Falsely decreased total cholesterol

HOSP #	Req#452050196	**WARD**	Pathcare Private
CONSULTANT	John Stanfliet / Jody Rusch	**DOB/AGE**	Unknown

Abnormal Result

Presenting Complaint

This was a case discussed in consultation with a private consultant:

The patient was admitted with SARS-CoV-2.

History

The clinician was contacted regarding an extremely low LDL-cholesterol, not comparable with the other measurements.

Medication history was unknown at the time when these results became known and had to be authorized.

Examination

Not applicable and information not available.

Laboratory Investigations

Test	Result
Lipaemia	Absent
Total Cholesterol	< 0.5 mmol/L

Triglyceride	0.38 mmol/L
HDL Cholesterol	1.9 mmol/L
Non-HDL-Cholesterol	-1.40 mmol/L
Cholesterol:HDL ratio	0.3
LDL-cholesterol (calculated)	Not done
LDL-cholesterol (measured)	3.0 mmol/L
Glucose (fasting)	5.0 mmol/L

Other Investigations

From the results above it becomes clear that there are some discrepancies in the results. The total cholesterol, as measured on the Abbott Allinity (<0.5 mmol/L) does not compare against the measured LDL-cholesterol (3.0 mmol/L), which should be lower than the total cholesterol.

Other investigations to perform on this sample would perhaps be to run it on a different analyser.

Final Diagnosis

The clinician was phoned and it was found that the patient was on high doses of Vitamin C intravenously.

Take Home Message

When there’s a big discrepancy between LDL (measured – directly with a homogenous assay) and the total cholesterol, the cause should be determined, or at least investigated.

The Total cholesterol, LDL-cholesterol, Triglycerides and HDL-cholesterol all use Trinder reactions.

Vitamin C is a quencher in the reaction (likely due to its high anti-oxidant activity). Since COVID has been around,

there are quite a lot of protocols of treatment with Vitamin C IV. It is likely that patients infused with IV N-acetylcysteine, also a potent anti-oxidant, will also cause spuriously low total cholesterol. Or perhaps spuriously low results in any reaction employing the trinder reaction.

It is also clear from this case how important it is to discuss results which do not make sense with clinicians.

Summary of the Trinder reaction

> *A few decades ago, Emerson presented a new color test reaction (Emerson 1943), which is still in common use for the determination of phenolic compounds (e.g. Ettinger et al. 1951; Fiamegos et al. 2002). Later, Trinder adapted this reaction for the determination of blood glucose using horseradish peroxidase (HRP), coupling the hydrogen peroxide produced from the glucose oxidase reaction, to the Emerson indicator reaction (Trinder 1969; Barham & Trinder 1972). For this reason, this reaction is also known as the Trinder reaction. The so-called Emerson–Trinder reaction, is now routinely used as a spectrophotometric indicator reaction in clinical chemistry, in which a quinoneimine dye product is produced by oxidative condensation of a phenol with 4-aminoantipyrine (4-AAP) (Emerson 1943). This indicator reaction was subsequently used for the spectrophotometric assay of a large number of substrates or enzymes (Burtis & Ashwood 1994) such as uric acid (Kabasakalian et al. 1973), cholesterol (Allain et al. 1974), free hemoglobin (Bauer 1981) or triglycerides (Fossati & Prencipe 1982) and also by using different organic hydrogen-donor compounds such as different substituted (ortho, meta and para) chloro or bromophenols, 4-hydroxybenzene-sulfonic acid (Wang et al. 1992), 2,4-dichlorophenol (Klose et al. 1978), 3,5-dichloro-2-hydroxybenzensulfonic acid (Fossati & Prencipe 1982; Fossati et al. 1980) or different aniline derivatives (Tamaoku et al. 1982).*

Farzad Deyhimi, Massoud Arabieh & Lida Parvin (2006) Optimization of the Emerson–Trinder enzymatic reaction by response surface methodology, Biocatalysis and Biotransformation, 24:4, 263-271, DOI: 10.1080/10242420600661943

A case of high HDL-cholesterol

HOSP #		**WARD**	GP Clinic
CONSULTANT	John Stanfliet / Jody Rusch	**DOB/AGE**	73 year Female

Abnormal Result

Abnormal lipid profile (see below)

Presenting Complaint

A 73 year old female was investigated with a full lipid profile after presenting with an increased total cholesterol upon routine screening at her general practitioner.

History

The patient had an increased Total Cholesterol, but was otherwise not unwell. Medication history unfortunately not available.

Examination

Not available

Laboratory Investigations

Test	Result
Urea	7.2 mmol/L
Creatinine	105 umol/L
eGFR	46 ml/min/1.73m2
Fasting Lipid profile (lipemia index - turbidity- on sample was absent):	
Total Cholesterol	6.7 mmol/L
Triglyceride	0.6 mmol/L
HDL Cholesterol	> 4.7 mmol/L
Non-HDL Cholesterol (calculated)	< 2.0 mmol/L
LDL Cholesterol (calculated)	< 1.7 mmol/L
LDL Cholesterol (direct – measured)	1.3 mmol/L
Glucose Fasting	5.5 mmol/L

Table 1 – Full lipogram with other routine chemistry tests.

Other Investigations

To rule out the possibility of interferents, the following tests were performed.

Test	Value
Apo A1	4.24 g/L (424 mg/dL) (Ref. >140 mg/dL)
Apo B	0.52 g/L (52 mg/dL) (Ref. < 130 mg/dL)
Apo B : Apo A1 ratio (calculated)	0.12

Table 2 – ApoA1 and ApoB by immunoassay. ApoA1: the major lipoprotein in HDL particles. ApoB: the major lipoprotein in Non-HDL particles.

Final Diagnosis

Increased HDL which may likely be an APOC3 deficiency.

Take Home Message

Although not present in this case, elevated apolipoprotein B (ApoB) confers increased risk of atherosclerotic cardiovascular disease, even in a context of acceptable LDL cholesterol concentrations. Extremely low values of ApoB (<48 mg/dL) are usually related to malabsorption of food lipids and can lead to polyneuropathy. Reduced apolipoprotein A1 (ApoA1) confers an increased risk of coronary artery disease. Extremely low ApoA1 (<20 mg/dL) is suggestive of liver disease or a genetic disorder. Elevated ApoB:ApoA1 ratio confers increased risk of atherosclerotic cardiovascular disease, independently of LDL and HDL cholesterol concentrations.

If the inverse of the above is true, then this lady is likely destined to live forever, but that's the whole conundrum in lipid metabolism – the inverse of one's theories does not always hold true under randomized controlled studies, and due to the difficulty of finding a proper control group. It was however previously demonstrated that patients with ApoC3 deficiency (if this is the cause in this case) increases longevity.

> *APOC3 has been established as an inhibitor for lipoprotein lipase, a gene that hydrolyzes triglycerides to generate free fatty acids before their uptake by muscle and adipose tissue (reviewed in Jong et al). Mice with a high-level expression of human APOC3 on a background of Ldlr deficiency proved to be an excellent model for familial combined*

hyperlipidemia, because they are disturbed in the breakdown of triglycerides. In contrast, mice lacking Apoc3 show increased activity of LPL, which causes hypotriglyceridemia and protection from postprandial hypertriglyceridemia. From these mice studies, it became clear that a deficiency of APOC3 could cause a healthier lipoprotein profile, which is associated with protection from cardiovascular diseases. However, in the absence of APOC3-deficient subjects, this hypothesis was difficult to test directly.

Dodacki, A., Wortman, M., Saubaméa, B. et al. Expression and function of Abcg4 in the mouse blood-brain barrier: role in restricting the brain entry of amyloid-β peptide. Sci Rep ***7,*** *13393 (2017). https://doi.org/10.1038/s41598-017-13750-0*