MR. JOSE RIOJA (Orcid ID : 0000-0002-1196-0352)

DR. PEDRO VALDIVIELSO (Orcid ID : 0000-0003-4458-9833)

Article type : Original Paper

TITLE PAGE

Full title:

Evaluation of the Chylomicron-TG to VLDL-TG ratio for Type I Hyperlipoproteinemia diagnostic**.**

Author names and affiliations:

José Rioja[1], María-José Ariza[1], Natalia García-Casares[1], Inmaculada Coca-Prieto[2], Teresa Arrobas[3], Ovidio Muñiz-Grijalvo[4], Alipio Mangas[5], Daiana Ibarretxe[6], Miguel Ángel Sánchez-Chaparro[1,7], Pedro Valdivielso[1,7].

1. Department of Medicine and Dermatology. Lipids and Atherosclerosis Laboratory. Centro de Investigaciones Médico Sanitarias (CIMES). Instituto de Investigación Biomédica de Málaga (IBIMA). University of Málaga. Spain.
2. Internal Medicine Unit. Ciudad Real General Hospital. Ciudad Real. Spain.
3. Clinical Chemistry Unit. Virgen Macarena Hospital. Sevilla. Spain.
4. UCERV-UCAMI, Internal Medicine Department, University Hospital Virgen del Rocío. Sevilla. Spain.
5. Internal Medicine Department, University Hospital Puerta del Mar, School of Medicine, University of Cadiz, Institute of Research and Innovation in Biomedical Sciences (INiBICA), Cádiz, Spain.
6. Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, Sant Joan University Hospital, Universitat Rovira i Virgili, IISPV. CIBERDEM. Reus, Spain.
7. Lipid Unit. University Hospital Virgen de la Victoria. Málaga. Spain.

Corresponding Author:

Miguel Ángel Sánchez Chaparro.

Departamento de Medicina y Dermatología. Facultad de Medicina. Universidad de Málaga. Boulevard Louis Pasteur 32. 29010. Málaga. Spain. TLF: (+34) 952 13 1615; e-mail: masch@uma.es

ABSTRACT

Background: The aim of this study is to confirm the diagnostic performance of the Chylomicron to Very Low Density Lipoproteins Triglycerides (CM/VLDL-TG) ratio, the Triglycerides to Cholesterol ratio (TG/TC) and a dichotomic rule including the Tryglicerides to Apolipoprotein B (TG/APOB) ratio for the presence of Type I Hyperlipoproteinemia (HPLI) in patients with severe hypertriglyceridemia (sHTG) that were at high risk for Familial Chylomicronemia Syndrome (FCS).

Methods: Two cohorts (Derivation and Validation) of patients with sHTG were included in the study. Anthropometric, clinical, biochemical and genetic data were obtained. The CM/VLDL-TG, TG/TC and TG/APOB ratios were calculated. Finally, a diagnostic performance study was developed to establish Sensitivity, Specificity and cut-offs by a ROC curve analysis in the Derivation Cohort as well as Agreement and Predictive Values in the Validation Cohort.

Results: Patients with FCS in both cohorts showed an earlier presence in pancreatitis, greater number of acute pancreatitis episodes and lower BMI. FCS patients also showed higher ratios of CM/VLDL-TG, TG/TC and TG/APOB ratios, whereas their HDL-C, LDL-C and APOB levels were lower than in non-FCS patients.

Sensitivity and Agreement were low for both the TG/TC and TG/APOB ratios, although Predictive Values were good. The CM/VLDL-TG ratio showed greatest sensitivity, specificity, Agreement and Predicitve Values for cut-off of 3.8 and 4.5.

Conclusions: Our results suggest that in subjects at high risk of FCS a total serum TG/TC ratio or TG/APOB ratio are feasible to initially screen for HLPI; however, a CM/VLDL-TG ratio ≥ 4.5 is a better diagnostic criterion for HPLI.

MAIN TEXT

INTRODUCTION

Severe hypertriglyceridemia (HTG) is a lipid disorder with patients showing fasting triglycerides above 11.3 mmol/L (1000 mg/dL), due to hyperchylomicronemia that may be accompanied with normal (Type I Hyperlipoproteinemia) or high Very Low Density Lipoproteins levels (Type V Hyperlipoproteinemia) [1].

Meanwhile Type V Hyperlipoproteinemia (HPLV) includes a wide spectrum of genetic and acquired physiopathological conditions [2] grouped under the term Multifactorial Chylomicronemia (MCM), Type I hyperlipoproteinemia (HPLI) is associated with a lack of Lipoprotein Lipase (LPL) enzyme activity [1]. The presence of Familial Chylomicronemia Syndrome (FCS) [3], or autoantibodies against LPL [4] or Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) [5] underlie the enzymatic deficiency.

Differentiating between HPLI and HLPV is challenging for clinical laboratories. The diagnosis of HPLI is made through the lipidogram [6–8] or the analytical ultracentrifuge based Donner Method [7]. Because of the limited availability of both methods, the diagnosis of the HPLI remains difficult in clinical settings. Some groups have proposed that a serum TG/TC ratio > 2.2 (mmol/L or > 5 in mg/dL) [9] or an algorithm including a TG/APOB > 10 in the case of total APOB < 0.75 g/L [10] may suggest the presence of type I HLP and, as a consequence, the presence of FCS. Nevertheless, neither sensitivity and specificity nor predictive studies have been developed for any of these methodologies. Furthermore, some studies assumed the presence of HPLI without any consistent confirmation [11–13].

Preparative ultracentrifugation is considered the reference method to quantify Low Density Lipoprotein Cholesterol (LDL-C) [14] and High Density Lipoprotein Cholesterol (HDL-C) specially in hypertriglyceridemic patients [15]. Preparative ultracentrifugation also allows the isolation of the plasma chylomicron-like and VLDL sub-fractions [16,17]. A sequential ultracentrifugation protocol, isolating the chylomicron-like band in a first step [16], and the VLDL band in a second step [14,15] is also available.

The aim of this study is to confirm the diagnostic performance of the CM/VLDL-TG ratio, the TG/TC ratio and TG/APOB algorithm for the presence of HPLI in patients with severe hypertriglyceridemia at high risk for FCS.

MATERIALS AND METHODS

Patients and Clinical Data collection

First, a Derivation cohort (n = 20) with a personal history of severe Hypertriglyceridemia (TG> 11.3 mmol/L) was recruited for the prospective sensitivity and specificity study. Nine patients had been previously diagnosed with FCS (lack of postheparin LPL activity and two pathogenic variants at *LPL* or *GPIHBP1* genes). The rest of the patients (non-FCS, n = 11) showed normal or elevated LPL activity and did not have any FCS-causing variants. Both groups of patients were being treated with a very low-fat diet and/or fibrates or omega-3 fatty acids at the time of diagnosis. Patients were recruited consecutively from 2013 to 2018 from the lipid units of Málaga, Seville, Cádiz and Reus University Hospitals (Spain).

Second, a validation cohort (n = 38, FCS = 5, non-FCS = 33) previously reported (17) was included to perform a retrospective study of the Positive (PPV) and Negative (NPV) Predictive Values and Agreement by Cohen's Kappa of the CM/VLDL-TG ratio, the TG/TC ratio and the TG/APOB ratio.

The number of pancreatitis and age of the first episode (years), weight (Kg), height (m), body mass index (BMI), presence of diabetes (DM), Cardiovascular Disease (CVD) or Hypertension (HBP) and triglycerides-lowering treatment at the beginning of the diagnosis were collected in all patients.

Laboratory methods

The mutations in *LPL* and *GPIHBP1* harboured by the FCS patients in both Derivation and Validations Cohorts were reported [18,19]. The absence of pathogenic variants in the non-FCS patients from the derivation cohort was confirmed by NGS sequencing (Illumina). Fasting serum and EDTA-K2 plasma samples were collected from all patients. Plasma total LPL activity and mass were measured after an injection of heparin 100 U/kg according to Olivecrona et al. [19,20]. Samples for all laboratory methods were obtained in the same day.

Total cholesterol and triglycerides were measured in fasting serum samples. Also, fasting Apolipoprotein B and C-II were measured by immune turbidimetry (Spinreact) in the BS-380 analyzer (Mindray) using a 1/6 sample dilution in order to minimize triglycerides interferences.

The lipid profile and the CM/VLDL-TG ratio was obtained by a two-step sequential ultracentrifugation as follows:

In a First step, 1 mL of serum was pipetted into a polycarbonate thick wall ultracentrifuge tube of 13X63 mm (Beckman). In order to preserve the sample osmolality, the sample was slowly covered with 1 mL of NaCl 0.9% w/v (d = 1.006 g / ml) and then, same mode, the 1.006 density layer was covered again

with 0.6 mL of distilled water (d = 1.000 g / ml), getting a discontinuous density gradient. Subsequently, a centrifugation at 105,000 x g for 37 minutes at 4° C with maximum acceleration and medium brake was performed in a L90 K centrifuge (Beckman). Once finished, the infranatant was aspirated from the bottom of the tube using a 230mm Pasteur glass pipette with a gum propypter and deposited in a second ultracentrifuge tube. In the first ultracentrifuge tube a creamy top layer corresponding to the chylomicron-like subfraction was obtained (Figure 1). This sub-fraction was diluted and re-suspended with a vortex to a final volume of 1 mL in NaCl 0.9 %. w/v.

In a second step of the sequential ultra-centrifugation, the second tube containing the infranatant of the above ultracentrifugation was covered to a final volume of 2.6 mL with NaCl 0.9 % w/v and centrifuged at 105000 x g for 18 hours at 4° C. After this, the infranatant was removed with a 230 mm glass Pasteur pipette. The remaining supernatant was then re-suspended, recovering the isolated VLDL subfraction. Finally, the triglycerides and cholesterol contents in serum and in both chylomicron-like and VLDL subfractions were quantified using enzymatic endpoint methods (Spinreact). All stoichiometric calculations were performed to get fasting triglycerides serum levels for every lipoprotein sub-fraction. The CM/VLDL-TG, TG/TC, and TG/APOB ratios were then calculated.

Ethical issues:

All participants signed the informed consent. The study was approved by the local Ethics Committe of Malaga, on the meeting held in November, 17th, 2011.

Statistical analysis

A comparison between groups was carried out by the Student t, U Mann-Whitney or the χ^2 (IBM; SPSS 25.0). In addition, a Diagnostic Performance Study of the CM/VLDL-TG and TG/TC ratios was carried out. For this purpose, a Receiver-Operating Characteristic (ROC) curve was performed in the Derivation Cohort and the Area Under the Curve (AUC); and bilateral significance coordinates for the sensitivity and specificity were calculated for both ratios (IBM SPSS Statistics, New York, USA). Sample size was performed using the software MedCalc V.19.0.3 (MedCalc, Ostend, Belgium). A sample size of 14 patients (7 patients with FCS + 7 non-FCS) was considered to obtain an area under the curve (AUC) of 0.95 with a type I error of 0.05 and a type II error of 0.1, considering a null hypothesis of an AUC of 0,5 and a ratio 1:1 positive:negatives. Cut-off values for the CM/VLDL-TG and TG/TC ratios were selected using the maximum values of the unweighted Youden's *J* index [21] or cut-off values previously

suggested in other studies [9]. Finally, positive and negative Predictive Values, and Agreement by Cohen's Kappa were calculated in the Validation Cohort in both ratios [22] for the obtained cutoffs.

Discrete matches (Y/N) for FCS and non-FCS patients between the TG/APOB algorithm and the reference methodology were also established in both cohorts. Then, Sensitivity, Specificity, positive and negative Predictive Values and agreement were calculated using the www.openepi.com web [22].

Reporting of the study conforms to broad EQUATOR guidelines [23]. The full study protocol can be accessed at the Lipids and Atherosclerosis Laboratory of the University of Malaga with the Study Code *EGHG*.

RESULTS

Clinical data

Clinical data from both the Derivation and Validation Cohorts are provided in Table 1. Patients with FCS, in the Derivation and Validation Cohorts had lower BMI and higher number of hypertriglyceridemic pancreatitis episodes at a younger age than non-FCS.

Laboratory data

Biochemical data from both the Derivation and Validation Cohorts as well as the *APOE* genotypes are also provided in Table 1. Regarding the Derivation cohort, patients with FCS had significantly higher total cholesterol, total triglycerides and triglycerides in the Chylomicron-like sub-fraction and significantly lower HDL-C, LPL activity, LPL mass and Apo B than non-FCS patients. In the Validation Cohort, patients with FCS also had higher total triglycerides, triglycerides in the Chylomicron-like sub-fraction and significantly lower levels of HDL-C, LDL-C, LPL activity, LPL mass and Apo B. The CM/VLDL-TG and the TG/TC ratios were significantly higher in patients with FCS than in non-FCS in both cohorts (Figure 2). The TG/APOB ratio (mg/dL/mg/dL) and the percentage of matches for this algorithm were also higher in FCS patients in both cohorts (Table 1).

Diagnostic performance data

The ROC curve analysis for sensitivity and specificity estimation of the CM/VLDL-TG and TG/TC ratios was carried out in the Derivation Cohort (figure 3). The AUC was close to 1.000 and statistically significant for the CM/VLDL-TG ratio (AUC = 0.990; $p < 0.001$). Conversely, the AUC for the TG/TC ratio was lower and did not reach statistical significance.

Table 2 describes the diagnostic performance of the CM/VLDL-TG and TG/TC ratios as well as the TG/APOB algorithm, showing sensitivity and specificity values (calculated from the Derivation Cohort) as well as Predictive Values and Agreement by Cohen's Kappa (calculated from the Validation Cohort) for the selected cutoffs of both ratios and the TG/APOB ratio. Briefly, in the case of the three selected cutoff points for the CM/VLDL-TG ratio all of them showed high sensitivity and specificity, but the maximum specificity was obtained for the 4.5 cutoff value. The highest Negative and Positive Predictive Values were also obtained for the 3.8 and 4.5 cutoff values. Agreement was moderate but close to strong (near to 0.80), for both 3.8 and 4.5 cutoff values.

As for the TG/TC ratio, lower Positive Predictive values where shown for both 3.9 and 5.0 cutoffs compared to those achieved with the CM/VLDL-TG ratio. Agreement was weak (< 0.60) for the TG/TC ratio compared with the CM/VLDL-TG ratio. Results from the TG/APOB ratio showed similar results compared to the CM/VLDL-TG ratio.

DISCUSSION

The diagnosis of FCS, a rare disease, in subjects with severe hypertriglyceridemia is challenging for clinicians as well for laboratories. Several clinical features point to FCS, such as low body-mass-index and recurrent acute hypertriglyceridemic pancreatitis especially at young age [9,18,24,25]. In fact, in one study the age at first symptom, body-mass index and serum levels of gamma-glutamyl transferase (GGT) were predictors of FCS in univariate and age- and sex- adjusted analyses [25]. For laboratories the diagnosis relay in the finding of two pathogenic variants at target genes, the absent serum postheparin lipase activity or the finding of an isolated hyperchylomicronemia (HLPI).

The Spanish Arterioclerosis Society recommends to refer patients with fasting TG > 1000 mg/dL to Lipid Units to increase the recognition of this disease [26]. Clinical laboratories may play a major role, marking so high levels of TG and suggesting to refer patients to specialized units. A correct diagnosis of FCS is especially relevant because Volanersorsen, a new drug targeting the apoC3 RNA, is very effective reducing triglycerides in phase II and III clinical trials [27].

Our study describes the evaluation of the CM/VLDL-TG ratio to diagnose HPLI. A cut-off value of 4.5 showed 100% specificity for the HPLI diagnostic and a 3.8 cut-off performed with a 100% sensitivity for the FCS screening.

The TG/TC, CM/VLDL-TG and the TG/APOB ratios were higher in patients with FCS than in non-FCS, since those patients have more chylomicron but moderate to normal VLDL-TG levels [28]. Furthermore, the non-FCS patient group showed a median TG lower than patients with FCS presumably in part because of the positive effect of the lipid lowering therapy, which is not effective in patients with FCS [18].

The total TG/TC ratio > 11.5 mmol/mmol has recently been proposed as a diagnostic criterion for FCS since it may reflect high circulating levels of chylomicrons and Very Low Density Lipoproteins [9]. Unfortunately, in our study the Sensitivity and Specificity in the ROC curve showed a poor and non-statistically significant AUC (Figure 3). In addition, Agreement was weak for the TG/TC ratio in the Validation Cohort (Table 2). Despite these results, the TG/TC ratio showed high Negative and Positive Predictive Values (Table 2). The use of total TG/TC ratio is supported by the fact that it can be calculated from fasting serum and does not need any additional manipulation in the laboratory. Considering these results, further studies need to be performed in order to clarify if the TG/TC ratio may fit as a diagnostic or screening criterion for the presence of HPLI.

Similar results were obtained for the dichotomic rule in the algorithm proposed by Sniderman et al. 2010, where sensitivity and specificity were lower than those for the CM/VLDL-TG, although specificity was acceptable. The main limitations of algorithm in our hands was that 20-22% of patients having HLPI had a serum apolipoprotein B $\geq$ 75 mg/dL (Table 1), being the reason for the very low Positive Predictive value and Agreement.

As we mentioned, the CM/VLDL-TG ratio was three fold higher in patients with FCS than in non-FCS in both the Derivation and Validation Cohorts. It is especially interesting that, despite some patients with FCS in both cohorts had TG < 11.3 mmol/l (n =10), their CM/VLDL-TG ratio remained $\geq$ 3.8 in 90% of them (6.1 (3.9 – 9.9)). Alternatively, to the TG/TC ratio, the CM/VLDL-TG ratio showed better diagnostic performance in the ROC curve for Sensitivity and Specificity (Table 2). Youden's *J* was maximum for cut-offs equal to 3.8 (100% sensitivity), 4.0 (grey zone) and 4.5 (100% specificity), suggesting that those values might work as potential suitable cut-offs. Although the ROC curve analysis showed that a cut-off value of 4.5 for the CM/VLDL-TG ratio showed the best specificity, a cut-off value of 3.8 showed the best sensitivity. These results suggest that the 3.8 cut-off can be considered a suitable screening value for the subsequent clinical diagnostic of FCS, involving both genetic and

enzymatic studies, while a cut-off value in 4.5 can be used as a diagnostic criterion for the presence of a HPLI clinical phenotype.

Despite the fact that sensitivity and specificity showed that the CM/VLDL-TG ratio performs correctly, little information can be obtained from these statistics on the significance of a result from this ratio for an individual patient. This last question was then confirmed by the calculation of the PPV (percentage of patients with the ratio > cut-off having FCS), NPV (percentage of patients with the ratio < cut-off being non-FCS) and Agreement in the Validation Cohort. Although Predictive Values were best for the 4.5 cut-off, all of the above three cut-offs yielded good Positive and Negative Predictive Values (Table 2). The 3.8 and 4.5 cut-offs also yielded Agreement values (77% and 75% respectively) close to strong. Although it is already known that Positive and Negative Predictive Values depend on prevalence, which is very low in rare diseases [29], we must highlight that our cohorts were populations with high pre-test probability for FCS. In this sense, the clinical and biochemical phenotype of patients with severe HTG increases the high risk of FCS, selected as the study population. Therefore, the Positive and Negative Predictive values showed in this work are related to subjects with severe HTG at high risk of FCS, not to the general population.

As a strength we can mention that, in order to achieve robust conclusions, the study has included a consistent number of FCS and non-FCS patients. As a limitation, we should have an external validation cohort.

In conclusion, in subjects with sHTG and therefore at high risk for FCS, a total serum TG/TC ratio and the algorithm based TG/APOB are feasible approach to screen for HLPI; however, the CM/VLDL-TG ratio ≥ 4.5 is a better diagnostic criterion for the presence of HPLI. Due to its high sensitivity, a CM/VLDL-TG ratio ≥ 3.8 might be used as screening for HLPI and confirmation of FCS diagnostic should be addressed by measuring the LPL postheparin activity and/or identifying pathogenic variants in the candidate genes for FCS.

ACKNOWLEDGEMENTS

The authors would like to thank the patients for participating in the study and Servicio de Traducción e Interpretación de la Fundación General de la Universidad de Málaga for their assistance in the English editing of this manuscript. We also thank Dr. Mario Gutierrez Bedmar (Preventive Medicine and Public Health Department, School of Medicine, University of Malaga) for his constructive comments on the statistical issues.

This study was financed by funds from the CTS-159 group (José Rioja, María-José Ariza, Miguel A Sánchez-Chaparro and Pedro Valdivielso) of the "Plan Andaluz de Innovación e Investigación (Andalusian Plan for Research and Innovation) (PAIDI).

CONTRIBUTIONS

José Rioja: Collected data, Validation, Formal analysis, Investigation, Writing – original draft**. María José Ariza:** Collected data, Investigation**,** Writing – review and editing **Natalia García Casares:** Investigation, Writing – review and editing. **Inmaculada Coca Prieto:** Methodology, Formal analysis, Investigation, Writing – review and editing. **Teresa Arrobas:** Investigation, Writing – review and editing. **Ovidio Muñiz Grijalvo:** Investigation, Writing – review and editing. **Alipio Mangas:** Investigation, Writing – review and editing. **Daiana Ibarretxe:** Investigation, Writing – review and editing. **Miguel Ángel Sánchez Chaparro:** Formal analysis, Investigation, Writing – original draft. **Pedro Valdivielso:** Formal analysis, Investigation, Writing – original draft, Supervision, Designed Study.

References

1. Fredrickson D, Lees R. a System for Phenotyping Hyperlipoproteinemia. 1965;Mar.(31):321–7. Available from: http://circ.ahajournals.org/
2. Gotoda T, Shirai K, Ohta T, Kobayashi J, Yokoyama S, Oikawa S, et al. Diagnosis and Management of Type I and Type V Hyperlipoproteinemia. J Atheroscler Thromb. 2011;19(1):1–12.
3. Brunzell JD, Bierman EL. Chylomicronemia Syndrome. 1982;66(2):1982.
4. Pruneta V, Moulin P, Labrousse F, Bondon PJ, Ponsin G, Berthezene F. Characterization of a new case of autoimmune type I hyperlipidemia: Long-term remission under immunosuppressive therapy. J Clin Endocrinol Metab. 1997;82(3):791–6.
5. Hu X, GM D-T, GK H, SY C, NP S, TLP1 D, et al. GPIHBP1 autoantibodies in a patient with unexplained chylomicronemia. J Clin Lipidol. 2017;11(4):964–71.
6. Donald S. Frederickson, Levy RI, Lees RS. FAT TRANSPORT IN LIPOPROTEINS - AN INTEGRATED APPROACH TO MECHANISMS AND DISORDERS. N Engl J Med. 1967;276(1):34–44.
7. Fredrickson DS, Levy RI, Lindgren FT. A comparison of heritable abnormal lipoprotein patterns as defined by two different techniques. J Clin Invest. 1969;47(11):2446–57.

8. Tanaka S, Ueno T, Tsunemi A, Nakamura Y, Kobayashi H, Hatanaka Y, et al. Lipoprotein lipase deficiency arising in type V dyslipidemia. Intern Med. 2019;58(2):251–7.

9. Stroes E, Moulin P, Parhofer KG, Rebours V, Löhr JM, Averna M. Diagnostic algorithm for familial chylomicronemia syndrome. Atheroscler Suppl. 2017;23:1–7.

10. Sniderman A, Couture P, De Graaf J. Diagnosis and treatment of apolipoprotein B dyslipoproteinemias. Nature Reviews Endocrinology. 2010.

11. Pingitore P, Lepore SM, Pirazzi C, Mancina RM, Motta BM, Valenti L, et al. Identification and characterization of two novel mutations in the LPL gene causing type I hyperlipoproteinemia. J Clin Lipidol [Internet]. 2016;10(4):816–23. Available from: http://dx.doi.org/10.1016/j.jacl.2016.02.015

12. Caddeo A, Mancina RM, Pirazzi C, Russo C, Sasidharan K, Sandstedt J, et al. Molecular analysis of three known and one novel LPL variants in patients with type I hyperlipoproteinemia. Nutr Metab Cardiovasc Dis [Internet]. 2018;28(2):158–64. Available from: https://doi.org/10.1016/j.numecd.2017.11.003

13. Chokshi N, Blumenschein SD, Ahmad Z, Garg A. Genotype-phenotype relationships in patients with type i hyperlipoproteinemia. J Clin Lipidol [Internet]. 2014;8(3):287–95. Available from: http://dx.doi.org/10.1016/j.jacl.2014.02.006

14. Bachorik PS, Ross JW. National cholesterol education program recommendations for measurement of low-density lipoprotein cholesterol: Executive summary. Clin Chem. 1995;41(10):1414–20.

15. Warnick G russell, Peter D. Wood, for the National Cholesterol Education Program Working Group on Lipoprotein Measurement. National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol: Executive Summary. Clin Chem [Internet]. 1995;41(10):1427–33. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7586511

16. Havel BRJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest. 1955;34(9):1345–53.

17. Havel BRJ, Fredrickson DS. The metabolism of chylomicra. I. The removal of palmitic acid-1-C14 labeled chylomicra from dog plasma. J Clin Invest. 1956;35(9):1025–32.

18. Ariza MJ, Rioja Villodres J, Ibarretxe D, Camacho A, Díaz-Díaz JL, Mangas A, et al. Molecular

basis of the familial chylomicronemia syndrome in patients from the National Dyslipidemia Registry of the Spanish Atherosclerosis Society. J Clin Lipidol. 2018;12(6):1482-1492.e3.

19. Coca-Prieto I, Valdivielso P, Olivecrona G, Ariza MJ, Rioja J, Font-Ugalde P, et al. Lipoprotein lipase activity and mass, apolipoprotein C-II mass and polymorphisms of apolipoproteins E and A5 in subjects with prior acute hypertriglyceridaemic pancreatitis. BMC Gastroenterol. 2009;9.

20. Holm C, Olivecrona G, Ottosson M. Assays of Lipolytic Enzymes [Internet]. Vol. 155, Adipose Tissue Protocols. 2001. 97–117 p. Available from: http://link.springer.com/10.1385/1592592317

21. Carter J V., Pan J, Rai SN, Galandiuk S. ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. Surg (United States) [Internet]. 2016;159(6):1638–45. Available from: http://dx.doi.org/10.1016/j.surg.2015.12.029

22. Dean AG, Sullivan KM, Soe MM, Mir RA, Kulkarni. H. Openepi Web Page [Internet]. 2004 [cited 2019 Sep 18]. Available from: https://www.openepi.com/Menu/OE_Menu.htm

23. Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. European Journal of Clinical Investigation. 2010.

24. Coca-Prieto I, Kroupa O, Gonzalez-Santos P, Magne J, Olivecrona G, Ehrenborg E, et al. Childhood-onset chylomicronaemia with reduced plasma lipoprotein lipase activity and mass: identification of a novel *GPIHBP1* mutation. J Intern Med. 2011 Sep;270(3):224–8.

25. Paquette M, Bernard S, Hegele RA, Baass A. Chylomicronemia: Differences between familial chylomicronemia syndrome and multifactorial chylomicronemia. Atherosclerosis. 2019;

26. Sánchez-Chaparro MÁ, Pérez-Martínez P, Ibarretxe D, Suárez-Tembra M, Valdivielso P. Criteria for referring patients to Spanish Atherosclerosis Society lipid units. Clin e Investig en Arterioscler. 2019;31(1):26–30.

27. Fogacci F, Norata GD, Toth PP, Arca M, Cicero AFG. Efficacy and Safety of Volanesorsen (ISIS 304801): the Evidence from Phase 2 and 3 Clinical Trials. Curr Atheroscler Rep. 2020;22(5).

28. Hegele RA, Berberich AJ, Ban MR, Wang J, Digenio A, Alexander VJ, et al. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol [Internet]. 2018;12(4):920-927.e4. Available from: https://doi.org/10.1016/j.jacl.2018.03.093

29. Altman DG, Bland JM. Diagnostic Tests 2: predictive values. 1994. p. 309:102.

KEYWORDS.

Chylomicrons, Hyperlipoproteinemia, Diagnosis, Triglycerides, Ultracentrifugation.

FIGURE LEGENDS

Figure 1. Creamy top layer of the centrifuge tubes corresponding to the chylomicron-like sub-fraction. 1A: patient with HPLI (flute-peak edge of the chylomicron-like subfraction). 2A: patient with HPLV (plain-edge).

Figure 2. Barr and dot plots for the CHM/VLDL-TG (2A) and TG/TC (2B) ratios for non-FCS patients in the Derivation Cohort (DnF), FCS patients in the Derivation Cohort (DF), non-FCS patients in the Validation Cohort (VnF) and FCS patients in the Validation Cohort (VF). Plots show means single points and standard deviations. *: Statistically significant difference ($p < 0,05$). ns: Statistically non-significant difference ($p > 0,05$).

Figure 3. Receiver Operating Characteristic curves for both CHM/VLDL-TG and TG/TC ratios in the Derivation Cohort. Area Under de curve (**AUC**), 95% of Confidence Interval (**95% C.I.**) and statistical signification of the curves (**p**) are also displayed.

Table 1. Clinical and Biochemical features of the subjects.

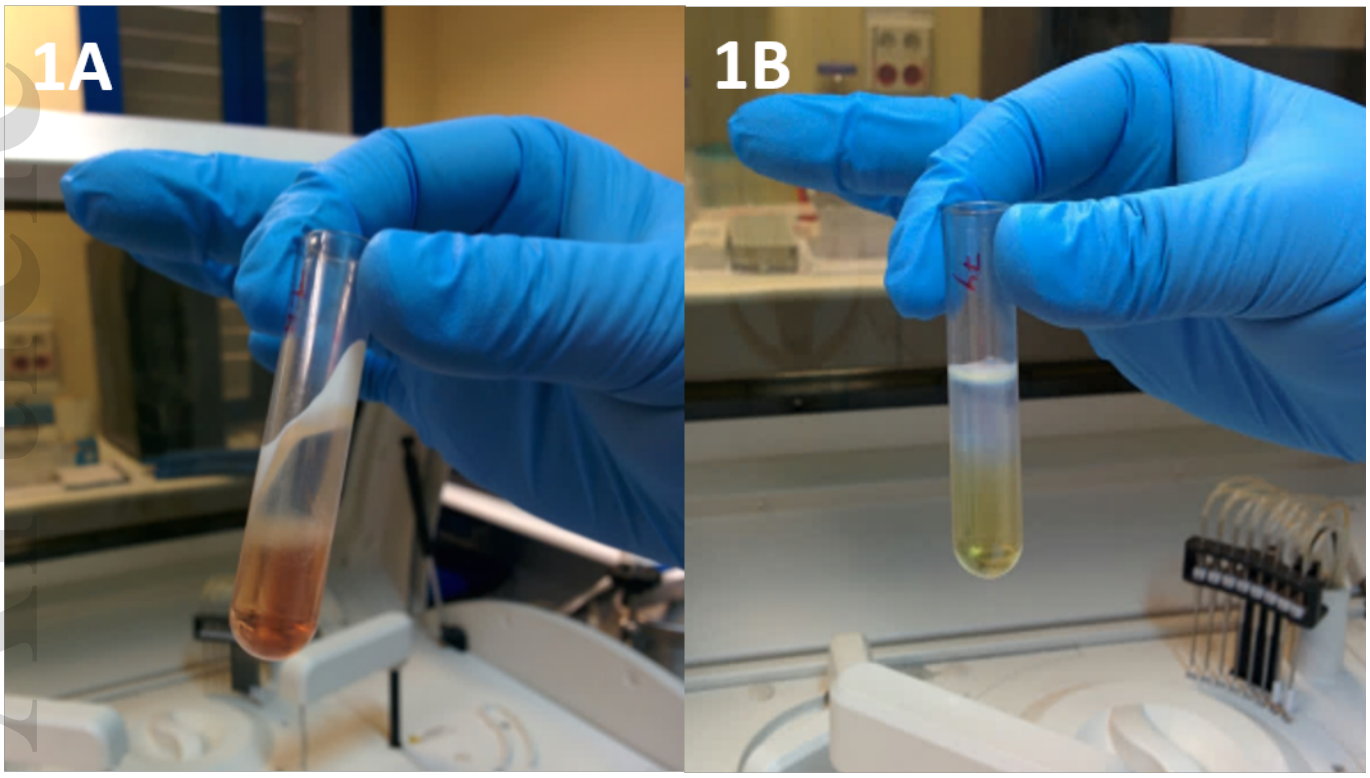
	(Derivation cohort)			(Validation cohort)		
	non-FCS (n=11)	**FCS (n=9)**	**p[a,b,c]**	**Non-FCS (n=33)**	**FCS (n=5)**	**p[a,b,c]**
Age (years)	48 ± 12	42 ± 15	0.390	47 ± 8	23 ± 6	0.000[a]
Men/Women	4 (36%)/7 (64%)	2 (22%)/7 (78%)	0.492	31 (94%)/2 (6%)	3 (60%)/2 (40%)	0.021[c]
BMI (Kg/m^2)	26.3 ± 3.8	22.4 ± 2.6	0.023[a]	29.5 ± 3.7	21.4 ± 1.6	0.000[a]
Alcohol consumption	1 (9%)	1 (11%)	0.943	16 (48%)	0 (0%)	0.041[c]
Alcohol consumed (g/day)	0 (0 – 3)	0 (0 – 24)	0.941	0 (0 – 20)	0 (0 – 0)	0.084
Smokers	4 (36%)	2 (22%)	0.492	23 (70%)	0 (0%)	0.004[c]
Diabetes	5 (45%)	3 (33%)	0.582	12 (36%)	1 (20%)	0.472
Hypertension	4 (36%)	2 (22%)	0.405	12 (36%)	1 (20%)	0.472
Pancreatitis	6 (55%)	9 (100%)	0.095	13 (39%)	4 (80%)	0.089
No. episodes	1 (0 - 3)	9 (4 - 14)	0.002[b]	2 (1 – 2)	5 (2 – 9)	0.079
Age 1st episode	32 ± 14	18 ± 11	0.058	41 ± 10	6 ± 6	0.000[a]
Fibrates/Omega 3 treatment*	7 (64%)	8 (88%)	0.671	25 (76%)	0 (0%)	0.001[c]
TC (mg/dL)	184 ± 64	272 ± 140	0.078	256 ± 56	335 ± 265	0.541
TG (mg/dL)	583 (301 – 796)	1054 (536 – 1262)	0.025[b]	425 (268 – 586)	1180 (682 – 2637)	0.003[b]
CM-TG (mg/dL)	352 (122 – 576)	676 (374 – 939)	0.020[b]	221 (7 – 327)	752 (465 – 2109)	0.001[b]
VLDL-TG (mg/dL)	113 (100 – 190)	93 (69 – 111)	0.056	141 (110 – 206)	172 (129 – 286)	0.424
LDL-C (mg/dL)	73.3 ± 50.4	41.9 ± 22.1	0.101	124.8 ± 45.0	56.5 ± 49.6	0.003[a]

HDL-C (mg/dL)	19.3 ± 8.6	11.0 ± 4.8	0.018[a]	34.9 ± 14.9	15.4 ± 4.7	0.000[a]
CM-TG/VLDL-TG ratio	1.9 (1.1 – 3.4)	6.3 (5.5 – 10.0)	0.000[a]	1.5 (0.1 – 2.1)	5.1 (3.4 – 7.0)	0.001[b]
TG/TC ratio	2.9 (2.5 – 3.8)	3.0 (2.8 – 4.3)	0.295	1.6 (1.2 – 2.7)	5.0 (3.6 – 5.6)	0.002[b]
TG/APOB alg. (%Matches)	10 (91%)	7 (78%)	0.002[c]	28 (85%)	4 (80%)	0.001[c]
TG/APOB (mg/dL/mg/dL)	5.2 (3.3 – 10.3)	19.5 (11.9 – 29.9)	0.001[b]	4.1 (2.8 – 7.3)	19.6 (12.4 – 34.0)	0.004[b]
APOB ≥ 75 mg/dL	2 (22%)	7 (64%)	0.064	26 (79%)	1 (20%)	0.007[c]
LPL activity (mU/mL)	125.0 (68.0 – 161.0)	3.6 (1.0 – 10.8)	0.000[b]	89.5 (71.4 – 131.3)	0.3 (0.0 – 1.0)	0.000[b]
LPL mass (ng/mL)	212.6 (139.0 – 393.2)	61.2 (28.3 – 90.4)	0.022[b]	404.2 (278.7 – 593.9)	50.0 (39.4 – 80.4)	0.000[b]
Apolipoprotein B (mg/dL)	91.0 ± 33.1	53.7 ± 25.4	0.014[a]	97.2 ± 27.2	55.8 ± 37.4	0.005[a]
Apolipoprotein C2 (mg/dL)	5.9 ± 1.0	5.3 ± 1.8	0.522	13.5 ± 5.2	9.0 ± 5.0	0.080
APOE Genotype**			0.252			0.831
ε3ε3	7 (64%)	9 (100%)		22 (67%)	4 (80%)	
ε2ε3	2 (18%)	0 (0%)		6 (18%)	1 (20%)	
ε3ε4	1 (9%)	0 (0%)		3 (9%)	0 (0%)	
ε4ε4	1 (9%)	0 (0%)		0 (0%)	0 (0%)	
ε3ε4	0 (0%)	0 (0%)		2 (6%)	0 (0%)	

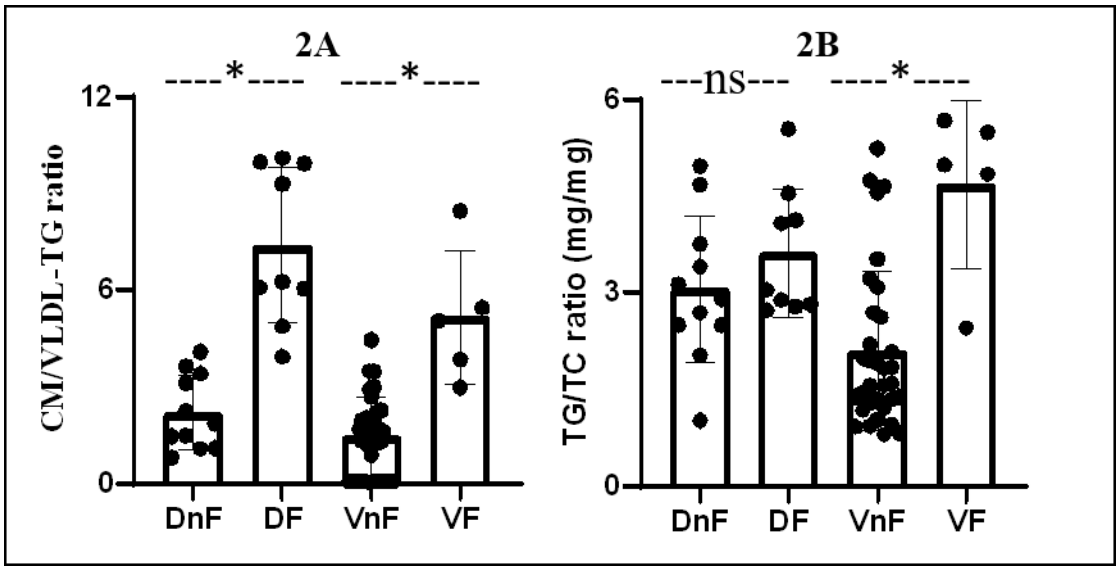
BMI: body mass index. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. TC: total cholesterol. TG: total triglycerides. CM-TG: triglycerides in chylomicrons. VLDL-TG: triglycerides in very low density lipoproteins. LDL-C: low density lipoproteins cholesterol. HDL-C: high-density-lipoprotein cholesterol. LPL: lipoprotein lipase. Data expressed as mean ± SD, number of subjects and percentages in brackets or median and interquartile range in brackets. [a]Bilateral statistical significance according to Students T test. [b]Bilateral statistical significance according to Mann-Whitney U test. [c]Bilateral statistical significance according to Chi-square test. *before non-FCS or FCS diagnostic. ** ε2: rs7412. ε4: rs429358.

Table 2. Diagnostic performance for HPLI of the CM/VLDL-TG and TG/TC ratios and the TG/APOB algorithm.

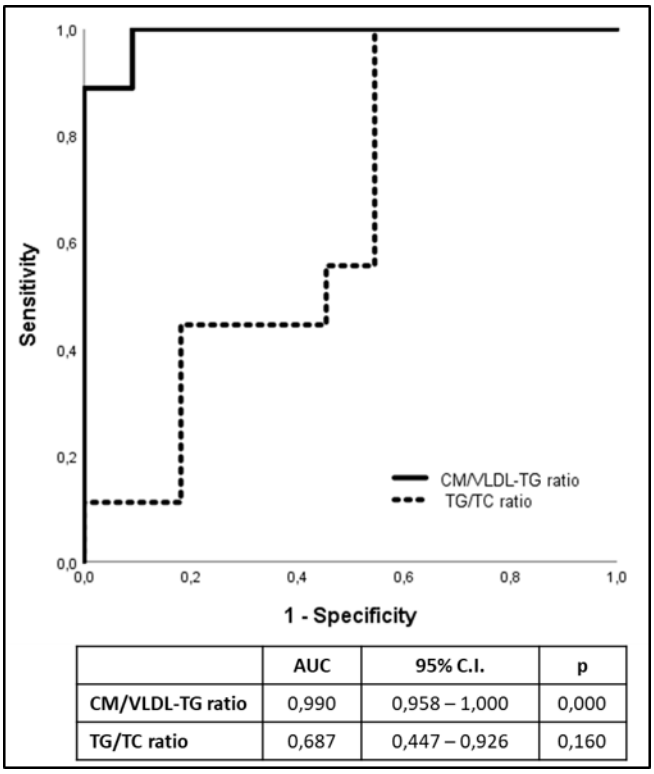
	(Derivation cohort)		(Validation cohort)		
CM/VLDL-TG Ratio	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Cohen's Kappa**
Cutoff = 3.8	100%	91%	80 (38 – 96)	97 (85 – 99)	0.77 (0.45 – 1.09)
Cutoff = 4.0	89%	91%	75 (30 – 95)	94 (81 – 98)	0.62 (0.31 – 0.94)
Cutoff = 4.5	89%	100%	100 (43 – 100)	94 (81 – 98)	0.75 (0.42 - 1.03)
TG/TC Ratio	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Cohen's Kappa**
Cutoff = 3.9	44%	82%	50 (22 – 78)	97 (85 – 99)	0.55 (0.26 – 0.84)
Cutoff = 5.0[a]	11%	96%	67 (21 – 94)	91 (78 – 97)	0.45 (0.14 – 0.75)
TG/APOB algorithm	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Cohen's Kappa**
	78%[b]	91%[b]	44 (19 – 73)	97 (83 – 99)	0.48 (0.19 – 0.78)



eci_13345_f1.tif



eci_13345_f2.tif



	AUC	95% C.I.	p
CM/VLDL-TG ratio	0,990	0,958 – 1,000	0,000
TG/TC ratio	0,687	0,447 – 0,926	0,160

eci_13345_f3.tif