharge from the hospital in August 2019, she was not readmitted again. She was discharged from the hospital with a low steriod dose (Prednisolon 2.5/5 mg in alternation) only till December 2019. As the TG concentrations were normal without any significant concentrations of GPIHBP1 antibodies together with a good medical condition, we decided, to hold further RTX administrations and to follow her up.

An adequate therapeutic B-cell depletion could be detected in our patient even after > 2 years after the last RTX administration. That is why we strongly believe that our patient has reached a state of remission after RTX treatment, which was also reported in other autoimmune diseases (72-77).

Reducing or sometimes also complete cessation of corticosteroids count for the advantages of RTX therapy (67). Studies have proven that patients can tolerate long-term RTX therapy well with no cumulative side effects (63).

In 2018, Béliard et al. reported the first case of a male patient with autoimmune HTG due to anti LPL autoantibody, who was successfully treated with Rituximab. After effective lowering of TG, the patient was then switched to RTX maintenance therapy every 6 to 12 months (35).

In the study of Beigneux et al (29), one patient out of six with GPIHBP1 autoantibody syndrome, was treated with Rituximab (375 mg/m2 once weekly for four weeks) after recurrent HTG. She also received TPE and cyclophosphamide. Unfortunately, this patient died. The immediate cause of death was not mentioned, however, Huntington's disease was claimed as an underlying cause of death (29).

Alternative therapeutic options

Mycophenolate mofetil (MMF)

MMF (78) is MPA (Mycophenolic acid) 2-morpholinoethyl ester, whereas MPA is a formed by fermentation of Penicillium species. MMF, as a potent immunosuppressant and anti-inflammatory agent to prevent rejection reactions, has been also used to treat chronic inflammatory diseases. It is also widely used in organ transplantation (to prevent rejection reaction). MMF has shown great efficacy in the treatment of autoimmune diseases for example

SLE. The mechanism of MPA action is believed to be through inhibition of the proliferation of B- und T-lymphocytes and of antibody production, as well as T lymphocyte-mediated immunopathogenic process (79)

MMF successfully lowered plasma TG levels in a 9 years old child with GPIHBP1 autoantibody syndrome after increasing the usual dosis (MMF 1250 mg daily) and switching prednisolon to hydroxychloroquine (29, 80). There is unfortunatly no follow up reported for this case. Another patient in this study (20 years old female) received also MMF, while this did not result in TG-lowering.

Thus, it is not clear so far whether MMf can be used to treat GPIHBP1 syndrome.

Volanesorsen

Apo C-III is a lipopprotein that inhibits both lipoproetein lipase and hepatic lipase, which hinder the clearance of TGs (81). Volanesorsen, an antisense oligonucleotide apolipoprotein C-III inhibitor (16, 81), can lower TG levels up to > 70%. This has been proven in patients with FCS and is approved in Europe for these patients after failure of conventional therapy (81). However, also in patients without a specific genetic defect in TG metabolism this inhibitor could effectively reduce HTG (37). It can also be used to treat chylomicronemia due to mutations in the APOC2, GPIHBP1, APOA5 or LMF1 loci independently of any other lipid lowering therapies (16, 37). The mechanism by which Volanesorsen reduce TG in FCS is thought to be through increasing TG rich lipoproteins clearance by binding to Apo C-III mRNA and accelerating its degradation (16, 81). Volanesorsen results in a reduced number of pancreatitis episodes, improvement in insulin sensitivity and a better quality of life, where patients receiving volanesorsen reported improvemnet in their physical and psychological life in many aspects, as well as in their work life through less sick leaves (82). So far no patients with GPIHBP1 autoantibody syndrome were treated with Volanesorsen. (82). However, it could be an alternative if an immunosuppresive therapy would not be effective or would be contraindicated.

Alipogene tiparvovec

Alipogene tiparvovec was a trial gene therapy intended to treat familial LPL deficiency through expressing LPL in muscles and subsequently decreasing TG (83). Here also patients treated with Alipogene tiparvovec reported less pancreatitis attacks and better dietary flexibility, but it is no longer widely used because of its very short-term efficacy (83). Also treatment with

Alipogene tiparvovec is difficult as it is given under spinal anaesthesia due to very high number (more than 40) of intramuscular injections (16, 53).

Reported immunosuppressive strategies in autoimmune mediated HTG

Beigneux et al (29), reported 6 cases with GPIHBP1 autoantibody syndrome, who received different immunosuppresive therapy except for a baby girl born to a diseased mother. This neonate did not need any immunosuppresseive therapy, because her TG returned gradulally to normal after disapperance of her mothers' antibodies. The mother was diagnosed with GPIHBP1 autoantibody syndrome during pregnancy, which limited her therapeutic options to plasmaphersis and corticosteriods. After delivery she was on low dose steriods (5 mg Prednisolon).

Another patient (53 years old female) was on prednisolone (10 mg/day) and Salazosulfapyridine (1000 mg/day), however her TG were in the follow up still high and antibodies could still be detected. (29). The other patients (the one, who received RTX and the two, who received MMF) were reported above.

Immunsuppressive therapy was also effectivly used in patients with anti LPL antibodies (32, 33)

Association of the GPIHBP1 autoantibody syndrome with other autoimmune diseases

GPIHBP1 autoantibody syndrome can be accompagnied by other autoimmune diseases either at the same time or independently (15). Interestingly, there is no direct correlation found between the levels of GPIHBP1 autoantibodies and levels of TG. As it was observed that some patients have very high TG levels despite having low autoantibody levels and vice versa (19).

Many studies proved that hyperchylomicronaemia is strongly associated with autoimmune diseases such as SLE, Graves disease, thrombocytopenic purpura, rheumatoid arthritis, Sjogren's syndrome, Hashimoto's disease, anti-phospholipid syndrome, haemolytic anaemia (19). SLE, at least in the known cases till now, remains the most common associated autoimmune disease with GPIHBP1 autoantibody syndrome (19, 33). As discussed above in the study by Beigneux et al. of the six patients with chylomicronemia due to GPIHBP1 autoantibodies, four were diagnosed with autoimmune diseases (three with SLE and one with Sjögren's syndrome). Patients with SLE have a significant decrease in their LPL activity, which may be due to modulation by autoantibodies or inhibition by inflammatory pathways. Carvalho

et al. proved that about 40 % of the patients with SLE had anti-LPL antibodies. LPL antibodies have not only been identified in patients with SLE but also in patients with autoimmune rheumatic diseases such as rheumatoid arthritis and systemic sclerosis (33).

Moreover, anti-LPL antibodies were detected in patients with HTG, who have a positive family history for dyslipidaemia or genetic defects (33). These IgG antibodies could been detected by ELISA and were confirmed by immunoblotting (33). Yet Carvahlo (33) concluded that HTG can also occur in some patients due to anti-LPL antibodies without associated autoimmune diseases. Another study of 33 patients with unexplained chylomicronemia revealed a patient with autoantibodies against GPIHBP1 who does not have any other autoimmune disease (30). Examining the 22 patients with GPIHBP1 revealed that most of them have past history and/or serological evidence of autoimmune diseases (19). Especially noticeable was that 50% of the patients have antiphospholipid syndrome, which also makes the prognosis worse as these patients have higher risk of developing blood clots. Whether there is a relation between antiphospholipid antibodies and antibodies against GPIHBP1 remains so far unclear.

Our patient also had different autoimmune disease such as: Graves' disease, antiphospholipid antibody syndrome and autoimmune myocarditis). By our patient the clinical chemistry analysis revealed no signs of an SLE or a vasculitis while ANAs and a positive ENA screen for CENP-B were detectable.

Analysing the results from other studies and by our patient, we could conclude that the majority of patients with GPIHBP1 autoantibody syndrome have associated autoimmune diseases.

Miyashitaa et al. (19) have collected the data of the 22 patients documented worldwide with GPIHBP1 autoantibody syndrome (including our patient). Analysing this data revealed that most of the patients are females (17 out of 22). 16 patients out of 22 had positive ANA. The age of the patients in this sample was very variable ranging from 3 years old to 53 years old, but more than 90% of the patients where above 10 years old and below 40 years old. Five patients have received treatment with Rituximab with a very good response. Unfortunately we do not have enough information regarding there TG-levels after treatment and if there is relapses under this therapy. Our patient reamins the first patient with well documented long-standing follow up.

Conclusion

Dyslipidaemia is an important clinical issue, due to its high prevalence and high impact on morbidity and mortality of the community. Among different forms of dylipidemia there are rare forms with an autoimmune pathological background as described here in this patient with GPIHBP1 antibodies, the first one in Germany out of 22 patients worldwide. Loss of GPIHBP1 function, whether through autoimmne antibodies or through its deficiency, leads to severe chylomicronemia. In all patients with a newly diagnosed chylomicronemia of unknown origin, the GPIHBP1 autoantibody syndrome should be excluded. The diagnosis relies on the detection of hypertriglyceridemia (usually >1500 mg/dl), low LPL concentrations and GPIHBP1 autoantibodies. Therapy should concentrate on a long-term reduction of autoantibody levels. Here rituximab was successful in the describedpatient. However, alternative immunosuppressive regimen might be also successful, which has to be shown in the furure.

Summary

Severe chylomicronemia is a serious clinical condition as it can lead to severe, in some patients even to fatal, episodes of pancreatitis. GPIHBP1 autoantibody syndrome is an important differential diagnosis in patients with newly diagnosed unexplained severe chylomicronemia. Searching for associated autoimmune diseases is an important step after diagnosing GPIHBP1 autoantibody syndrome. So far, 22 patients have been described worldwide. Thus, experience with therapies is also limited. Conventional lipid lowering drugs have only marginal effects if any; this applies also for different forms of diets. Extracorporal therapies to lower triglycerides as well as autoantibody levels have only short term effects.

According to our data and based on the successful treatment and the relatively long-time follow up period of this patient, as the first case with GPIHBP1 autoantibody syndrome in Germany, Rituximab can be considered as a therapeutic option for the treatment of GPIHBP1 autoantibody syndrome as it can be safely applied and has long lasting effects on autoantibody levels. It can also be used as a maintenance therapy for long-term control of the disease. However there is no sufficient data regarding RTX as only very few patients have been treated with this substance so far and the follow up data scarce in these patients. In the future more patients with this syndrome need to be identified in order to determine the best treatment options as well as the duration of treatment. Furthermore, this will also shed some light on the nature of the disese and its tendency to reoccur after successful treatment.

Zusammenfassung

Eine ausgeprägte Chylomikronämie infolge einer massiven Hypertriglyceridämie kann zu schweren, bei manchen Patienten sogar tödlichen Episoden einer akuten Pankreatitis führen. Die Chylomikronämie kann infolge metabolischer Störungen oder im Rahmen einer Ernährungsstörung auftreten. In seltenen Fällen kann eine Chylomikronämie auch genetische aber auch autoimmunologische Ursachen wie z.B. bei dem GPIHBP1-Autoantikörpersyndrom haben. Dabei verhindern Autoantikörper gegen das Transportprotein GPIHBP1 die regelrechte Funktion der für den Triglyzeridmetabolismus wichtigen Lipoproteinlipase (LPL). Die etablierten Behandlungen sind dabei unzureichend. Lipidsenker haben, wenn überhaupt, nur eine minimale Wirkung. Dies gilt ebenso für eine Umstellung der Ernährung. Extrakorporale Eleminationsverfahren wie die Plasmapherese, LDL-Apharese oder Immunadsorption mit dem Ziel, die Triglycerid- und Autoantikörperkonzentrationen zu senken, haben nur kurzfristige Wirkungen. Bisher sind weltweit erst 22 Patienten mit dieser Erkrankung diagnostiziert worden. Daher sind auch die Erfahrungen mit Therapien dieser Patienten bisher begrenzt.

In der vorliegenden Untersuchung werden Möglichkeiten einer erfolgreichen Behandlung bei der ersten in Deutschland mit einer durch GPIHBP1-Autoantikörper vermittelten Chylomikronämie diagnostizierten Patientin aufgezeigt, deren Krankheitsverlauf am Beginn durch eine schwere akute Pankreatitis gekennzeichnet war. Nach zunächst erfolglosen Behandlungen mit konventionellen Substanzen bzw. Verfahren war erst die Behandlung mit dem gegen B-Zellen gerichteten CD20-Antikörper Rituximab (RTX) auch über einen längeren Verlauf erfolgreich. Dies zeigte sich vor allem an einer deutlichen Verbesserung des Allgemeinzustands sowie einer nachhaltigen Reduktion der Autoantikörper und einer Normalisierung der LPL-Konzentrationen. Eine erfolgreiche Behandlung mit RTX mit einer längeren Verlaufsbeobachtung wurde bisher nicht dokumentiert.

In Zukunft sollte das GPIHBP1-Autoantikörpersyndrom bei Patienten mit unklarer Chylomikronämie differenzialdiagnostisch abgeklärt werden. RTX kann dabei eine Grundlage für eine erfolgreiche Behandlung sein.

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Acknowledgement

Throughout the writing of this thesis I received a great deal of support and assistance.

I would like to thank my main supervisor for his patient support, persistent encouragement and for all of the opportunities I was given to complete this work.

I would also like to thank our secretary and the dialysis team for providing me with all the data I needed.

Finally, I would like to thank every member in my family for their continuous support and understanding.

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