

LIPID CASE 274 Know the science or just treat?

This case, shared with me by a lipidologist from Atlanta is a real challenge and should be quite an exercise for lipidologists. We are very fortunate in that usually these case discussions are me, myself and I. I was fortunate to have insight from three of lipidology's most respected folks, Allan Sniderman (Dr apoB), Russ Warnick (Dr Lipoprotein) and Joe McConnell (Dr Lab). If those names are not familiar, just Google them. Since this is a very unusual case some of the pathophysiology may get a bit ugly! For all of you real world every day clinicians, as you go through the case do what you always do: 1) Establish risk, 2) establish if possible the exact lipid/lipoprotein diagnosis and then 3) establish treatment regimen. For the geeks among you please try and establish the disorder using Fredrickson's diagnostic classification. Luckily the patient had a very thorough work up at Health Diagnostic Labs in Richmond, VA (www.myhdl.com) so we will have lots of information to ponder

The patient is a 43 year old normotensive black male with a BMI of 27 with no known atherosclerosis and no history of pancreatitis who exercises 4 days a week. Father is ~ 72 y/o, T2DM, HBP, no events, no other diagnosis that patient is aware of; 1 brother, 37, & w; 7 sisters, all living and well, no known disease. Mom 70, has elevated triglycerides, on fenofibrate and no events. Knows nothing about grandparents except they lived to be "pretty old". He presented with the following lipid & lab panel (at time of the draw he was taking OTC niacin 500 mg per day, Fish oil 2 caps a day, and Centrum multivitamin).

Hgb A1c = 5.7, FBS 87

TC = 420 LDL-C = 26 (direct) HDL-C = 20 TG = 1315

hs-CRP = 1.42 Fibrinogen elevated at 473 (high risk)

Lp-PLA2 195 (PLAC test or lipoprotein associated phospholipase A2)

Before we start analyzing this case, remember chylomicrons are intestinally produced very large apoB48 containing lipoproteins that traffic mostly triacylglycerols (TG) and phospholipids with some free cholesterol and cholesteryl ester (CE). VLDLs come out of the liver: Basically they are much smaller chylomicrons trafficking TG and phospholipids except their structural apoprotein is apoB100. VLDLs lose their core TG in the lipolytic process (hydrolysis of TG) as well as surface phospholipids they shrink in size and become intermediate density lipoproteins. However these TG-rich particles also can swap their core TG for CE with other lipoproteins including HDLs and thus become cholesterol-rich. A chylomicron or VLDL remnant lipoprotein is simply a chylomicron or VLDL that has lost some TG and acquired some CE: they are fairly large cholesterol rich apoB particles and are considered very atherogenic. Part of the atherogenicity is also due to their carrying apolipoprotein C-III (which delays their catabolism), but we will leave that for another day. You should understand if a patient has a lot of remnants he likely will have elevated TG and apoB-cholesterol values (especially VLDL-C). NCEP suggests using VLDL-C to diagnose the existence of remnants. Since these lipoproteins are so big, even a few of them can create severely abnormal lipids without doing much to an apoB or LDL-P level. Since remnants are less than 70 nm in diameter they can enter the arterial endothelium, just like the much smaller LDLs do.

Well, using the ten year old, practically defunct, NCEP ATP-III, the man has a very high risk. As Chylomicron and TG value and qualifies for treatment. He may or may not be at risk for CHD but he is certainly at risk for pancreatitis. NCEP would advise getting his TG to < 500 mg/dL with lifestyle and if needed TG-modulating drugs. Once that is achieved non-HDL-C would become his goal of therapy. A TG > 500 mg/dL is the one lipid/lipoprotein disorder where a statin is not a first line drug. After lifestyle most would start with fenofibrate and/or 4000 mg of omega-3 FA (Lovaza being the FDA approved therapy). The new AHA statement also lists niacin as a potential therapy for very high TG. If needed, the two most powerful statins at lowering TG are Crestor 40 and Lipitor 80 mg.

Does this man have Familial Hypertriglyceridemia (FHT)? This is an autosomal dominant trait with reduced penetrance. Classically it has been taught that these folks have pancreatitis risk, but little coronary risk (however the new statement advises these folks can also have CAD risk). They typically have normal LDL-C but may have reduced HDL-C and show metabolic abnormalities of insulin resistance. Or does this man have Familial Combined Hyperlipidemia (FCHL) which is usually associated with severe CAD risk. Some might not recognize that because the LDL-C is not high, in fact it is spectacularly low! So does he have FHT or FCHL? Something else? Let's turn to other lipid measurements which were present in the HDL report.

sdLDL = 24 mg/dL (moderately elevated)
%sdLDL = 90% (very high)
HDL2 = 3 mg/dL (very low)

This creates great confusion even among lipidologists. At first glance one suspects they are looking at concentrations of small, dense LDL and large HDL. Nothing could be further from the truth. These tests would be more accurately labeled:

sdLDL-C Meaning all of this persons small LDLs per dL are carrying 24 mg of cholesterol
HDL2-C Meaning all of this persons large HDL particles in a dL are carrying 3 mg of cholesterol
%sdLDL Refers to the percentage of LDL-C that is trafficked in the small LDLs

After seeing the above, I presume many are thinking this person has a very high small LDL-P and if that is true total LDL-P would also have to be high. One might also think there are no large HDL particles and that is usually a significant risk factor (in reality only in drug naive patients). The Vertical auto-profile centrifugation assay reports lipoprotein subparticle cholesterol counts like this, and everyone assumes they are getting small LDL-P or large HDL-P and they are not. Never, EVER, confuse cholesterol measurements with lipoprotein measurements. They are lipid, not lipoprotein concentrations. They may or may not correlate with each other and in IR folks there is often great discordance.

Let's turn to what we really need to make the diagnosis: lipoprotein measurements. From what you already know, in view of the direct LDL-C of 26 mg/dL does anyone think this person is going to have a high apoB or LDL-P. If not can the person be at risk for CHD? Are not we all taught 90% or more of apoB particles are LDLs because of their long half life. Well here we go. Health Diagnostic Labs is an NMR based lab but they also report apolipoprotein B which is essential for this case. Some docs question doing both LDL-P and apoB as duplicative. I, being a true lipoproteinologist, love it.

The apolipoprotein B = 88 (35th percentile cut point in Framingham Offspring study). Using the ADA/ACC Lipoprotein Guidelines for patients with cardiometabolic risk, he would be at goal with a value < 90. He does not meet criteria for the very high risk category where the goal would be < 80.

Apolipoprotein A-I = 107 which is very low
ApoB/apoA-I ratio is 0.82 is high (this ratio was the best predictor of risk in the global InterHeart study)
ApoE genotype is E3/E3 (normal)
Total LDL-P = 458 nmol/L (very low)
Small LDL-P = < 90 nmol/L (very low)
Total HDL-P = 4.1 umol/L (nearly absent)
Large HDL-P = < 0.7 umol/L (virtually none)
Large VLDL-P = 32 nmol/L (99th percentile)
VLDL size = 49.9 nm (around the 65th percentile)
LDL size cannot be determined
HDL size < 8.3 nm (extremely small)
LP-IR score = 67 (> 50 indicative of insulin resistance)

So my fellow lipidologists and lipoproteinologists: what is the diagnosis and what is the CV risk? We need to go into the library stacks and dust off the 1967 New England Journal of Medicine and get the classic treatise: Fredrickson DS, Levy RI, Lees RS. Fat Transport in Lipoproteins - An Integrated Approach to Mechanisms and Disorders. New Eng J Med 1967;276:32-44,94-103,148-156,215-226,273-281. This paper started our specialty. From this came the five types of lipid disorders: Types I to V with Type II having an A and B group. These are the authors that invented the term "lipoproteinology" which they preferred to lipidology and in this paper hailed the passing of lipid concentration measurements. LOL

Type I Chylomicronemia Extreme glyceridemia
Type II Hypercholesterolemia (II) with glyceridemia (IIB) LDL excess
Type III Broad beta disease as it was called then (now remnant disease or dysbetalipoproteinemia)
Type IV VLDL with or without LDL excess
Type V VLDL and Chylomicrons (far less chylols than in Type I)

The new AHA statement has an excellent discussion of these conditions, but no longer refers to them as type I to V.

Recall that Chylomicrons and VLDLs are very large and large (respectively) TG and phospholipid-trafficking lipoproteins. LDLs (and HDLs to some extent) typically carry most of the cholesterol in plasma in normal folks. Remnants carry both TG and cholesterol: remnants are simply what is left after large TG-rich chylomicrons and VLDLs have lost their TG through lipolytic (hydrolysis of TG) actions of lipoprotein lipase and also exchange of TG for cholesteryl ester (CE) with other lipoproteins mediated by cholesteryl ester transfer protein (CETP). Lipolysis makes lipoproteins small and CETP exchange makes chylomicrons and VLDLs TG-poor and CE-rich. VLDL-C is actually the cholesterol content of these TG-rich lipoproteins and thus

VLDL-C = chylomicron-C + VLDL-C + Remnant-C.

In a normal patient who is fasting there should be no remnants or chylomicrons and thus VLDL-C is the cholesterol in all of the VLDLs that exist in a dL of plasma. Dr Friedewald (his classic equation was coauthored by Robert Levy and Donald Fredrickson in Clinical Chemistry 1972;18:499-502) of course stated that in a fasting state all of the TG are pretty much in the VLDLs (not in LDLs and HDLs to any appreciable extent) and since a normally composed VLDL carries five times more TG than cholesterol, one could calculate VLDL-C by dividing TG by 5. Once you calculate VLDL-C and use the lab assays HDL-C and TC, one can calculate LDL-C by:

LDL-C = TC - [HDL-C + VLDL-C] or LDL-C = TC - [HDL-C + TG/5] or more exactly
LDL-C = TC - HDL-C - TG/5

Thus please note as TG elevate, LDL-C should go down, or if a very high TG is reduced LDL-C will rise! We often see that when treating very high TG with fibrates or high dose omega 3 FA.

Paradoxically despite the rise in LDL-C, risk reduces as non-HDL-C gets better.
Non-HDL-C = TC - HDL-C or = VLDL-C + LDL-C

Reducing TG will lower VLDL-C much more than it raises LDL-C and thus despite increasing LDL-C, the goal of therapy, non-HDL-C improves.

So have you figured out what Fredrickson classification does the patient under discussion fall into? Does it matter? Sure: Types I, III (rare), IV and V and can be associated with pancreatitis. Types II,III, some IVs and Vs are at risk for CHD. Since TG back then (and today) are assayed in the labs by converting TG (triacylglycerol) to glycerol and then measuring glycerol (not the fatty acids in the TG), high TG disorders were referred to as glyceridemia. All of the above Fredrickson

phenotypes except IIA have some degree of hypertriglyceridemia. All IIB, Type IIIs, IVs and all Vs have high total cholesterol and TG. Anyone with high TG might have a low HDL-C due to CETP exchange of CE for TG. What helps to distinguish these phenotypes, although unknown to the discoverers, is the apoB level. All are clearly apoB (Chylo/VLDL, remnants and/or LDL disorders but the number of apoB particles characteristic in each phenotype enables us to make a diagnosis. Since 1967 several authors have come up with various ratios or algorithms to phenotype the patients. The best is that developed by Allan Sniderman (Journal of Clinical Lipidology 2007;1:256–263).

Before we use the Sniderman algorithm: lets try and solve it using lipoprotein and lipid knowledge:

Note Type IVs can have two different lipoprotein makeup's. The so called "pure" Type IV has very large VLDLs with delayed catabolism - not a lot of conversion to IDLs or LDLs. They have high TG, high TC (driven by the VLDL-C) but normal LDL-C. This is called Familial Hypertriglyceridemia (FHT) and the risk is mostly pancreatitis. The other Type IVs have large VLDLs but many are converted to LDLs and they have high TG, high TC and may have high LDL-C and is referred to as Familial Combined Hyperlipidemia (FCHL). This is typical of IR and type 2 diabetes. This person has a high TG and normal LDL-C, so does he have FHT and not FCHL? Type IIIs overproduce VLDLs which are converted to IDLs but they are not converted to LDLs. > 90% of Type III patients have the apoE2 allele, but it is not present in 100% of patients. The E2 allele is not well recognized by the hepatic LDL receptor related protein (LRP) which clears TG-rich particles (chylos, VLDLs, remnants, IDLs) that often have multiple copies of normal apoE. If those particles, because of their "faulty apoE2" cannot be cleared then both TG and cholesterol levels (due to the high remnant-C) will rise. Defective clearing of these cholesterol loaded remnant particles would deny the liver a source of cholesterol, and hepatic LDL receptors (LDLr) would be upregulated enhancing clearance of LDL particles (explaining why such patients do not have high LDL-C or high LDL-P). The patient under discussion has the apoE3/E3 genotype. Another characteristic of Type IIIs is that the TC and TG levels are fairly similar, usually in the 300-400 range. They have abnormal cholesterol-rich chylomicron-VLDL remnant like particles (of variable sizes, but all larger than an LDL) but few LDLs. So I guess Type III is unlikely, right? Pure Type Vs have an excess of chylomicrons and VLDLs, IDLs but usually do not have a lot of LDLs. This is often but not always seen in poorly controlled diabetics or in Type IVs with a lot of fat consumption. There is delayed clearance of post prandial lipoproteins. These patients usually have severe hypertriglyceridemia based on the TG-rich particles and high TC based on high chylo-C and VLDL-C. Their pancreatitis and CV risk is high.

Finally the Type IIBs are patients with high TC, high LDL-C and high TG, but not usually super high (> 500 mg/dl). They have high apoB. In effect they also qualify as Familial Combined Hyperlipidemia.

Note all persons with high TG (due to CETP, exchange of CE for TG between apoB and apoA-I particles) can have low HDL-C, so HDL-C levels or apoA-I or HDL particle sizes do not help differentiate these disorders. The HDLs become TG-rich and CE-poor and are subject to catabolism by hepatic and endothelial lipase and renal excretion of apoA-I. This person has almost no large or for that matter any size HDLs left.

So I am not so sure understanding lipoprotein possibilities by guessing can really help one classify the phenotypes. There is much more overlap of these phenotypes than Fredrickson, Levy and lees recognized in 1967. Using the info at hand, this man could have Type IIB (TG a bit too high and LDL-C too low), Type III (no apoE2, TG much higher than TC), Type IV or Type V. The only Types we know with certainty he does not have is I and IIA and IIB.

CALLING, DR SNIDERMAN! (and I did)

Using the Sniderman et al algorithm (referenced above): When following this algorithm be sure not to confuse TC with TG (as I did when I first ran the equations which of course will lead to an

erroneous diagnosis). Luckily for me I shared the case with Allan and he and his brilliant brain did not screw up and he alerted me to my math error and cinched the diagnosis

TG is > 75th percentile: this excludes Type IIA

TC/apoB ratio $420/88 = 4.7$

If it was > 6.2 it would be Type IIA or I

If < 6.2 it could be Types I, III or V

TG/apoB ratio is > 10.0 at 14.7 That means he has a type I or V.

Since I is incredibly rare and he is an adult (Type I manifests in childhood) and he has no lifelong history of hypertriglyceridemia or pancreatitis he is almost certainly a Type V.

Well, I wanted more info so working with the provider I actually got a hold of the full NMR spectral analysis on this patient: we obtained the following:

Large VLDL-P is very high but not horrific at 32 nmol/L

Medium VLDL-P extremely high at 911.4 nmol/L

Small VLDL-P 30 nmol/L

Total VLDL-P high at 973 nmol/L

IDL-P extremely high at 407 nmol/L

NMR technology cannot differentiate a chylomicron from a VLDL. Unless an unusual lipoprotein disorder is present (as in the current case) the vast majority of fasting patients will not have circulating chylomicrons. With a TG of 1315 I would expect a patient to have a much higher large VLDL-P. This man has a high level of large VLDL-P but much higher levels of medium VLDLs. So he does have some lipoprotein lipase activity converting many large VLDLs and chylomicrons to smaller particles (remnants) which register on NMR as medium VLDLs. He is also converting large and medium VLDLs to IDLs but is not creating small VLDLs or converting his IDLs to LDLs. Is this some hepatic lipase issue? The apoE genotype is E3/E3 and the liver LDL receptor-related protein (LRP) and LDLr should be clearing his TG- rich lipoproteins (also apoE enriched) as well as his LDLs. The disorder could also be explained by a VLDL/chylo overproduction.

Interestingly on the HDL report form the Lp(a) mass is elevated at 74 but the Lp(a)-cholesterol could not be done and the lab noted: Interfering VLDL peak present on electrophoresis. Unable to accurately quantitate Lp(a) cholesterol. So we have additional evidence of cholesterol-rich remnants which obscure the Lp(a) peak on the electrophoretic tracing.

As discussed above, He surely has high CETP activity as the TG are going from his VLDLs and IDLs to his HDLs. The TG-rich HDLs are undergoing rapid catabolism (? endothelial lipase) with the excretion of apoA-I (thus no large HDLs and near absent total HDLs: However some unlipidated apoA-I is still circulating with an apoA-I of 107 mg

So a patient with very high TG, low direct LDL-C, extremely low HDL-C, with high VLDL-P, high IDL-P, slightly high apoB and normal LDL-P: so instead of speculating could this be a Type III, pure IV or V - thanks to Allan Sniderman's incredible algorithm we know it is a V. The CV risk in this man is driven by the very high remnants and IDL-P (almost like a Type III).

Treatment is as follows: Very high TG: reduce it to < 500 (take out the pancreatitis risk) and then normalize whatever excess apoB particles (apoB-cholesterol or non-HDL-C). So aggressive lifestyle and aggressive glycemic control followed by very high dose (4000 mg or more of omega-3 FA: I'd use Lovaza as I know what I am getting and I know there are no contaminants) with fenofibrate or fenofibric acid. Once TG are < 500 NCEP simply states to get non-HDL-C to goal, but I'd want to be sure I have eliminated the very high VLDL-P and IDL-P and did not create a high LDL-P, so I'd repeat the NMR. If any of those particle counts were still high, one would add a

statin. Once apoB, and VLDL-P, and IDL-P and LDL-P (if present) are good, I'd try to raise total HDL-P and that might require high dose Niaspan. If when the TG are < 500, there are no longer major increases in remnants (VLDL-P, IDL-P) or LDL-P, then treatment is done.

Allan Sniderman added the following: The number of VLDL particles depends on: a) the rate at which they are secreted and b) the rate at which they are cleared. The number of LDL particles depends on the number of VLDL particles converted to LDL particles (vs those cleared directly) and the rate at which LDL particles are cleared. From the number of VLDL particles, you cannot infer the secretion rate or the conversion rate. I think your error is assuming that a large number of VLDL particles should produce a large number of LDL particles. That is not necessarily the case. That is why apoB (and LDL apoB/LDL P) do not have to be increased in type V- or type IV for that matter since the same considerations apply there.

Does the lack of an extremely high large VLDL-P or LDL-P elevation exclude V (a chylomicron disorder?).

Well I went to the source: In Fredrickson, Levy and Lee's classic paper where they first described Type V, they note that on electrophoresis prebeta (VLDL) and chylo fractions (at origin) were high but the beta-lipoproteins (LDL) were low. Alpha lipoprotein (HDLs) are (as expected) also low in Type Vs.

How about some more expert opinion: The current Godfather of lipoproteins Russ Warnick (Chief Scientific officer of HD Lab) and co-author of the classic text, Handbook of Lipid and Lipoprotein Testing commented: *"This exercise is bringing back memories of my early years. I am one of the few who has been around long enough to remember when the phenotypes came out with the intention of simplifying the categorization of hyperlipidemias. I was running the Northwest Lipid Research Center Core Lipoprotein Lab at the time supporting all the U of WA research as well as UWA and Harborview Medical Center's lipid clinics. Initially we used the phenotypes to categorize patients but we gradually realized that the phenotypes were not exclusive with each of the six patterns representing differing combinations of genetic and metabolic states. At the time we were interested especially in dysbetalipoproteinemia and familial combined and even the former was quite heterogeneous. We gradually recognized it was impossible to unambiguously assign many patients to a particular phenotype and finally discontinued trying to assign the phenotypes. In my view now the phenotype names are useful in conveying a sense of the combinations of lipoproteins present, but they do not represent discrete disorders and even categorize patients well for treatment. So trying to decide whether this patient is a Type III or Type V is interesting, but as you say Tom, both patterns would be treated the same."*

Finally a comment from expert laboratorian Joe McConnell (Lab Director at HD Lab): *"I ran lipoprotein metabolism profiles for a number of years at Mayo. Using the Fredrickson classification scheme, with some modifications developed by Ralph Ellefson at Mayo (which I studied and believe are quite good), I ultimately came to my own decision that type IV and V in many cases are likely due to then same underlying disorder. I tracked the diagnoses of all Fredrickson phenotypes that we defined at Mayo and when patient samples were sequentially sent, many times they converted from a type V to a type IV and vice versa (sometimes they typed as IV, sometimes as V). I attributed this to fasting status or diet. Type IV with good fast and V with poor fast or bad diet. If ApoB or LDL-P were high in these patients I would have called them a Fredrickson type IIB, and recommended testing for the metabolic syndrome and/or diabetes. Admittedly I did not get apoB or LDL-P in most of these, but rather relied on LDL-C, and we know we can have normal LDL-C and high LDL-P or apoB. Type IIB patients tended to have values rise over time and maintain until treatment. Type IV and V tended to come on quickly associated with some other condition like thyroid disease, drug reaction (often steroids), alcohol abuse, or new onset diabetes, etc. If the secondary cause was corrected, many times the hyperlipoproteinemia*

resolved. I'm not sure if this is consistent with your thinking, but these were my thoughts based on lipoprotein metabolism testing at Mayo."

Finally: Did you really have to have the in depth understanding of the issues outlined above or could you simply have said: the TG is too high and I am treating it no matter what the lipid phenotype! Perhaps but I close with Alan Sniderman's wisdom and comment about that: *"I do not think it is meaningless to accurately diagnose a problem. The error is assuming clinical and genetic heterogeneity in the phenotypes. The error has nothing to do with the advantage. As to treatment being the same, perhaps. But one reason we have learned so little about treatment of specific disorders is that we have not examined the outcome of therapy when they were separated."*

That's it friends - hope at least a few of you stayed with me and enjoyed the discussion.