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

Correcting the Myth of Calcium Correction

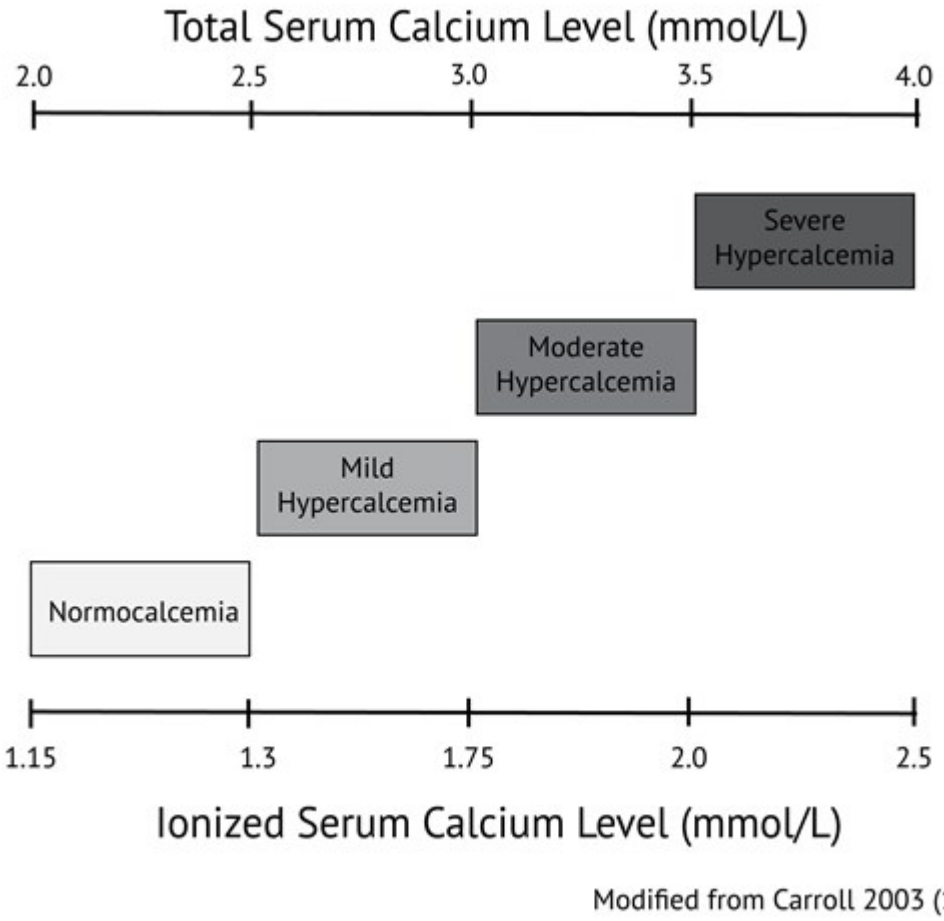
By Dr. Janet Simons on June 26, 2019

By Dr. Janet Simons (biography, no disclosures)

What gap I have noticed

Calcium levels are commonly ordered in both primary and acute care in patients with a variety of signs and symptoms. Hypocalcemia (total calcium concentrations generally below 2.0 mmol/L or ionized calcium below 1.15 mmol/l) is usually related to dietary deficiencies or disorders of the parathyroid axis, such as in patients with previous surgery or autoimmune destruction of the parathyroid gland. Hypercalcemia (above 2.5 mmol/L total calcium or 1.3 mmol/L ionized) in primary care is commonly associated with dehydration, primary hyperparathyroidism, and malignancy such as multiple myeloma. When hypercalcemia is severe, generally defined as total calcium above 3.5 mmol/L or ionized calcium above 2.0 mmol/L, therapy should be initiated immediately. Values of calcium below this threshold but above 3.0 mmol/L total calcium or 1.75 mmol/L ionized are considered moderate hypercalcemia and patients with calcium values in this range may not need immediate therapy but should be monitored closely. Precise

values of normal ranges and cut offs may vary between laboratories.



Modified from Carroll 2003 (17)

It remains common practice to apply the Payne formula (usually expressed as albumin-adjusted calcium (mmol/L) = total calcium (mmol/L) + 0.02 [40 – albumin (g/L)]) (1) to adjust total calcium. This correction is intended to enhance the ability of the total calcium concentration to serve as a marker of the physiologically relevant parameter, ionized calcium, in patients with hypoalbuminemia.

Since the original Payne paper, clinical use of this correction formula has spread such that many clinicians routinely apply this ‘correction’ to all total calcium measurements. This observation is supported by data available from Vancouver Coastal Health and Providence Health Care laboratories. In 2018, total serum calcium and albumin were ordered together **72%** of the time, suggesting that many clinicians believe that serum albumin measurement is required

in order to interpret total calcium concentrations.

There are a number of problems with the Payne formula. This formula was derived in 200 patients whom Payne considered to be **unlikely to have abnormalities of ionized calcium**, however 20% of the patients had **hyperproteinemia secondary to multiple myeloma**. Payne et al relied upon results from a single laboratory which used methodologies for the measurement of albumin and total calcium which are different from methods in routine use today. The formula was designed to transform the calcium results in those patients who had hypoalbuminemia so that the distribution of results would match the distribution of calcium results in the patients with normal serum albumin concentrations. There was no validation of the formula using ionized calcium, which was not measured.

What data addresses this gap

There is considerable evidence (2-12) that application of the Payne formula tends to misclassify the calcium status of patients and performs less well than simply evaluating uncorrected total calcium. Payne himself recently wrote a letter to the editor (13) in which he acknowledged that his original formula is not universally applicable, requiring modification for the specific albumin assay in use by a laboratory, and that any albumin-based adjustment will likely overestimate calcium in patients with renal failure. In renal failure, the albumin concentration is underestimated when uremia induced carbamylation of albumin reduces its detection by the assay (14). Attempts to derive a new formula (10-12) to improve upon the performance of the Payne formula have failed to find a correction which performs significantly better than unadjusted total calcium.

The physiological basis for the albumin adjustment is the theory that when albumin is reduced, the amount of calcium bound to albumin is also reduced, such that the total serum calcium may be low despite a normal ionized calcium

concentration. However, this physiologic basis is belied by evidence that in hypoalbuminaemic states, the binding constant between albumin and calcium changes, and more calcium binds to each available gram of albumin (15). Formulae such as the Payne formula which assume a constant relationship between albumin concentration and the fraction of calcium which is bound to albumin are thus expected to overestimate ionized calcium in patients with low albumin. Several studies have borne out this tendency of correction formulae to overestimate ionized calcium.

Steen et al (2) found that in patients with albumin <30 g/L, 75% of patients classified as normocalcemic using the Payne formula in fact had hypocalcemia based on ionized calcium levels. Another study (3) found that adjusted calcium values derived by applying the Payne formula agreed with ionized calcium levels in only 55-65% of patients. In contrast, unadjusted total calcium correctly categorized 70-80% of patients. Agreement between adjusted calcium and ionized calcium was even worse for patients with renal impairment (eGFR<60 min/mL/1.73m^2). The adjustment significantly overestimated calcium concentrations in these patients. A similar trend has been documented in critically ill patients in both the medical and surgical ICU settings (4-6).

The poor performance of the calcium correction has also been observed in the hypoalbuminemic geriatric population (7). Again, the correction impairs the sensitivity of the corrected result to detect true hypocalcemia. The more severe the hypoalbuminemia, the poorer the performance of the adjustment formula. This has also been demonstrated in stable hemodialysis patients (8-9).

Other studies (10-11) have sought to derive new formulae for the purpose of correcting calcium for albumin concentration. James et al (10) considered many possible formulae but ultimately concluded that if any adjustment is to be made to

calcium to account for hypoalbuminemia, the adjustment formula must be locally derived.

Many of the studies above were done in hospital inpatients. Less data is available in outpatients, as ionized calcium is more difficult to measure in this population due to the requirement that specimens for ionized calcium be analyzed promptly after collection (16). However, a study which examined results from both inpatients and outpatients of a hospital and excluded critically ill patients (12) confirmed that unadjusted total calcium performs better than any of the available correction formulae (including those put forth by Payne and James) in ROC analysis compared to the ionized calcium gold standard.

What I recommend (practice tips)

Formulae to adjust total calcium for the albumin concentration should be abandoned. The use of these formulae overestimates ionized calcium in patients with hypoalbuminemia, causing false negatives for hypocalcemia and false positives for hypercalcemia. Measurement of ionized calcium is now relatively inexpensive and is available in most hospitals and many outpatient settings.

1. Measurement of ionized calcium is recommended over total calcium when calcium homeostasis is in question.
2. If calcium is ordered as a ‘screening’ test without specific clinical suspicion for a disorder of calcium homeostasis, it is reasonable to assess unadjusted total calcium. If this level is abnormal, confirmation with ionized calcium may be sought prior to further workup or therapy.
3. Where ionized calcium is not available, total calcium should be assessed without the application of any correction formula.
4. Order serum albumin only if clinically indicated for reasons other than adjusting total calcium.

References

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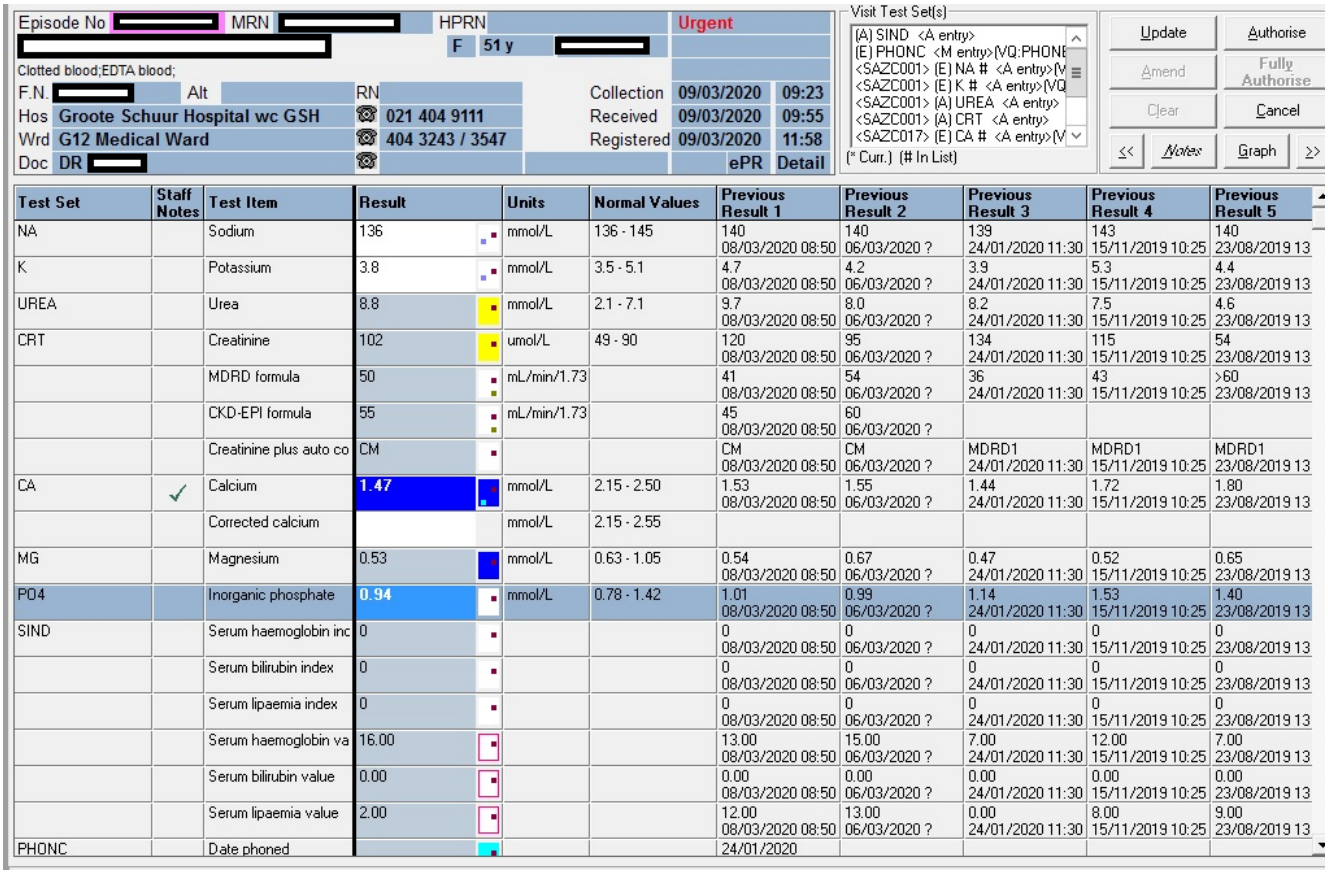
A case of persistent hypocalcemia

HOSP #	MRN63985901	**WARD**	Medical Ward
CONSULTANT	Dr. Heleen Vreede	**DOB/AGE**	51 year Female

Abnormal Result



Episode No [redacted] MRN [redacted] HPRN Urgent
[redacted] F 51 y
Clotted blood;EDTA blood;
F.N. [redacted] Alt RN
Hos Groote Schuur Hospital wc GSH 021 404 9111 Collection 09/03/2020 09:23
Wrd G12 Medical Ward 404 3243 / 3547 Received 09/03/2020 09:55
Doc DR [redacted] Registered 09/03/2020 11:58
ePR Detail

Visit Test Set(s)
(A) SIND <A entry>
(E) PHONC <M entry>{VQ:PHONE
<SAZC001> (E) NA # <A entry>{V
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(* Curr.) (# In List)

Update | Authorise | Amend | Fully Authorise | Clear | Cancel | << | Notes | Graph | >>

Test Set	Staff Notes	Test Item	Result	Units	Normal Values	Previous Result 1	Previous Result 2	Previous Result 3	Previous Result 4	Previous Result 5
NA		Sodium	136	mmol/L	136 - 145	140 08/03/2020 08:50	140 06/03/2020 ?	139 24/01/2020 11:30	143 15/11/2019 10:25	140 23/08/2019 13
K		Potassium	3.8	mmol/L	3.5 - 5.1	4.7 08/03/2020 08:50	4.2 06/03/2020 ?	3.9 24/01/2020 11:30	5.3 15/11/2019 10:25	4.4 23/08/2019 13
UREA		Urea	8.8	mmol/L	2.1 - 7.1	9.7 08/03/2020 08:50	8.0 06/03/2020 ?	8.2 24/01/2020 11:30	7.5 15/11/2019 10:25	4.6 23/08/2019 13
CRT		Creatinine	102	umol/L	49 - 90	120 08/03/2020 08:50	95 06/03/2020 ?	134 24/01/2020 11:30	115 15/11/2019 10:25	54 23/08/2019 13
		MDRD formula	50	mL/min/1.73		41 08/03/2020 08:50	54 06/03/2020 ?	36 24/01/2020 11:30	43 15/11/2019 10:25	>60 23/08/2019 13
		CKD-EPI formula	55	mL/min/1.73		45 08/03/2020 08:50	60 06/03/2020 ?			
		Creatinine plus auto co	CM			CM 08/03/2020 08:50	CM 06/03/2020 ?	MDRD1 24/01/2020 11:30	MDRD1 15/11/2019 10:25	MDRD1 23/08/2019 13
CA	✓	Calcium	1.47	mmol/L	2.15 - 2.50	1.53 08/03/2020 08:50	1.55 06/03/2020 ?	1.44 24/01/2020 11:30	1.72 15/11/2019 10:25	1.80 23/08/2019 13
		Corrected calcium		mmol/L	2.15 - 2.55					
MG		Magnesium	0.53	mmol/L	0.63 - 1.05	0.54 08/03/2020 08:50	0.67 06/03/2020 ?	0.47 24/01/2020 11:30	0.52 15/11/2019 10:25	0.65 23/08/2019 13
PO4		Inorganic phosphate	0.94	mmol/L	0.78 - 1.42	1.01 08/03/2020 08:50	0.99 06/03/2020 ?	1.14 24/01/2020 11:30	1.53 15/11/2019 10:25	1.40 23/08/2019 13
SIND		Serum haemoglobin inc	0			0 08/03/2020 08:50	0 06/03/2020 ?	0 24/01/2020 11:30	0 15/11/2019 10:25	0 23/08/2019 13
		Serum bilirubin index	0			0 08/03/2020 08:50	0 06/03/2020 ?	0 24/01/2020 11:30	0 15/11/2019 10:25	0 23/08/2019 13
		Serum lipaemia index	0			0 08/03/2020 08:50	0 06/03/2020 ?	0 24/01/2020 11:30	0 15/11/2019 10:25	0 23/08/2019 13
		Serum haemoglobin va	16.00			13.00 08/03/2020 08:50	15.00 06/03/2020 ?	7.00 24/01/2020 11:30	12.00 15/11/2019 10:25	7.00 23/08/2019 13
		Serum bilirubin value	0.00			0.00 08/03/2020 08:50	0.00 06/03/2020 ?	0.00 24/01/2020 11:30	0.00 15/11/2019 10:25	0.00 23/08/2019 13
		Serum lipaemia value	2.00			12.00 08/03/2020 08:50	13.00 06/03/2020 ?	0.00 24/01/2020 11:30	8.00 15/11/2019 10:25	9.00 23/08/2019 13
PHONC		Date phoned				24/01/2020				

Total calcium of 1.47 mmol/L (2.15 – 2.50)

Presenting Complaint

The patient has been having persistent hypocalcemia despite supplementation with calcium.

History

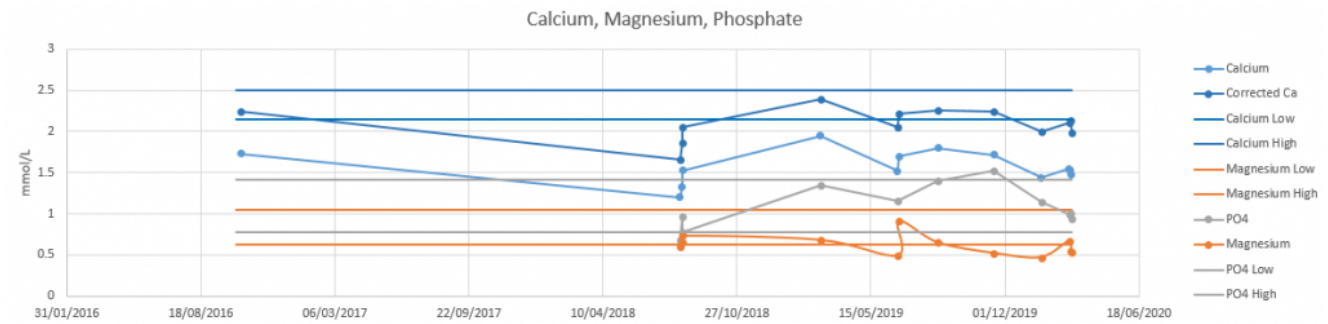


Figure 1 – Illustration of the patient's CMP over time: Calcium: blue; Magnesium: orange; Phosphate: grey Reference ranges are the horizontal lines without dotted markers

Examination

Not available.

The typical findings in a patient with true hypocalcemia (low ionised calcium) are

Trouseau’s sign

Chvostek’s sign

Laboratory Investigations

Arguably, the first important consideration in patients with low calcium is the albumin. The patient had a mean albumin of 12 g/L, significantly lower than normal (40-50g/L). Arguably, the calcium can be corrected with the well known Payne’s formula to then be 1.47 + (0.02 x (40-12) = 2.03 mmol/L:

> *Albumin-adjusted calcium (mmol/L) = total calcium (mmol/L) + 0.02 [40 – albumin (g/L)])*
>
> *Payne RB, Little AJ, Williams RB, Milner JP. Interpretation of serum calcium in patient with abnormal serum proteins. Br Med J. 1973;4:643-646. DOI: 10.1136/bmj.4.5893.643. (View)*

Measurement of serum intact parathyroid hormone (PTH) should be performed in all patients with hypocalcemia; it is the most valuable laboratory test for determining the etiology of hypocalcemia:

	2019/11/15	2019/06/28	2018/08/03
PTH (pmol/L)	21,8 H	15,5 H	25,8 H

Reference interval: (1.6-6.9 pmol/L)
Vitamin D

	09/09/2020	15/11/2019	03/08/2018

Total Vitamin D (25-OH VitD)	20.5 nmol/L	45.4 nmol/L	23.2 nmol/L

Guidelines for assessment of Vitamin D status:
<30 nmol/L <12 ng/mL Deficient
30-50 nmol/L 12-20 ng/mL Insufficient
>50 nmol/L >20 ng/mL Sufficient
125-150 nmol/L 50-60 ng/mL Safe upper limit
Reference: Revised South African Clinical Guideline for the diagnosis and management of osteoporosis (NOFSA 2017), endorsing the institute of Medicine Dietary Reference intakes for calcium and vitamin D (2010). Note regarding conversion of units:
Divide result in nmol/L by 2.496 to convert to ng/mL
Multiply result in ng/mL by 2.496 to convert to nmol/L

Other Investigations

Anti-Tissue Transglutaminase antibodies: **Negative**: repeated 3 months apart, with sufficient IgA levels in the serum): 0.9 & 0.8 U/mL (EliA c/o: 6.9)

Anti-Gliadin antibodies: **Equivocal**: 7.8 & 9.6 U/mL (EliA c/o: 6.9)

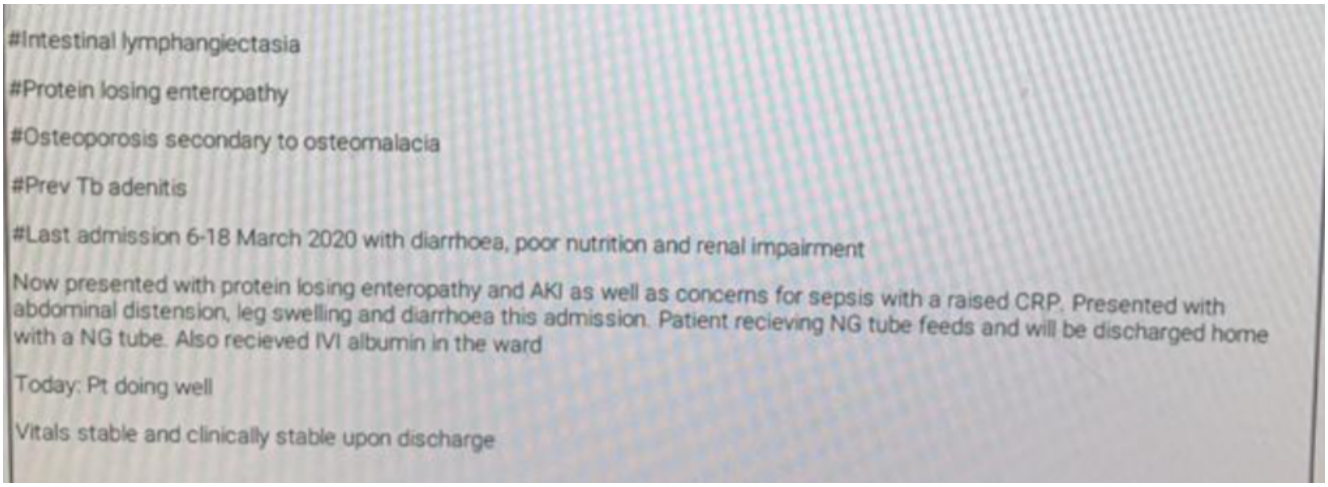
Anti-endomysial antibodies: **Negative**

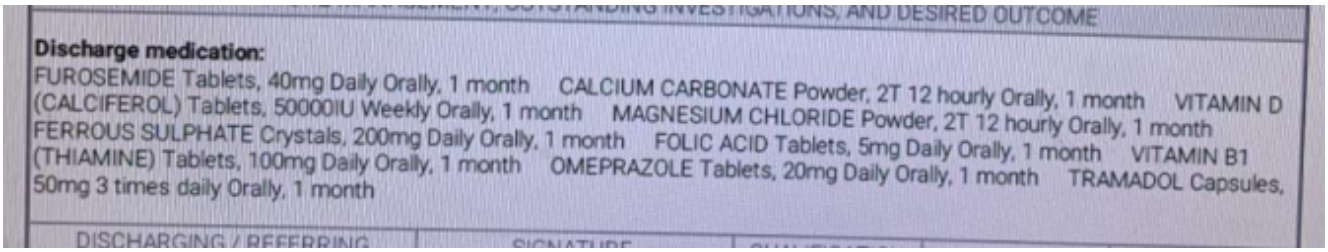
HLA-DQ2: **Positive**

HLA-DQ8: **Negative**

Final Diagnosis

Hypocalcemia likely due to malabsorbtion (telangiectasia stated by the clinicians).





Take Home Message

According to International guidelines the following association is expected for patients with Coeliac Disease:
Positive for HLA-DQ2 (HLA-DQA1*05, DQB1*02)
Positive for HLA-DQ8 (HLA-DQA1*03, DQB1*03:02)

Considering the fact that the albumin was high with an increased PTH, the calcium very likely was physiologically also low (bioactive Ca). The Payne's formula also failed to correct the calcium to the normal reference range.

Cumulative History:Download