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CASE REPORT

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Evolocumab and lipoprotein apheresis combination therapy may have synergic effects to reduce low-density lipoprotein cholesterol levels in heterozygous familial hypercholesterolemia: A case report

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A 49 years old woman (weight 68 kg, BMI 27.3 kg/m^2) with heterozygous familial hypercholesterolemia (HeFH) and multiple statin intolerance with muscle aches and creatine kinase elevation, presented at the Outpatient Lipid Clinic of Verona University Hospital in May 2015. Hypercholesterolemia was firstly diagnosed during adolescence, followed in adulthood by a diagnosis of Cogan's syndrome, a rheumatologic disorder characterized by corneal and inner ear inflammation. No xanthomas, corneal arcus, or vascular bruits were detectable at physical examination. Screening for macrovascular complications did not reveal relevant damages. Ongoing medical therapy included salicylic acid, methylprednisolone, methotrexate, and protonic-pump inhibitor. In the absence of specific lipid-lowering therapy, plasma lipid levels at first visit were: total-cholesterol = 522 mg/dL, LDL-cholesterol = 434 mg/dL, HDL-cholesterol = 84 mg/dL, triglycerides = 120 mg/dL, Lp(a) = 13 mg/dL. On December 2015, evolocumab 140 mg sc every 2 weeks was initiated. After a 24-week treatment, the LDL-cholesterol levels decreased by an average of 21.2% to 342 ± 22 mg/dL (mean ± SD). On May 2016, LDL-apheresis (H.E.L.P.system) was started as add-on therapy. Compared to the average levels obtained during the evolocumab monotherapy period, the LDL-cholesterol was reduced by 49.4%, thus reaching an inter-apheresis level (mean ± SD) of 173 ± 37 mg/dL. This report suggests that a combination therapy with evolocumab and lipoprotein-apheresis may have synergic effects on circulating lipid levels. Its relevance as a highly effective treatment option for hyperlipidemia in HeFH patients warrants further investigation in larger datasets.

KEYWORDS
anti-PCSK9 antibody, evolocumab, heterozygous familial hypercholesterolemia, lipoprotein apheresis, statin intolerance

1 | INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of lipid metabolism, resulting from mutations that most frequently involve the genes encoding for the low-density lipoprotein (LDL) receptor, apolipoprotein B, or the pro-protein convertase subtilisin/kexin type 9 (PCSK9) .[1,2] The considerable increase of plasma LDL cholesterol levels and the extra-plasmatic lipid depots are accounted among the most characteristic phenotypic hallmarks of the disease. The increased LDL cholesterol (LDL-C) levels per se and the life-long exposure to inappropriately high lipid concentrations are amongst the leading factors that significantly increase the risk of atherosclerosis and overt cardiovascular diseases (CVD) in patients with FH.[2]The phenotypic diagnosis of FH stands on the finding of LDL-C concentrations above 190 mg/dL (4.9 mmol/L) in individuals with a family history of hypercholesterolemia and/or early coronary heart disease.[2,3]

As recently reported in the consensus statement of the European Atherosclerosis Society, a specific lipid-lowering therapy targeted to effectively reduce the LDL-C levels toward the normal range (or lower) could potentially reduce the individual CVD risk to the same level of un-affected individuals from the general population.[2] The advocated target levels for both homozygous and heterozygous familial hypercholesterolemia (HeFH) pointed to lowering LDL-C to <3.5 mmol/L (<135 mg/dL) in children and to <2.6 mmol/L (<100 mg/dL) in adults, or < 1.8 mmol/L (<70 mg/dL) in those at the highest risk.[2,4]

The pharmacological treatment of HeFH recognizes statins as pivotal therapeutic option. However, statin therapy may be either not sufficiently effective, a situation also referred to as "statin resistance," or it may be accompanied by side effects that are labeled as "statin intolerance." Among the latter, it should be accounted a phenomenon of relatively rare occurrence, also known as statin-induced myositis, and characterized by muscle aches accompanied by substantially elevated serum creatine kinase (CK) concentrations.[5] In these cases, the use of ezetimibe may be considered as a second-line therapy, potentially followed by bile acid sequestrants or fibrates, alone or in combination.

The recent advent of new classes of lipid-lowering agents, which act by promoting the LDL catabolism via monoclonal antibody-mediated inhibition of the activity of pro-protein convertase subtilisin/kexin 9 (PCSK9), represents a new opportunity for patients with HeFH and multiple statin intolerance.[6,7]

A further therapeutic option stands on the physical removal of cholesterol-rich lipoproteins from the blood stream, a procedure dubbed as "lipoprotein apheresis," which has been demonstrated as a very effective strategy to treat individuals with severe hyperlipidemia, including FH patients resistant or intolerant to statin therapy.[8,9]

We herein report the clinical case of a HeFH patient with intolerance to both statins and ezetimibe, in whom a combination therapy with evolocumab and lipoprotein apheresis obtained a remarkable decrease of circulating lipid levels toward the recommended ranges. Our observations point to further studies investigating whether the combination of evolocumab and lipoprotein apheresis may act on hyperlipidemia with additive and synergic effects, thus eventually supporting the relevance of this treatment strategy as a useful therapeutic option in FH patients.

2 | CASE REPORT

A 49 years old female patient presented in the Outpatient Lipid Clinic of the Verona University Hospital in May 2015. A clinical diagnosis of HeFH was made in her adolescence at 17 years of age, according to extant plasma LDL-C concentrations above 300 mg/dL and to a positive family history of hypercholesterolemia and early coronary heart disease (CHD) (father and paternal uncles). A genetic test conducted in 2016 revealed a heterozygous mutation (c.1646 G>A; p. Gly528Asp) in the exon 11 of the LDL-receptor (LDLR) gene, pathogenic for HeFH.

With regard to the specific lipid-lowering therapy, a cholestyramine p.o. bid treatment was promptly started at diagnosis in 1985 and it was continued until 1991. Starting from 1991, pravastatin 20 mg/die was initiated in place of the bile acid sequestrants and this therapy was continued until 2000 with sporadic interruptions due to disabling muscle pain. In 2000, she was diagnosed as being affected by Cogan's syndrome, a rare rheumatologic autoimmune disorder characterized by corneal and inner ear inflammation. The medical therapy from this point onward also included salicylic acid 100 mg/die, methylprednisolone 4 mg/die, methotrexate 10 mg/week; pantoprazole 20 mg/die. In 2001, the lipid lowering therapy was potentiated by introducing atorvastatin 20 mg/die.

In 2002, during an intense physical training session, she experienced acute muscle pain, rhabdomyolysis with massive myoglobinuria and concomitant acute renal failure, which required immediate hospitalization and hemodialysis. The laboratory measurements at that time were as follows: myoglobin = 159.8 ng/mL (normal range: 0–85 ng/mL), CK > 100 000 U/L (normal range: 40–300 U/L), Lactate dehydrogenase (LDH) = 7656 U/L (normal range: 122–222 U/L), aspartate aminotransferase (AST) = 2469 U/L (normal range: 5–50 U/L), alanine aminotransferase (ALT) = 646 U/L (normal range: 5–50 U/L). After a full clinical and laboratory recovery from the incumbent situation was reached, the patient was switched to an alternative combination therapy with fluvastatin and ezetimibe. Unfortunately, muscle symptoms and CK elevation reappeared. For these reasons, a nutraceutical treatment with substances characterized by complementary lipid-lowering properties was started in 2003, including red yeast rice, policosanol, and berberine combined with folic acid, astaxanthin, and coenzyme Q10 (Armolipid Plus® 1 cp/die).[10]

The patient came to our attention in December 2015. At the physical examination: body weight = 68 kg, height = 158 cm, BMI= 27.3 kg/m^2, ambulatory blood pressure = 120/84 mm Hg. No signs of xanthomas, corneal arcus, or vascular bruits were detectable. The US-Doppler scan of the carotid arteries showed bilateral punctiform calcific atherosclerosis without hemodynamic significant plaques at the level of both internal and external carotid vessels. The estimated calcium score (46.9 Agatston Units) at the CT-scan of coronary arteries corresponded to the 97th percentile of individuals of same age, gender and ethnicity, free of cardiovascular disease, and treated diabetes. Ultrasound scan of abdominal aorta and arterial leg vessels did not reveal any pathologic lesion. At the laboratory measurements on

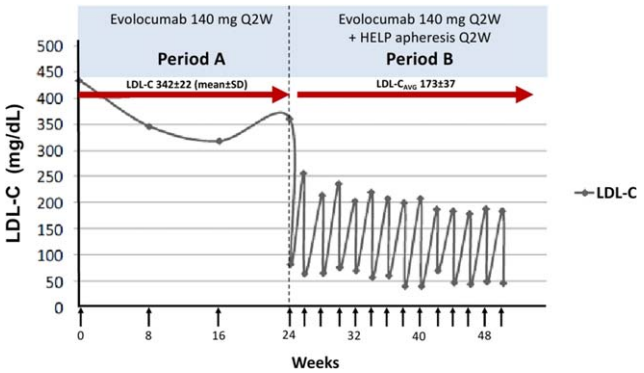

FIGURE 1 LDL-C plasma levels during monotherapy treatment with evolocumab (140 mg QW2) and during combination therapy with evolocumab and HELP apheresis (Q2W). Blood sampling was performed at baseline (0) and every 8 weeks during period A (evolocumab monotherapy) and every two weeks (immediately before and after each apheresis session) for period B (evolocumab + HELP apheresis combination therapy). The average LDL-C levels are displayed on top of treatment period A as mean ± SD; the LDL-C_{AVG} is displayed on top of period B and represents the time-averaged mean concentrations (C_{AVG}) of LDL-C across the apheresis sessions over 6 months

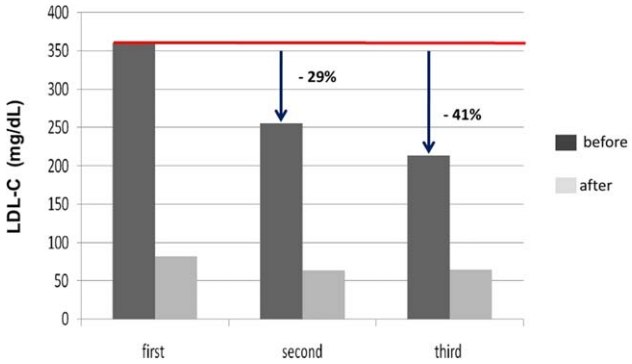

FIGURE 2 LDL-C plasma levels immediately before and after the first, second and third HELP apheresis session (Q2W). The graph shows the results obtained during the first three HELP apheresis sessions and highlights the considerable reduction of the pre-apheresis LDL-C concentration (dark grey columns) that is achieved within the third apheresis session. As clearly shown, compared to the basal (first apheresis) pre-apheresis levels, the concentrations of pre-apheresis LDL-C displayed a remarkable reduction of 29% and 41% at the second and third apheresis session, respectively

nutraceutical treatment: total-C = 522 mg/dL, LDL-C = 434 mg/dL, high-density lipoprotein cholesterol (HDL-C) = 84 mg/dL, triglycerides = 120 mg/dL, Lp(a) lipoprotein = 13 mg/dL.

Since the nutraceutical treatment did not obtain satisfactory plasma lipid levels, a therapy with evolocumab 140 mg sc every two weeks was started according to the scheme outlined in Figure 1 (period A). Total cholesterol, LDL-C, HDL-C, and triglycerides concentrations were measured every 8 weeks, as reported in Table 1. In this patient, suffering from a rheumatic disease, PCSK9 inhibitor treatment was well tolerated. However, despite the evolocumab treatment, the LDL-C plasma levels (mean ± SD) remained above the recommended target (342 ± 22 mg/dL; −21.2%).

Starting from May 2016, a treatment with lipoprotein apheresis was added to the evolocumab therapy (Figure 1, period B). Lipoprotein apheresis sessions were performed every two weeks with heparin-induced LDL precipitation apheresis (H.E.L.P. system Plasmat Futura-Braun, Melsungen, Germany),[11] treating 3000 mL of plasma for each session (corresponding to 1.1 patient plasma volume). Anticoagulation was performed with heparin (3000 IU as a bolus and 2000 IU/h continuously). Plasma levels of total cholesterol, LDL-C, HDL-C, and triglycerides were measured before and immediately after each apheresis session. Evolocumab was administered immediately after apheresis (140 mg every 2 weeks). In period B, lipoprotein apheresis obtained a pronounced and rapid reduction of circulating LDL-C levels at each session [pre-apheretic LDL-C (mean ± SD):

TABLE 1 Lipid and liver profile during evolocumab treatment in monotherapy (140 mg every two weeks)—Period A

	Baseline	8 weeks	16 weeks	24 weeks
Total-C (mg/dL)	522	468	392	446
HDL-C (mg/dL)	84	81	70	67
LDL-C (mg/dL)	434	346	318	361
Triglycerides (mg/dL)	120	87	88	97
Lp(a) (mg/dL)	13	11	ND	ND
AST (U/L)	25	19	29	13
ALT (U/L)	23	24	36	22
CK (U/L)	69	59	60	78

ND = not determined.

216 ± 47 mg/dL; post-apheretic LDL-C: 57 ± 13 mg/dL; Δ% −73.2%], with a progressive decline of pre-apheretic LDL-C spanning from 29% to 41% after two and four weeks of treatment, respectively (Figure 2). From this point onward the inter-apheresis LDL-C levels remained substantially constant around 161 ± 15 mg/dL. No adverse effect was reported in any of the apheresis sessions.

Inter-apheresis LDL-C levels represent the most accurate measurement of the individual total exposure to LDL-C and it is obtained by calculating the time-averaged concentration between consecutive sessions of lipoprotein apheresis.[12] Therefore, the time-averaged mean concentrations (C_{AVG}) of LDL-C herein presented were calculated by applying the following formula devised by Kroon et al.[12]

$$C_{AVG} = C_{MIN} + 0.73\ (C_{MAX} - C_{MIN})$$

where C_{MAX} is the pre-treatment level and C_{MIN} the levels immediately after apheresis.

Accordingly, as shown in Figure 1 (period B), the mean ± SD pre-apheresis and post-apheresis LDL-C levels were 216 ± 47 mg/dL (LDL-C_{MAX}) and 57 ± 13 mg/dL (LDL-C_{MIN}), respectively. Overall, the resulting LDL-C inter-apheresis levels (LDL-C_{AVG}) over 6 months of combination therapy were 173 ± 37 mg/dL, corresponding to a 49.4% reduction, as compared to the average LDL-C levels obtained during the evolocumab monotherapy (period A).

3 | DISCUSSION

The inhibitors of PCSK9 can be offered as a valuable and alternative therapeutic option in patients with statin intolerance or resistance to the lipid lowering therapy. In the ODYSSEY ALTERNATIVE trial, the 24-week administration of alirocumab, a human PCSK9 monoclonal antibody, obtained a reduction of mean LDL-cholesterol levels by 45% in patients with well-documented statin intolerance.[6] In the GAUSS-3 trial, the mean percent change in LDL-C levels after 24 weeks with evolocumab was −52.8% among patients with statin intolerance.[7] In our case report, in a patient with severe hypercholesterolemia and a rheumatic disease, evolocumab given for 24 weeks reduced LDL-C levels by 21.2%. Therefore, since the LDL-C levels decreased <25%, this patient could be defined "hyporesponder," according to similar clinical experience recently described by Galema-Boers et al.[13]

Lipoprotein apheresis is currently the best treatment option to bring patients with severe forms of hypercholesterolemia (such as HeFH) closer to target LDL-C levels.[8] In patients on lipoprotein apheresis therapy, the most accurate measurement of the individual total exposure to LDL-C is obtained from calculating the interval mean or time-averaged concentration between consecutive procedures,[12] whereas the pre-apheresis concentrations reflect the maximal values reached during this form of treatment.[14] Using pre-treatment levels, the documented long-term reductions in LDL-C in HeFH patients were approximately 30% after 6 months of therapy.[14] In our case report, the pre-apheresis LDL-C levels showed a remarkable reduction of about 41% after 4 weeks of combination therapy, as compared to the LDL-C obtained during the evolocumab monotherapy (Figure 2). Lipoprotein apheresis, along with its intrinsic lipid lowering action, also exerts effects on the individual inflammatory profile as well as on the coagulative system, hemorheological functions, and fibrinolysis.[15] These pleiotropic effects may have long-term effects on the individual cardiovascular risk profile, particularly by reducing the pro-inflammatory status that usually accompanies metabolic disorders.

The striking reduction of LDL-C levels obtained in this patient affected by Cogan's syndrome may suggest that the combination of lipoprotein apheresis with evolocumab therapy enhances the effects of the PCSK9 inhibitor on LDL catabolism and may have dampened the lipid rebound effect of the first three lipoprotein apheresis sessions. However, it should be noted that, despite the remarkable response to the combination therapy, the recommended LDL-C target in this high-risk individual was not reached. Although it would be advisable to intensify the treatment by weekly apheresis sessions, this was not practicable as they would have conflicted with the working schedule of our patient. With regard to other pharmacologic treatment options currently available for the treatment of FH in our country, it should be pointed out that lomitapide, a small molecule inhibitor of microsomal triglyceride transfer protein, is currently licensed for homozygous FH, but not for HeFH. Nonetheless, despite the cardiovascular risk profile in this patient remains high, it is reasonably expectable that the combination therapy of lipoprotein apheresis with evolocumab will eventually be effective to relent/prevent the development of a major cardiovascular event.

This case report taught us that evolocumab treatment in an individual with HeFH and statin intolerance was effective at remarkably reducing the LDL-C levels without side effects, particularly when coupled with lipoprotein apheresis. Evolocumab may not be sufficient to reach the recommended LDL-C target neither as monotherapy, nor in combination with lipoprotein apheresis, at least in this clinical case. As a possible explanation for the relatively low LDL-C percent reduction with evolocumab monotherapy (–21.2%), we may point to the concomitant immunosuppressive therapy for the Cogan's syndrome and to the hyperlipidemic effect of methylprednisolone.[16] However, although we did not test the effect of lipoprotein apheresis alone in our patient, it is possible that the combination of evolocumab and lipoprotein apheresis may have a synergic effect on the resulting circulating lipid levels. Further ad hoc studies are needed to

specifically test this hypothesis by administering lipoprotein apheresis alone, evolocumab alone, and then combine the two treatments. It should also be noted that the costs associated with increased frequency of apheresis sessions is considerably higher than the combination therapy herein proposed. Our observations should also be interpreted in light of the individual risk profile of this specific patient. In absolute terms, the combination therapy with evolocumab and lipoprotein apheresis was able to reduce the LDL-C by approximately 60%, a result that is particularly relevant, given the considerably high basal LDL-C levels detected in this patient at the time of first evaluation at our center.

In light of the relevance of this combination therapy as a useful therapeutic option to reach the recommended lipid targets in high risk patients with HeFH, further investigations are needed to confirm our observations in larger datasets and to test its cardiovascular benefits and the potential systematic application in high-risk patients resistant or intolerant to conventional therapies.

CONFLICT OF INTERESTS

None to disclose.

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