A case of amenorrhoea in a 17-year old female

A case of hyperprolactinemia with amenorrhoea

Laughing spells and precocious puberty in a child

A case of laughing spells with precocious puberty

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*

Glucagon Stimulation C-peptide testing

HOSP #		**WARD**	Endocrinology ward
CONSULTANT	Dr. Heleen Vreede	**DOB/AGE**	22 y Male

Abnormal Result

Patient presented with Diabetic Ketoacidosis and a glucose value of 27.4 mmol/L.

Presenting Complaint

Signs and symptoms typical of Diabetic Ketoacidosis

History

Patient was diagnosed with diabetes 7 years ago after presenting with diabetic ketoacidosis. Upon diagnosis he was given insulin in the hospital. Upon discharge he was given Metformin and Glimeperide (oral hypoglycemic medication – reason for oral agents unknown – likely because of his young age?). Defaulted Rx completely. Presented with DKA again. Restarted about 2 y ago on insulin.

The differential diagnosis at the current presentation is thus one of:

1. Ketosis prone diabetes
2. LADA (Latent auto-immune diabetes of the adult)
3. Type1 – went into honeymoon phase after diagnosis and now relapsed

To differentiate – the clinicians prompted to do antibodies, insulin levels and a glucagon stimulation c-peptide dynamic test.

Examination

N/A

Laboratory Investigations

Date	05/02/2021	02/02/2021	28/08/2018	25/01/2018	05/06/2017	03/03/2017	03/03/2017	24/02/2017	16/09/2016	14/04/2015
Na		134 L					137.000			133 L
K		4,6					4.890		4,5	UOLD2
Cl		93 L								
Urea		13,4 H					5.000			1,9 L
Creat		91					69.000		66	34 L
Glu Random				27,4				21.860		
HbA1c (NGSP)		12,7	>14	13,7	>14			12,8	13,7	
Total chol	5,04						ბ- 4,98			
Triglyceride	1,74						1,25			
HDL chol	1,35						1,16			
LDL chol (calc)	2,89						3,25			
Total chol									8,99	
U creat	4,1						1,9			
U albumin	32.70						<3			
U alb : creat	8.0 H						UTC			
Test referred						Anti-IA2 Antibody Positive; Anti-GAD antibody Positive				

Other Investigations

A glucagon-stimulated C-peptide level was drawn.

0 min	1.5 ug/L	0.5 nmol/L
1 min	2.0 ug/L	0.67 nmol/L
2 min	1.9 ug/L	0.63 nmol/L
3 min	1.9 ug/L	0.63 nmol/L

Final Diagnosis

LADA – latent autoimmune diabetes of the adult

Take Home Message

Serum c-peptide has traditionally been thought to be an inconvenient method as immediate lab analysis is required before degradation (when collected in serum gel or plain sample tubes). This is because c-peptide is a small linear peptide, which is susceptible to enzyme proteolytic cleavage.

Gel tubes are therefore traditionally required to be transported to the lab on ice, promptly centrifuged and separated, then stored in frozen conditions unless lab analysis is possible at that center.

However, c-peptide sample collection for c-peptide determination in whole blood in EDTA prepared tubes is stable at room temperature for up to 24 h. Venous blood c-peptide levels can be measured in the random, fasting, or stimulated scenarios. Random samples are taken at any time during the day without consideration of recent food intake, whereas fasting samples are taken after an 8- to 10-h fast.

Stimulation methods include using

- glucagon
- intravenous/oral glucose
- tolbutamide
- sulfonylurea
- glucose-like peptide 1
- amino acids
- a mixedmeal

In this case a glucagon stimulation yielded sufficient results to assist the clinicians in making the diagnosis, indeed a case of atypical diabetes presentation.

An uncommon cause of unconjugated

hyperbilirubinemia

HOSP #		WARD	Red Cross Endocrinology
CONSULTANT	Dr Jody Rusch	DOB/AGE	27 day female

Abnormal Result

TSH > 100

Free T4: 0.5 pmol/L

Presenting Complaint

Patient was brought to the ER being lethargic.

History

Term Neonate; Had a history of profound jaundice after birth, with unconjugated hyperbilirubinemia.

The patient’s mother lives in Athlone, gave birth at Carl Bremer hospital where a cord blood TSH was done, but results not available at the time.

Examination

No overt abnormalities on examination was found, except the single sign of jaundice.

No defects at the base of the tongue was observed.

No abnormalities in the neck was observed.

Laboratory Investigations

TSH > 100

Free T4: 0.5 pmol/L

Other Investigations

The patient had an ultrasound of the abdomen done (since it was the first occurence of hyperbilirubinemia, and in fact is termed pathological jaundice).

Cord blood TSH was retrospectively reviewed as being 178 uIU/ml.

Final Diagnosis

Congenital hypothyroidism

Take Home Message

Congenital hypothyroidism (**CH**) is thyroid hormone deficiency present at birth. If untreated for several months after birth, severe congenital hypothyroidism can lead to growth failure and permanent intellectual disability. Infants born with congenital hypothyroidism may show no symptoms, or may display mild symptoms that often go unrecognized as a problem. Significant deficiency may cause lethargy, hypotonia, hoarse cry, infrequent bowel movements, significant jaundice, and hypothermia.

Causes of congenital hypothyroidism include

- iodine deficiency (most common cause)
- developmental defect in the thyroid gland, either due to a genetic defect or a biochemical defect in thyroxine production
- pituitary defects – congenital hypopituitarism (present at birth) may be the result of complications around delivery, or may be the result of insufficient development (hypoplasia) of the gland, sometimes in the

context of specific genetic abnormalities.

Hypoglycemic seizures

HOSP #	MRN90378429	**WARD**	Endocrinology Ward
CONSULTANT	Jody Rusch	**DOB/AGE**	14 y girl

Abnormal Result

Fingerprick glucose 2.9 mmol/L

Presenting Complaint

Hypoglycemic seizure

History

The patient is a known type 1 diabetic patient who presented to the Internal Medicine Paediatric specialist OPD during two occasions of hypoglycemic seizures before.

The pateint had, according to the mothed, no post-ictal state.

She was admitted to the Endocrinology ward for a fast provocation test. At two hours, the glucose measured 2.9mM on point-of-care glucometer – glucose and other parameters on laboratory values however is illustrated below.

2 weeks after this presentation patient presented again with hypoglycemic seizures – mother is a nurse – puts in drip after which the patient’s condition normalizes.

IGF-1 normal, Ketones raised (quantitative beta-

hydroxybutyrate, Insulin: 6 nmol/L, glucose: 3.5mM, hGH: 1.9 ug/L.

Examination

On examination the patient had no signs and symptoms of hypoglycemia (during the provocative test). And after the hypoglycemic seizure there were no “post-ictal” symptoms identified.

Laboratory Investigations

Glucose 3.5 mmol/L /L
Insulin: **6** nmol/L
Lactate 1.5 mmol/L (0.5 – 2.2)
Beta-hydroxybutyrate 1855 umol/L (20 – 270)
Ammonia 56 umol/L (11 – 35)
Cortisol 367 nmol/L
Cortisol reference intervals (when performed on a Roche Cobas analyzer):
Levels in adults: Morning (06:00-10:00) 133 – 537 nmol/L ; Afternoon (16:00-20:00) 68 – 327 nmol/L
Human growth hormone 1.9 ug/L

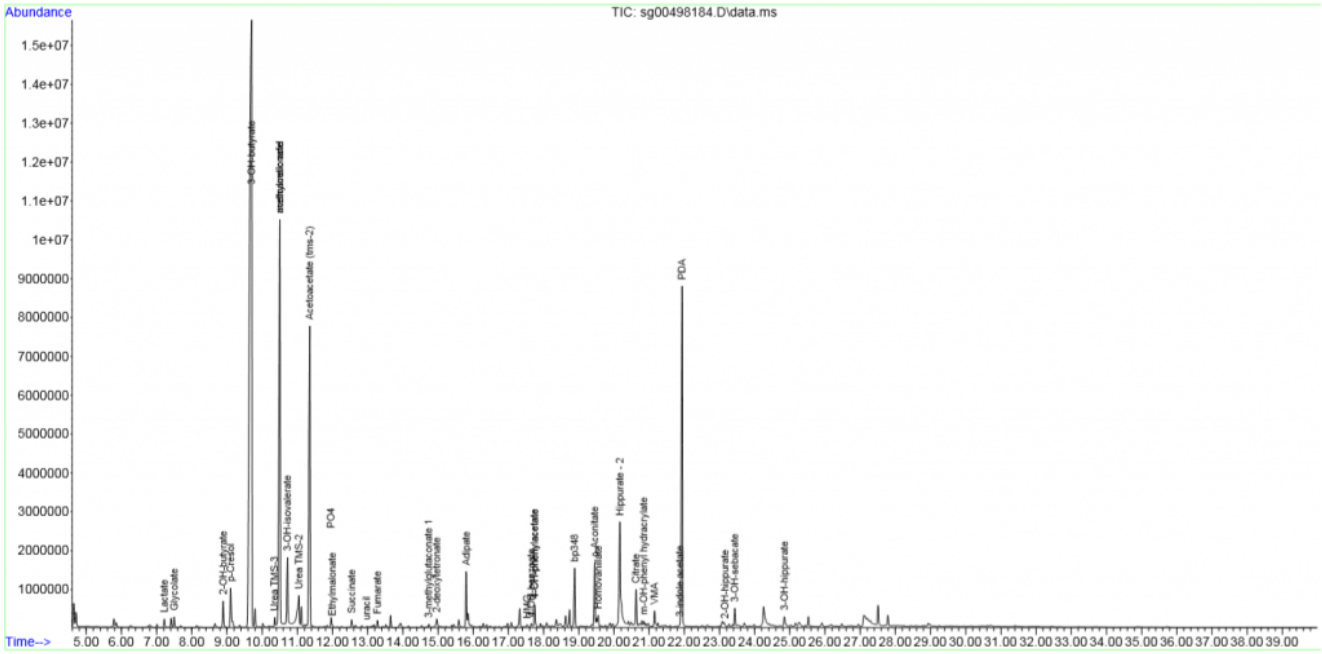
IGF-1 (Insulin-like growth factor I) @ 22/02/2021 09:30 : **366.0** ug/L (170.0 – 527.0)
Tanner stages Boys vs Girls:
Stage I 63 – 271 ug/L ; 71 – 394 ug/L
Stage II 114 – 411 ug/L ; 122 – 508 ug/L
Stage III 166 – 510 ug/L ; 164 – 545 ug/L
Stage IV 170 – 456 ug/L ; 174 – 480 ug/L
Stage V 161 – 384 ug/L ; 169 – 400 ug/L

Synacthen stimulation test:

Time on 22/02/2021	Cortisol (nmol/L)
14h00 (Baseline)	316

14h30	597
15h00	436

Other Investigations



Urine organic acid analysis profile: The 3 prominent peaks on the left are from left to right: B-hydroxybutyrate, Acetoacetate (with TMS derivative 1), Acetoacetate 2nd peak (with TMS derivative 2). TMS = trimethylsilyl derivative reagent, PDA = pentadecanoic acid (internal standard).

Final Diagnosis

Type 1 Diabetes with ketoacidosis and occasional episodes of hypoglycemia

Possible reasons for the hypoglycemia may be:

1. Ketogenic diet (fairly easily excludable I think).
2. Ketone utilisation disorder:
   - SCOT (succinyl-CoA:3-ketoacid CoA transferase) deficiency
   - Mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase) deficiency

Take Home Message

There are two predominant ketone utilisation disorders: SCOT deficiency and beta-ketothiolase deficiency. These disorders produce fairly continuous ketones, as they cannot be metabolised in the muscle and brain upon these deficiencies, which are autosomal recessive (as is most inherited metabolic diseases).

Giving the mother a urine dipstick home to measure urine at home mane before meals, midday just before meals and late afternoon or so before meals may be advised to assist with the diagnosis of one of the above disorders.

Urine organic acid analysis can sometimes pick up a marker to diagnose beta-ketothiolase deficiency:

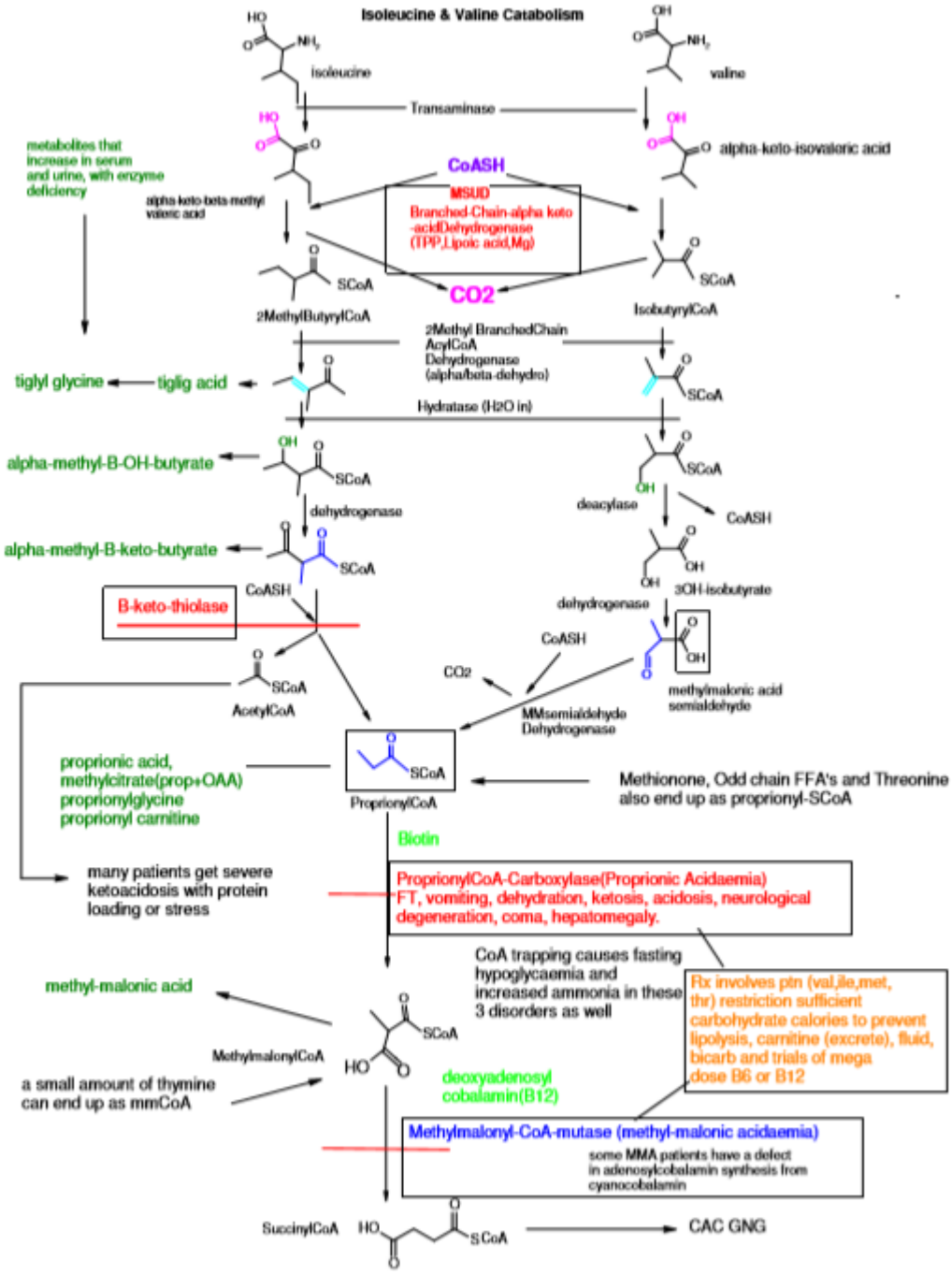


Figure 2 – The metabolism of Isoleucine and valine (credits to George van der Watt). In beta-ketothiolase deficiency, alpha-methyl-beta-keto-butyrate will accumulate, and can be detected on urine organic acid analysis by GC-MS.

A classic case of Cushing Disease

A classic case of Cushings Disease

An interesting cause of hyponatremia

HOSP #		**WARD**	Red Cross Hospital Oncology ward
CONSULTANT	Dr Amith Ramcharan / Dr Jody Rusch	**DOB/AGE**	11y Female

Abnormal Result

Persistent hyponatremia

2018 supracellar JPA (Astrocytoma)

Seizures – phenobarb.

Chemo @ 8 y of age.

Vincristin and Carboplatin administration

Craniospinal radiation – leptospinal

Presenting Complaint

Seizures – controlled with Phenobarbital

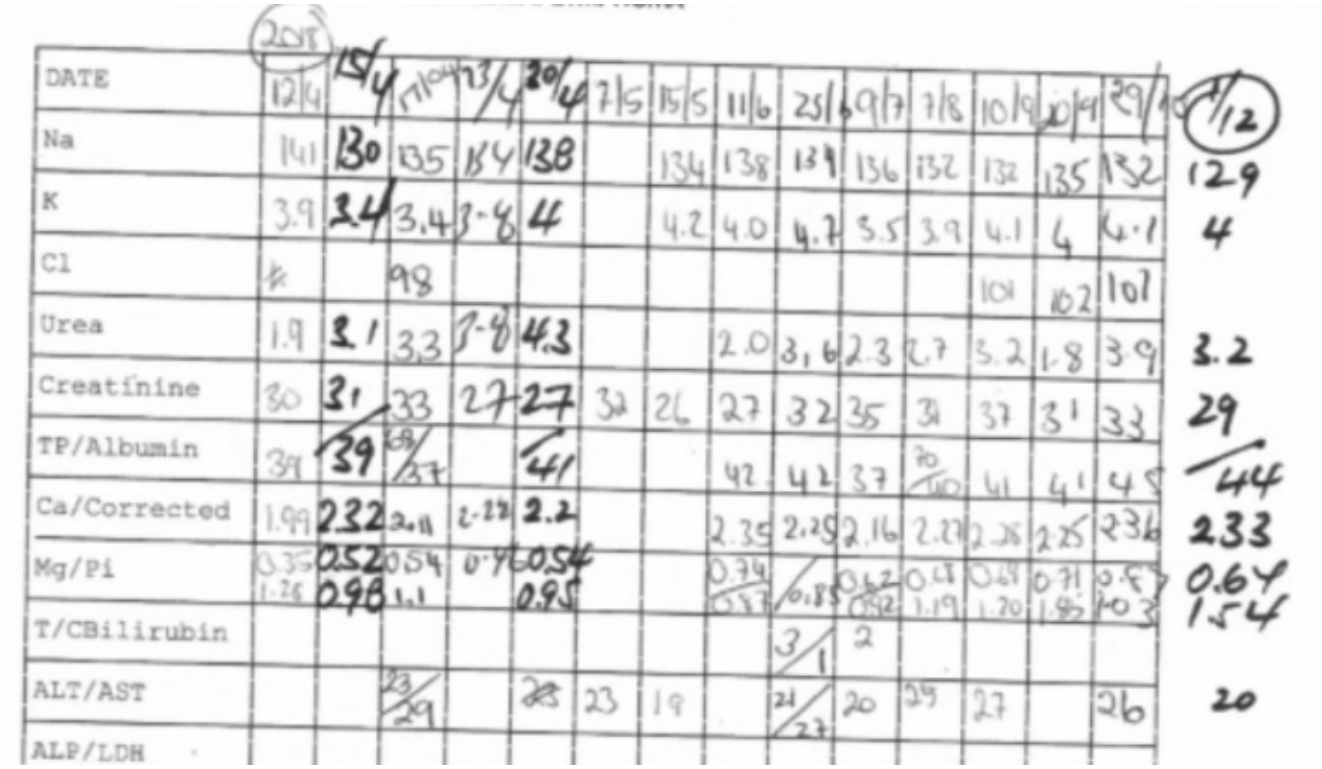
History

This is an 11 year old patient with a suprasellar JPA (Juvenile Pilocytic Astrocytoma). The tumour was diagnosed at 8y of age, upon which chemotherapy with Vincristine and Carboplatin was initiated. The pituitary was close to the area of radiation therapy as well.

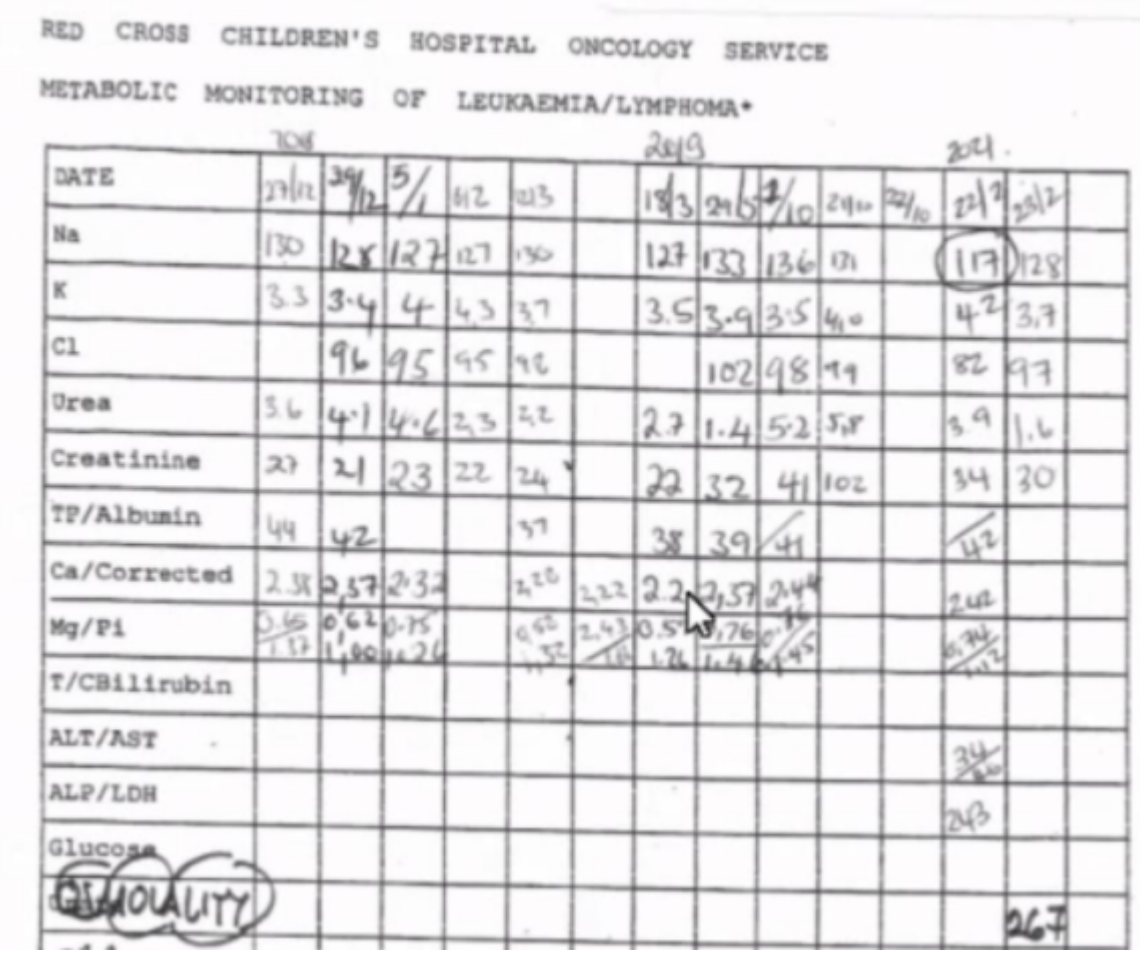
Examination

The patient’s hydration status was normal and there was no cerebral edema.

Laboratory Investigations



2018 – Electrolytes relatively stable



RED CROSS CHILDREN'S HOSPITAL ONCOLOGY SERVICE

METABOLIC MONITORING OF LEUKAEMIA/LYMPHOMA*

DATE	27/12	3/1/19	5/1	612	1013		18/3	29/5	1/10	29/10	24/10	22/12/20	25/12	
Na	130	128	127	127	130		127	133	136	131		117	128	
K	3.3	3.4	4	4.3	3.7		3.5	3.9	3.5	4.0		4.2	3.7	
Cl		96	95	95	98			102	98	99		82	97	
Urea	3.6	4.1	4.6	2.3	2.2		2.7	1.4	5.2	5.8		3.9	1.6	
Creatinine	27	21	23	22	24		22	32	41	102		34	30	
TP/Albumin	44	42			37		38	39	41			42		
Ca/Corrected	2.35	2.37	2.32		2.26	2.22	2.2	2.57	2.44			2.62		
Mg/Pi	0.65 / 1.17	0.62 / 1.80	0.75 / 1.26		0.92 / 1.32	2.43 / 1.0	0.5 / 1.26	0.76 / 1.46	0.9 / 1.45			0.34 / 1.12		
T/CBilirubin														
ALT/AST												34 / 30		
ALP/LDH												243		
Glucose														
[illegible] QUALITY													267	

2018-2019 – Hyponatremia and hypomagnesemia developing
The patient was found to have hypothyroidism and started on T4 replacement 50ug mane.

Other Investigations

Urine electrolytes on 23/02/2021:

- Na 54 mM
- K 31.3 mM
- Cl 110 mM
- Osmol 554 mOsmol
- Fractional reabsorption of phosphate: 85%

Final Diagnosis

Unknown – but likely indicates a tubular loss of sodium due to the chemotherapeutic agent(s).

Take Home Message

Chemotherapeutic agents does cause tubulopathy.

TMP/GFR is likely a better indicator of renal phosphate handling than only fractional reabsorption of phosphate. This can be calculated mathematically or read from a nomogram.

Falsely decreased glucose

A case of falsely low blood glucose values due to a pre-analytical error.

Hyponatremia with a urine sodium measurement

A case of hyponatremia with only a urine electrolyte measurement available

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Beta-HCG’s half life

A case of rapidly decreasing b-HCG

A serum albumin of 2 g/L

A case of severely low serum albumin

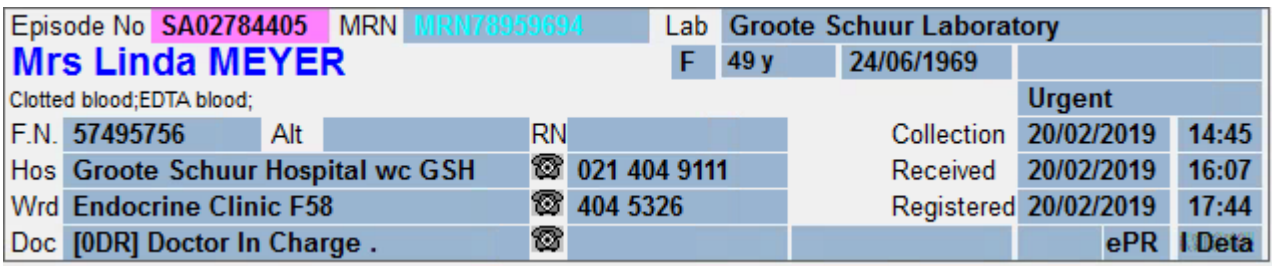
A case of elevated caeruloplasmin

A short case of elevated caeruloplasmin

Hypercalcemia with uric acid crystals

From other results it is also evident that:

HOSP #		**WARD**	Nephritic clinic
CONSULTANT	Dr. Heleen Vreede	**DOB/AGE**	49 y Female



Uric acid nephropathy with hypercalcaemia (Mrs. Linda Meyer) MRN78959694

Abnormal Result

The calcium on 20/02/2019 on bloods taken 14h45 was 3.29 (2.15-2.50 mmol/L).

Presenting Complaint

The patient presented with pain “from loin to groin” which is the typical presentation of passing a renal stone.

History

The patient has chronic renal failure (first creatinine was 362 umol/L with eGFR of 12ml/min – MDRD) on 12 December 2017. Creatinines relatively unchanged since then.

Upon re-evaluation of the case in 2020 it was seen that the baseline creatinine has risen to ~445 umol/L indicating a worsening of the chronic renal failure eGFR now 9 ml/min – by both CKD-EPI and MDRD formulas.

Examination

N/A

Laboratory Investigations

The patient is known with Hyperuricemia, first result 0.50 (0.16-0.36mmol/L) on 16 February 2018. The response to treatment appears poor due to continuing rising serum uric acid levels (considering whether the patient is on allopurinol).

2. Regarding the hypercalcemia:

Episode	SA04315821	SA03552076	SA03535628	SA02816641	SA02784405	SA02622825	SA02369770	SA02123812	SA01901592
Date	11/11/2020	11/12/2019	04/12/2019	04/03/2019	20/02/2019	12/12/2018	04/09/2018	23/05/2018	16/02/2018
Time	09:44	10:22	17:03	15:48	17:44	17:11	10:31	16:25	15:28

Na			135 L		139	138	139.000	138.000	137.000
K	5,3 H	4,7	4,8		4,8	4,5	4.320	4.400	4.780
Urea			17,2 H		14,3 H	16,2 H	11,3 H	18,8 H	17,1 H
Creat	443 H	484 H	434 H	444 H	446 H	475 H	334 H	408 H	415 H
MDRD	9	8	9	9	9	8	13	10	10
CKD-EPI	9								
Ca	**2,79 H**		**2,59 H**	**3,09 H**	**3,29 H**	**2,97 H**	**2.820 H**	**2.850 H**	**3,12 H**
Mg			0.94		1,05	1.00		1.060 H	.980
Phos			1,02		1,25	1,33	.980	1.240	1.110
PTH			**13,3 H**		4,3	4,6			

Cumulative history of UEC and CMP with PTH.
From above results a consistent hypercalcemia with a single raised PTH result can be seen – see “Final Diagnosis” and “Take Home Message” below.

Other Investigations

Uric acid crystals were seen on the urine microscopy reflecting uric acid nephropathy – a possible cause of the chronic renal failure, but I could not find any biopsy result or alternative explanation for the renal failure and assume it is uric acid nephropathy. The patient also appears to have been for a procedure at Urology (? Renal stone removal).

A serum protein electrophoresis with immunofixation (13/09/2018) showed no monoclonal peaks.

Final Diagnosis

Uric acid nephropathy with renal stones.

Hypercalcemia likely due to tertiary hyperparathyroidism.

Take Home Message

Uric acid nephropathy appears to be an uncommon cause of chronic kidney disease (ref. Up-to-date).

It should however be emphesized that clinicians consider the cause on a differential, as it is a manageable cause.

Hypercalcemia sometimes occur in Chronic Kidney Disease patients due to tertiary hyperparathyroidism. This is due to persistent hyperphosphatemia with resulting hyperparathyroidism leading to hypercalcemia (as opposed to the more commonly occuring **hypocalcemia** is renal failure).

——Commentary by Nephrologist- Dr. Erika Jones——

WRT the Uric Acid

Difficult to say if it is cause or effect of CKD. We can only really make a diagnosis of uric acid nephropathy on kidney biopsy. But it is definitely a cause that we see on occasion.

The good news is that the creatinine has remained fairly stable in the last couple of years, unlike the UA, but as kidney function deteriorates it is expected the UA will increase.

According to our buff records she had staghorn calculi and that was labelled as the cause of her CKD.

Allopurinol in CKD is challenging as it accumulates with side effects. We have had two patients with full on Steven’s Johnson Syndrome. So if she isn’t symptomatic I wouldn’t give it to her. She is recorded as having Sarcoidosis which explains the hypercalcaemia. I think this stage is too early to have tertiary hyperparathyroidism.

Query EDTA contamination

A case of likely EDTA contamination

Discrepant TFT’s

A case of discrepant TFT’s

Rapidly decreasing Prolactin result

HOSP #		**WARD**	Endocrinology Clinic
CONSULTANT	John Stanfliet	**DOB/AGE**	36 y Female

Abnormal Result

A low prolactin result was obtained in a patient in whom a macroadenoma was suspected:

Prolactin: 1.3 mIU/L

Presenting Complaint

The patient presented with headache and decreased visual acuity (more specifically peripherally).

History

There were bilateral galactorrhoea, amenorrhoea, and as noted above, headache and visual disturbances.

The patient had received Cabergoline (a dopamine receptor

agonist on D2 receptors) for the past 4 months.

Examination

As above

Laboratory Investigations

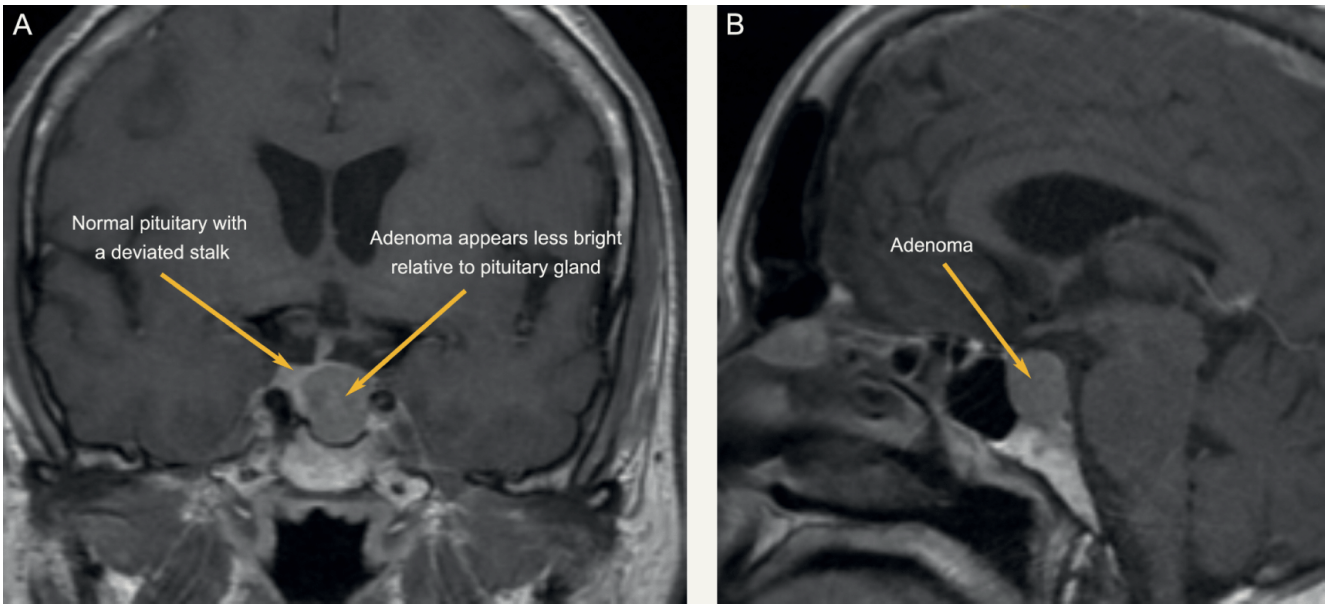
Date	**Prolactin** (mIU/L)
02/2019	106 (Recovery of 80% following PEG precipitation)
05/2019	135
06/2019	85
08/2019	1.3 (1.59 with a 1:10 dilution; 3.94 with a 1:50 dilution)

Prolactin Results

Other Investigations

MRI Head was booked for the following week. Interestingly, even in prolactin secreting tumours, the correlation between tumour size and prolactin level is limited. MRI head remains a vital investigation.

Final Diagnosis



Pituitary Macroadenoma

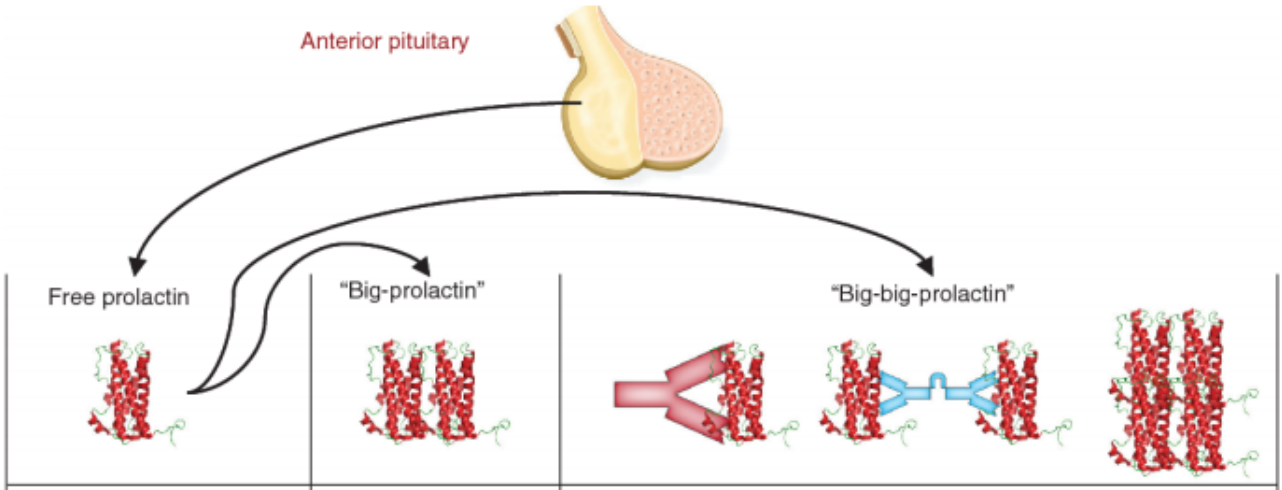
Take Home Message

During pregnancy the concentration of prolactin rises under the influence of elevated estrogen and progesterone production. The stimulating action of prolactin on the mammary gland leads post partum to lactation. Hyperprolactinemia (in men and women) is the main cause of fertility disorders. The determination of prolactin is utilized in the diagnosis of anovular cycles, hyperprolactinemic amenorrhea and galactorrhea, gynecomastia and azoo-spermia. Prolactin is also determined when breast cancer and pituitary tumors are suspected. As in this case, a pituitary tumour was suspected, hence the repeated prolactin results.

As was noted in another short case, our assay on the Roche platform does measure all forms of prolactin, and when a high result is obtained (above the gender-specific reference range) it is recommended to measure the recovery after PEG precipitation.

Roche Prolactin Package Insert (2013)Download
Clin-Biochem-Rev-2018_Prolactin-Biology-and-Lab-MeasurementDownload
Clin-Chem-2008-Macroprolactin-Reference Intervals-after-

PEGDownload



Monomeric	Dimeric	IgG-prolactin	IgA-prolactin	Prolactin aggregates
23 kDa	40–60 kDa	>100–150 kDa		
Active	Inactive	Inactive		
60%–90%	15%–30%	0%–8%	0%–2%	0%–3%

Figure 1. Structure of monomeric prolactin, "big-prolactin" and "big-big prolactin". Figure 1 adapted from reference 9 with permission.

Figure 1

Dr. John Stanfliet (pathologist at Pathcare) repied to the above case with very valuable comments:

- We use Beckman Coulter DxI, an immunoassay that is not affected by macroprolactin (I've include an article that shows this).
- Even in prolactin secreting tumours, the correlation between tumour size and prolactin level is limited. MRI head remains a vital investigation.
- Some prolactin secreting tumours also secrete other pituitary hormones such as growth hormone.
- I would ascribe the reduction in PRL to the Carbegoline and wonder whether the dose has been increased.
- Dr. Pete Berman would often suggest a mixing study: find a sample with high PRL, mix it 50/50 with this sample, and measure it to see whether there is some interferant in this sample.

The-Beckman-DxI-800-prolactin-assay-demonstrates-superior-specificity-for-monomeric-prolactinDownload

Iron deficiency anemia with a twist

An unexpected high TSH in an adolescent near Christmas.

A case of Cryptococcal meningitis with hypomagnesemia

A case of hypomagnesemia and accompanying hypocalcemia with cryptococcal meningitis

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A pepper-pot skull?

HOSP #		**WARD**	General Practitioner Practice in Robertson
CONSULTANT	Dr. Jody Rusch	**DOB/AGE**	83 year Male

Abnormal Result

Serum protein electrophoresis demonstrates a 4.4 g/L, IgG kappa monoclonal peak in the gamma region.

Presenting Complaint

Complains of bilateral hip pain and RUQ discomfort.

History

Atrial fibrillation on Xarelto.

2 x CABG

Examination

RUQ pain and tenderness

Hear rate regular

Laboratory Investigations

Urine protein electrophoresis: No Bence Jones protein

Serum free light chains:·

- Kappa 62.87 mg/L (3.30-19.40)·
- Lambda 19.63 (5.71-26.30)·
- K:L ratio 3.20 (0.26-1.65)

- Creatinine 108 (eGFR 56)
- Calcium 2.42 mmol/l
- Albumin 40 g/L
- Hb 12.7 (11.0-16.0)

Other Investigations

U/S shows gallstones.

X-Ray of pelvis shows “sclerotic changes to both hips and pelvis”

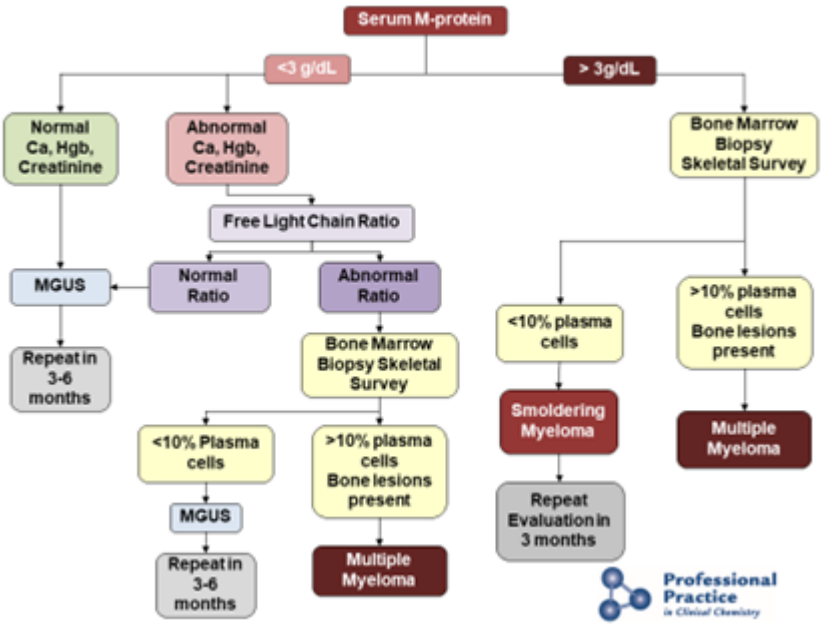
Final Diagnosis and Take Home Message:

1. What is the likely diagnosis

This 83 year old male with multiple co-morbidities presenting with signs and symptoms suggestive of multiple myeloma, confirmed on SPE as IgG Kappa.

- CRAB criteria before performing SPE: C- R+ A- B+ (2/4)
- Bony pain could be secondary to lytic bone lesions associated with MM, but also possibly to due sclerotic/ wear-and-tear when considering his age. RUQ pain is likely due to gallstones.
- Renal impairment – this is probably normal renal function for an 83 year old man
- In medicine generally an eGFR < 60 is representing renal impairment (stage 3)
- However, in monoclonal disease eGFR < 40 or serum Cr > 177 is the cut-point
- Bone lesions – myeloma classically causes lytic bone lesions, e.g. “pepper-pot skull”

It was suggested that the clinician talks to the radiologist as to whether the X-Rays were in keeping with myeloma.



2. Critically discuss whether this patient needs a bone marrow biopsy.

The patient's age along with co-morbidities would concern any drastic intervention:

- he will be an anesthetic risk for BM Bx to be performed in theatre (assuming that is standard procedure), and
- will the BM biopsy give add anything further to the already established IgG Kappa diagnosis, which can be treated accordingly.

The case should ideally be discussed with Oncology. A bone marrow biopsy is done under local anaesthetic. The bone marrow will allow the haematologist / oncologist to assess the degree of marrow clonal infiltration. The important cut-offs are 10 & 60%. This is important to decide on diagnosis, stage, prognosis, treatment and later, the response to treatment. The criteria for doing a bone marrow biopsy at our centre are:

- Positive CRAB·
- IgG monoclonal peak > 15 g/L·

- IgM or IgA monoclonal·
- FLC K:L > 10

Why is there a lower (10%) limit for degree of marrow clonal infiltration? Is there a link to immunoparesis? One likely always has some clonal expansion in bone marrow, probably a normal or a non-pathological finding.

3. Discuss the serum FLC in the setting of the renal impairment.

FLC are filtered and reabsorbed by the nephron under normal circumstances, along with other LMW proteins. During a plasma cell dyscrasia, the nephron is overwhelmed by the amount of FLC (stemming from monoclonal origin), can cause renal impairment. Hence, renal function being part of the CRAB criteria. Furthermore, renal impairment itself (in the absence of MM), can cause elevated Kappa and Lambda FLC – usually with a slight higher ratio =3.2.

In patients with renal failure, there is greater retention of serum free light chains. It is difficult to interpret ratios ranging between 1.65 – 3.0 in the context of renal insufficiency. In such cases, further investigation with a 24-hour urine protein electrophoresis and urine immunofixation helps to guide interpretation. If both of these subsequent studies are normal and the patient has no other symptoms suggestive of a plasma cell dyscrasia, then the increased ratio is likely due to the renal insufficiency.

4. Discuss electrophoresis briefly.

Electrophoresis is a general term that describes the separation of charged particles/ ions under the influence of an electric field – in this case the charge of proteins. Migration of proteins is based on their charge, size and

velocity (product of their mobility and field strength) Make sure you understand why the proteins are charged the importance of NET charge and how we keep those charges stable in the field. If I can take a crack at this: The overall NET charge of the molecule is based on the number of elements (incl. amino acids with varying side-chains moeities) (I think this is the confusion when some mention that electrophoresis is based on charge, and also size. I don't necessarily think that the two are synonymous), and each amino acid has different degrees of charge based on their differing R-group. The stability of the charges within the field is achieved by running the sample solution through a buffer. Right about the buffer. Remember that size and charge are two different physical aspects that you can use to separate molecules. For example, a DNA gel is a separation purely based on size. The net charge is the same on all the molecules. The net charge in proteins is from the side chains, which is why you have to learn about neutral, acidic and basic amino acids. The side chains have different pKa's and so are charged differently.

a. What is the difference between capillary and gel electrophoresis. Explain which your lab uses and why.

What I described in Q4 was basically the concept of gel electrophoresis where agarose gel is used as the medium in which the proteins are separated according to their size, charge, and interaction with the medium itself. At TBH we use gel electrophoresis, but will soon be getting a Minicap/ CZE. CZE: As with gel electrophoresis, CZE also separates ions based on their electrophoretic mobility with the use of an applied voltage – all dependent on the charge of the molecule, viscosity and particle size. CZE's voltage is much higher compared to GE – quicker results. The buffer/ mobile phase of CZE uses an electrolyte filled capillary, where eletro-osmotic flow (EOF) is generated: similarly sized and charged ions move

together and are subsequently separated and detected at different time intervals.The more voltage you apply the faster the separation occurs. However, the limiting factor is that applying high voltages generates a lot of heat which can denature proteins, thereby altering their conformational shape and changing their NET charge. Capillaries are much more effective at shedding heat as they are long and thin. Thus, very high voltages can be applied and the run time is much shorter. In gel electrophoresis, you measure how far the molecules travel in a set time, e.g. 1 hour. In capillary electrophoresis, the distance that the molecules move is set and so you measure the time it takes for the molecules to travel that set distance (like running a 100m race).

The way I reconcile how the CZE differential seperation works is by the

1. driving force of the buffer through the tubing (forward force)
2. negative charge on the side of the tube (retarding force)
3. NET charge on the molecule (many amino acids=higher charge, eg Albumin) (determine degree of retardation of flow)
4. Voltage powers the whole system

5. Why is the serum FLC abnormal but not the urine protein electrophoresis?

UPE’s sensitivity is limited due to the reabsorption of FLC in the renal tubules. FLC in urine will only be detected until loss of tubular function/ tubules are overwhelmed by FLC volumes. This patient’s Kappa FLC of 63 mg/L in serum should be detected on UPE, but tubular function is seemingly still intact with little being excreted.

Some are of the opinion that SPE and SFLC is the preferred method to screen for myeloma because of higher sensitivity and specificity, as opposed to SPE and UPE, which may have a slightly lower sensitivity.

It should however be noted that quoted sensitivities and specificities are usually based on retrospective audits of patients who eventually end up in a myeloma clinic. So, it is not sure what the sensitivity and specificity is if you just screen the general population, older people, people with some vague symptoms…

6. Against which epitopes are the FLC assay directed?

The FLC epitopes are located between the interface between the light and heavy chains and are "hidden" – when bound to Ig, they will not be detected. Only when these epitopes are "free", can they be detected, hence free light chains. They are directed at 2 hidden epitopes.

7. Why is the FLC assay polyclonal and not monoclonal?

The biggest decider many times is COST, but lets put that aside for now.

It appears that polyclonal assays are more robust and have higher yields in product during testing and easier to make. They are unfortunately less specific, but this is not the most critical when one wants to measure the FLC broadly, instead of particlularly specific sites.

Epitopes are three dimensional shapes that the antibody binds to. This is determined by the amino acid sequence. One drawback with polyclonal assays is that lot to lot will vary. The difficulty is to maintain consistency in further production and / or distrubution of the antibody – it is not a

simple process to ensure consistency.

8. Describe how a monoclonal antibody is made for use in an assay.

Inject a rabbit (or other animal) with the protein of choice. In three weeks, the rabbit will have produced antibodies to the protein. The rabbit is sacrificed (killed) and the spleen harvested. The spleen is ground up and the cells are put in a culture with a certain myeloma cell line. The culture medium contains colchicine that induces the rabbit cells and the myeloma cells to fuse. It also contains HAT medium: hypoxanthine, aminopterin and thymidine. This specific myeloma cell line cannot recycle thymidine in the presence of hypoxanthine.

So in the culture there are now three cell lines.

- Firstly, there are the rabbit cells that haven't fused; these will die because they are not immortal.
- Secondly, there is the myeloma cell line, this will also die because of the recycling problem.
- Finally, there is a fused cell line that will survive .

Each of these surviving cell lines will produce one Ig against one part of the protein. Now the researchers take the medium and put a tiny amount into a well. The amount is so small that *on average* each well will contain only one cell; some will of course contain nothing. Then, each well is targeted against the protein and the most promising ones are investigated further. An immortal Ig producing factory directed against one epitope and based on one cell line, a single clone, or as we'd call it a monoclonal, has been produced. Each manufacturer's produced immunoglobulin is different and may produce better or worse results.

9. The GP, in Robertson, wants some advice on how to proceed. What do you tell him?

A multidisciplinary approach would be best:

- Treatment for the lytic bone lesions (after opinion by radiology): bisphosphonate
- Assess overall medication and lifestyle to determine overall risk for worsening renal dysfunction (drugs, co-morbidities……always suggest stopping smoking/drinking)
- Prevention of thrombotic/infective episodes
- Treatment of any further abnormalities should they arise (hypercalcaemia, anaemia etc.)
- Specialist referral:
    - Haematoncologist for treatment of MM: UPE , Bone marrow biopsy
    - General surgery for gallstone

10. Is there any relevance for the RUQ/gallstone pain in myeloma specifically?

There are some reports where cholecystitis has presented in MM (mets etc), but it is not a separate entity on its own (such as in POEMS), this is simply the real world where elderly patients have more than one pathology.