The role of the Clinical Chemistry laboratory in facilitating earlier diagnosis of dyslipidaemia-associated inherited metabolic disease

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Sulaiman presents a best practice review for primary/monogenic dyslipidaemia, their clinical presentation and best use of molecular genetics for earlier diagnosis.[1] This review is important in the context of such patients, with multisystem issues, presenting to non-lipidology specialties to enable more timely diagnosis of dyslipidaemia-associated inherited metabolic disease (IMD). Efficient diagnosis ensures appropriate medical management (particularly important where disease-specific medications exist) and attenuates long-term sequelae.

Clinical guidelines, and best practice reviews such as this, are often not followed.[2 3] There may be a significant delay from clinical presentation to diagnosis of such IMDs. This is due to the diversity of symptoms and the breath of specialties to which such patients present, most notably and often in the first instance Primary Care. Patients may be asymptomatic with the incidental finding of abnormal lipids the only prompt for further investigation. Patients with non-specific or variable symptoms may progress down a winding road of referrals and inappropriate investigation, which can take years. Even patients with classical signs of primary dyslipidaemia, for example, eruptive or tuberous xanthoma and premature cardiovascular disease, will take time to be investigated and thus diagnosed in the absence of specialist knowledge or availability of multigene panel testing for monogenic dyslipidaemic mutations.

As suggested by the author, such a tool (a multigene panel) would expedite diagnosis for some of these rarer diseases.[1] The diagnostic yield of these dyslipidaemia next-generation sequencing panels will depend on a range of factors, such as pretest probability, the number of disorders and genes represented in the panel, analytical performance, such as coverage metrics, detection of non-coding and regulatory variants, availability of deletion/duplication (copy number variant) analysis in conjunction with sequencing and clinical interpretation of the findings.

Clinical Chemistry laboratories producing test results are well positioned to proactively direct and advise the non-lipidologist on how best to proceed with unusual lipid results.[4 5] First, the exclusion of all secondary causes of dyslipidaemia is required. In case of hyperlipidaemia, conditions such as diabetes mellitus, excessive alcohol consumption, thyroid dysfunction or renal disease should be excluded. Severe chronic liver disease, cystic fibrosis, hyperthyroidism, malabsorption and cachexia should be considered in the setting of marked hypocholesterolaemia. A finding of low high density lipoprotein-cholesterol (HDL-C) during acute infection or inflammation should be confirmed on recovery. HDL-C is a negative acute phase reactant and will decrease during an acute immune response. To guarantee follow-up of a low HDL-C, C reactive protein (CRP) could be reflexed by the laboratory information system (LIS) in response to low HDL-C concentrations, although the optimum HDL-C concentration is not well defined. Low HDL-C in the presence of an elevated CRP could further prompt addition of a comment to reports outlining the concern and requirement for repeat lipid testing on stabilisation. Medication review is essential in delineating secondary causes of dyslipidaemia. Based on all testing performed and the patterns observed, reflective comments could be added to reports by suitably competent clinical chemistry personnel to improve the medical care of patients.[6] Comments should ensure that any unusual or suspicious test findings are highlighted and could outline possible scenario-specific follow-up investigations. Dialogue between the requesting clinician and the laboratory will enhance the patients' management by ensuring that the tests to be requested are appropriate and possible.

As indicated in this review, it will be much more probable for a patient to have a secondary cause of dyslipidaemia or even a polygenic dyslipidaemia than one of the rarer monogenic disorders.[1] However, in the absence of more plausible aetiology, these rarer single mutation diseases should not be omitted from the differential.

A simple tool in investigating dyslipidaemia is the visual inspection of a sample for lipaemia. Lipaemia can be observed if the concentration of triglycerides is over 3.4 mmol/L, but in practice, is subjective and can go undetected until triglyceride concentrations exceed 11.3 mmol/L.[7] Due to this insensitivity and the sheer volume of samples received for testing by Clinical Chemistry laboratories, automated platforms can now produce an automatic Lipaemic-index (L-index) on each specimen by way of a pre-analytical check. This semiquantitative measurement uses calculations of absorbance measured at bichromatic wavelengths and is considerably more sensitive for detection of lipaemia than visual inspection. It is important to be mindful that a high L-index does not equal a high triglyceride concentration in a sample.[8] High L-indices have been associated with sample turbidity unrelated to triglyceride content, for example, elevated protein/immunoglobulins or total parenteral nutrition, inappropriate timing of sample collection, grossly haemolysed or hyperbilirubinaemic samples. The lack of index standardisation between manufacturers with reference to wavelengths used and reporting methods and units can also be problematic. Nonetheless, if a high L-index is noted in a patient for the first time and no lipids have been previously requested, a lipid profile could be automatically added by information technology (IT) rule in the middleware software and/or the LIS. This expedites the investigation of this incidental finding and may aid a clinical diagnosis down the line. It is not routine practice to examine specimens post-storage for floating chylomicrons. However, IT systems could prompt such visual inspection based on triglyceride concentration and/or the L-index. Furthermore, a test request code for this visual inspection of a specimen post-storage could be available to the requestor and allow perceptive physicians to proactively request such a check before disposal of the specimen from the laboratory.

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Lack of visible plasma turbidity and/or a normal L-index in the presence of raised serum/plasma triglycerides may suggest the presence of an artefactual elevation. Pseudohypertriglyceridaemia is observed in glycerol kinase deficiency (GKD), an X-linked recessive disorder which may occur in isolation or as part of a contiguous gene syndrome.[9] Contiguous gene syndrome is more severe and presents in the neonatal period or infancy with developmental delay, failure to thrive, congenital adrenal hypoplasia and/or Duchenne muscular dystrophy. Isolated GKD is, on the other hand, phenotypically very variable even within a single family. Presentations range from metabolic crisis during catabolic stress in early childhood, tendency to hypoglycaemia, to the incidental finding of pseudohypertriglyceridaemia in an asymptomatic individual.[9]

Triglycerides are typically measured by a fully automated enzymatic method based on glycerol oxidation, after the action of lipase, to dihydroxyacetone phosphate. The concentration of triglycerides is proportional to the rate of glycerol oxidation. Despite being recommended,[10] many current triglyceride assays do not include glycerol blanking or treatment with adsorbents to remove free glycerol as it is assumed that endogenous free glycerol is negligible.[11] High concentrations of free glycerol in plasma may result from glycerol contamination of the blood tube, excessive intake of beer containing large amounts of glycerol[12] or GKD.[13] Pseudohypertriglyceridaemia is supported by raised urinary or plasma glycerol (glycerol is measured qualitatively by urinary gas chromatography–mass spectrometry (GC-MS) in author IB's institution), absence of a chylomicron band or increased very low density lipoprotein (VLDL) band on lipoprotein electrophoresis and lack of triglyceride reduction on fibrate treatment. Genetic analysis of the GK gene confirms a definite diagnosis.

Hypertrigyceridemias are increasingly recognised in children and young adults due to more widespread screening and increasing prevalence of childhood obesity, sedentary lifestyle, type 2 diabetes, high-fat and high-carbohydrate diet, and use of medication including antidepressants, retinoids or oestrogens.[14] Secondary hypertriglyceridaemias associated with IMDs as highlighted by Sulaiman[1] are typically associated with other accompanying clinical and biochemical abnormalities which can point an astute physician in the right direction.

HMG-CoA synthase deficiency (HMGCS2D), an autosomal recessive disorder of ketone body synthesis, was not mentioned in the review[1] and could be highlighted as another condition associated with significant hypertriglyceridaemia in early childhood. HMG-CoA synthase mediates the formation of HMG-CoA, a required intermediate of ketone bodies and a precursor or mevalonate and cholesterol. The condition is characterised by episodes of hypoketotic hypoglycaemia, high anion gap metabolic acidosis with only a mild ketosis, transient hepatomegaly and acute encephalopathy during a crisis typically precipitated by an infection in the first years of life. Transient severe hypertriglyceridaemia and low HDL-C detected during an acute episode might be explained by impaired ketogenesis and hypoglycaemia, leading to a marked elevation of free fatty acids and triglycerides.[15] The diagnosis is suspected from typical, although not pathognomic, findings of urinary organic acid analysis by GC-MS and plasma acylcarnitine analysis by tandem MS during an acute episode. The diagnosis should be confirmed genetically.

Hypertriglyceridaemia is also a frequent feature of hemophagocytic lymphohistiocytosis (HLH), an aggressive syndrome of excessive immune activation which can occur as a familial or sporadic disorder and typically but not exclusively affects infants from birth to 18 months of age.[16] Primary and secondary HLH is precipitated by infections or other immune activating events. Typical findings in an acutely ill child are fever, splenomegaly, bicytopaenia (usually anaemia and thrombocytopenia), hypertriglyceridaemia with fasting triglycerides >3 mmol/L, abnormal liver function and coagulation, very high ferritin >500 µg/L and/or low to absent natural killer cell activity. Bone marrow aspirate and biopsy helps evaluate the cause of cytopaenias and/or detecting haemophagocytosis.[16]

Hypocholesterolaemia in contrast to hypercholesterolaemia may be underappreciated. The diagnosis of inherited hypobetalipoproteinaemia can be delayed until the clinical manifestations of vitamin E and A deficiency or progression of liver steatosis to fibrosis and eventually cirrhosis occurs.

Inherited hypobetalipoproteinaemia should be suspected if marked hypocholesterolaemia is associated with serum/plasma low density lipoprotein-cholesterol (LDL-C) and apolipoprotein-B (apoB) concentrations <5th percentile for age and gender with no obvious secondary cause. Abetalipoproteinaemia, homozygous familial hypobetalipoproteinaemia and chylomicron retention disease present in childhood with steatorrhoea and failure to thrive. Heterozygous familial hypobetalipoproteinaemia and loss of function mutations in PCSK9 are, on the other hand, asymptomatic. Loss of function mutations in ANGPTL3 result in combined hypolipidaemia, an autosomal recessive condition characterised by a reduction in all plasma lipids.

Laboratories routinely use automation and IT to appropriately manage thousands of test results daily. Clinical Chemistry laboratories could reflexively or reflectively add complimentary testing to samples already present in the laboratory based on available lipid results. We would recommend slowly rolling out such testing to get experience and see if there is any user feedback.[17] Low LDL-C could trigger the addition of the less routine test apoB, while a low HDL-C with normal LDL-C could trigger an apoA1 test request. Based on the patterns of specific total cholesterol and triglyceride results, as outlined by Sulaiman,[1] lipoprotein electrophoresis or other more specialist investigations could be recommended to define the phenotype, observe the lipoprotein pattern and look for evidence of complications.

Further to this, using in-built diagnostic algorithms, middleware and/or the LIS could be harnessed to facilitate advanced automatic management of abnormal test profiles. Intelligent ordering processes inputted to the LIS have proven effective in other disciplines for timely diagnosis and disease management.[18] Similarly in the realm of dyslipidaemia, the combination of biochemistry test results, demographics, such as age and ethnicity, detail on clinical presentation (signs and symptoms) and personal/family history would produce sufficient information on a patient to allow automatic reflexing of pertinent tests. Big data may help with this.[19] At a minimum, such detail would permit logical commentary (prompting referral perhaps) back to the requesting clinician, irrespective of their specialty. Order communications is a necessary prerequisite tool for establishment of intelligent test ordering. Order communications would prompt the requestor for the information required and advise on the appropriate sample requirements allowing the inputted algorithms to work optimally.

On identification of a dyslipidaemia-associated IMD in an index case, it will be necessary to roll out screening to family members based on lipid and genetic testing. Genetic counselling is recommended in all monogenic dyslipidaemias. No guidelines for monogenic dyslipidaemia outside of

familial hypercholesterolaemia and paediatric primary hypertriglyceridaemias exist. Given the rarity of some of these monogenic dyslipidaemic disorders, preparation of even a single guideline document encompassing all these conditions may be appropriate.

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