College of Chemical Pathologists of Sri Lanka

CCPSL NEWSLETTER

2020

Issue 04

Cover Story

5th Annual Academic Sessions of the CCPSL

Editors

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Dr H.W.Dilanthi

IN THIS ISSUE

President's Message

Dr Manjula Dissanayake

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Teaching Hospital Karapitiya, Galle

This is the 4th newsletter published by the College of Chemical Pathologists of Sri Lanka (CCPSL). I am happy to announce that this is the first time we are publishing a biannual newsletter, since the appointment of 2019/2020 office and it is a great step forward. On behalf of the CCPSL council, I am grateful to our two editors who made this a reality.

Newsletter is a wonderful opportunity for our members to share knowledge by means of publishing their research, articles on current affairs and express their talents in the form of poems, arts or short stories. We have many young bloods full of talents and creativity in our organization. I do hope they will use this opportunity by writing and expressing their ideas and views at their maximum. I make this an opportunity to invite all our members to cultivate the good habit of writing and sharing them with colleagues.

Our next goal is to commence our own journal and necessary groundwork for this is underway at the moment. I hope that our members would actively contribute in the very first journal of CCPSL and would maintain the enthusiasm continuously to make it a success.

Because of the COVID-19 pandemic almost all scheduled programmes by the CCPSL were cancelled. Nevertheless, we will soon return to our usual rhythm with a new, fruitful set of programmes in the coming months of this year in view of enhancing the educational opportunities for the membership.

Induction of 5th President of CCPSL
&
Inauguration Ceremony of Annual Academic Sessions of CCPSL 2020

The induction of the 5th President of College of Chemical Pathologists of Sri Lanka and the inauguration of the 5th Annual Academic Sessions, 2020 was held on 13th of February, 2020 at the Grand Ballroom, Hotel Hilton, Colombo.

The chief guest for the occasion was Professor Chandrika Wijeyratne, Vice Chancellor, University of Colombo. Dr Samuel Vasikaran, Consultant Chemical Pathologist, PathWest Laboratory Medicine, Western Australia was the guest of honour and Professor Khosrow Adeli, President-Elect, International Federation of Clinical Chemistry and Laboratory Medicine was the special guest for the evening.

Dr Manjula Dissanayake was inducted as the 5th President of CCPSL by the immediate Past President, Dr Gaya Katulanda. The presidential address was delivered by Dr Manjula Dissanayake, focusing on "testing related laboratory errors."

Dr Samuel Vasikaran and Professor Sumedha Wijeratne were awarded CCPSL fellowship in recognition for their service in the field of chemical pathology. Mr K.S. Ramyakumara and Ms Thilanie Wimala Ediriweera were felicitated for their valuable contribution in the field of chemical pathlogy.

The cultural performances by "Nruthyanjalee Dancing Academy" entertained the audience.

The ceremony concluded with a grand reception.


Award of CCPSL
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Dr. Gaya Katulanda
Past President - CCPSL
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5th Annual Academic Sessions of CCPSL

The 5th Annual Academic Sessions of College of Chemical Pathologists of Sri Lanka was held on 14th and 15th February, 2020 at Hotel Hilton, Colombo.

Academic Programme and Medical Laboratory Science Workshop were conducted parallely to address this years' theme of "Transcending Boundaries for Better Health Care". The sessions were attended by over 650 participants. There were 11 international and 31 local resource persons who shared their expertise and experiences. The sessions included 9 plenaries, 7 symposia, 21 guest lectures and a quiz competition. This year almost 40 posters and 6 free papers were presented. Many chemical pathology trainees and consultants participated in the breakfast symposium where overseas speakers participated as resource persons. Grand industrial exhibition was held on both days.

The sessions were concluded on 15th February evening following the award ceremony for the best poster presenters and quiz winners.


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Winners of the awards
5th Annual Academic Sessions of CCPSL

POSTER PRESENTATION (Research Category)

FIRST PLACE

RP 18: A comparison of Jaffe and creatininase methods for serum creatinine as a screening test for renal dysfunction

Siriwardene SC, Perera WNP, Senanayake SW

Department of Biochemistry, Lanka Hospitals Diagnostics, Colombo 5, Sri Lanka

SECOND PLACE

RP 16: Complaint-handling in chemical pathology: facing them is road to prevention

Siriwardene SC[1], Perera WNP[1], Liyanage S[2], Gunawardena S[2]

[1] Department of Biochemistry, Lanka Hospitals Diagnostics, Colombo 5, Sri Lanka

[2] Department of Quality Assurance, Lanka Hospitals Diagnostics, Colombo 5, Sri Lanka

THIRD PLACE

RP 15: The effect of gym training and cycling on albuminuria among gym trainees and professional cyclists – A study from Gampaha district

Kodagoda KIU[1], Wickramarachchi WKDSA[1], Weerarathne LRND[1], Senarathne UD[2,3], Dayanath BKTP[3]

[1] University of Sabaragamuwa, Sri Lanka

[2] University of Sri Jayawardenepura, Sri Lanka

[3] North Colombo Teaching Hospital, Sri Lanka

POSTER PRESENTATION (Case Report Category)

FIRST PLACE

CR 19: Thyrotoxicosis and renal tubular acidosis causing hypokalaemic periodic paralysis

Senarathne UD[1,2], Sajeethan P[3], Dayanath BKTP[2], Premawardhena A [3,4], De Silva ST[3,4], Thilakarathne PMYI[3,4]

[1] Department of Chemical Pathology, Colombo North Teaching Hospital, Ragama, Sri Lanka

[2] Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayawardenepura, Sri Lanka

[3] Professorial Medical Unit, Colombo North Teaching Hospital, Ragama, Sri Lanka

[4] Department of Medicine, Faculty of Medicine, University of Kelaniya, Sri Lanka

SECOND PLACE

CR 10: Acute pancreatitis in a young woman with eruptive skin lesions

Inthujah T[1], Veerendran P[1], Majitha SI[1], Hooper AJ[2], Burnett JR[2]

[1] Department of Biochemistry, Teaching Hospital Batticaloa, Sri Lanka

[2] Department of Clinical Chemistry, PathWest Laboratory Medicine, Royal Perth Hospital and Fiona Stanley Hospital Network, Perth, Australia

THIRD PLACE

CR 08: A young girl with autosomal recessive hypercholesterolaemia

Inthujah T[1], Veerendran P[1], Majitha SI[1], Hooper AJ[2], Burnett JR[2], Thirukkumar V[3]

[1] Department of Biochemistry, Teaching Hospital Batticaloa, Sri Lanka

[2] Department of Clinical Chemistry, PathWest Laboratory Medicine, Royal Perth Hospital and Fiona Stanley Hospital Network, Perth, Australia

[3] Paediatric Unit, Teaching Hospital Batticaloa, Sri Lanka

QUIZ COMPETITION

FIRST PLACE

- Ms Usha Nanthini Aiyadurai
  International Organization for Migration

SECOND PLACE

- Ms K.A.L.G. Karunarathne
  Durdans Laboratory, Kalubowila

THIRD PLACE

- Ms E. Lakmalee Sanjeevanie Zoysa
  Sachitra Hospital, Panadura

Importance of selective venous sampling in occult endocrinopathies

Dulani Jayawardana, Fasilaas Kiyamudeen
Department of Chemical Pathology, National Hospital, Kandy

Selective venous sampling serves as the gold standard tool for the diagnosis, pre-operative localization and lateralization of hypersecreting endocrine pathologies when conventional imaging failed to locate the lesion.[1] This makes minimal access surgeries possible and reduces peri and post-operative morbidity and mortality. This is a multi-disciplinary approach where departments of endocrinology, interventional radiology, surgery and chemical pathology work together. Although department of interventional radiology plays a major role, department of chemical pathology also has a critically important part in issuing accurate reports of these highly precious blood samples.

There are several situations in which selective venous sampling is helpful.

- Adrenal venous sampling (AVS) - localizes autonomous aldosterone secretion in primary aldosteronism and differentiates unilateral pathology from bilateral. [1,2]
- Parathyroid venous sampling (PVS) - locates ectopic or missed parathyroid adenoma. [1,2]
- Bilateral inferior petrosal sinus sampling (BIPSS) confirms Cushing disease and lateralizes the lesion. [1,2]
- Intra-arterial calcium stimulation (IACS) and hepatic venous sampling - localize occult insulinoma. [1,2]
- Ovarian venous sampling - used in the diagnosis of androgen secreting tumours. [1,2]

Herein we report three case summaries where selective venous sampling played a major role in the management of patients at National Hospital, Kandy.

1. Parathyroid venous sampling and primary hyperparathyroidism

A 23-year-old unmarried man was admitted to a surgical ward with acute abdomen and diagnosed to have acute necrotizing pancreatitis in June, 2017. It was his second admission with acute pancreatitis. He was non-alcoholic, had past history of constipation and was not on any continuous medication. His mental status and physical examination were unremarkable. The results of biochemistry tests in blood were as follows,

Test	Result	Reference range
Total calcium	3.12	2.15 – 2.55 mmol/L
Phosphate	0.75	0.81 – 1.45 mmol/L
Alkaline phosphatase	144	42 – 98 IU/L
Intact PTH (iPTH)	212	15 – 75 pg/mL

In this patient, hypercalcaemia with inappropriately elevated PTH level, absence of history of long term medication of drugs like thiazide or lithium and no history of renal disease suggest the diagnosis of primary hyperparathyroidism. Common causes for primary hyperparathyroidism are sporadic solitory adenoma (85%), multiglandular parathyoid hyperplasia (15%) and parathyroid carcinoma (<1%).[3] Adenomas may be found in ectopic locations in about 16% of cases- commonly the thymus, tracheo-oesophageal groove, mediastinum and the thyroid.[3] In our patient, ultrasound scan (USS) of the neck revealed a 6 mm sized nodule in the mid pole of the right lobe of thyroid gland and failed to locate any masses in the parathyroid gland. However, TC99 Sestamibi scan was reported as no scintigraphic

evidence of abnormal focal retention of tracer in parathyroid region of the neck or elsewhere in ectopic location. However, hyperplastic parathyroid glands could not be ruled out. Four gland exploration was done suspecting parathyroid hyperplasia. However, during surgery only three glands were identified except right superior gland. Two and a half glands were excised by the surgeon. Histology of the removed parathyroid glands revealed normal parathyroid tissue. Post-operative biochemistry revealed;

Test	Result	Reference range
Serum calcium	2.84	2.15 – 2.55 mmol/L
Intact PTH	104	15 – 75 pg/mL

Further evaluation was required as hypercalcaemia was persistent post-operatively. Repeat USS of the neck revealed a 5 mm sized hypo-echoic nodule within the right thyroid lobe and the repeat TC99 Sestamibi scan revealed a focal abnormal uptake in the region of the right lobe of thyroid bed. However, FNAC from the suspicious lesion showed only thyroid follicular cells.

This warranted the need for PVS. Selective catheterization was done in to superior, middle and inferior thyroid veins, internal jugular, subclavian, innominate, azygous, thymic, superior intercostal veins and in right atrium. PTH concentration was measured in each sample. In the interpretation of the results, elevation of iPTH in selected veins as compared to peripheral venous sample is helpful for regionalizing the parathyroid gland.[2] Ratio of 3-fold increase in PTH has a positive predictive value of 83%.[1,2]

Summary of our patient's biochemical test results are,

Site	PTH (pg/mL)
Right middle thyroid vein	5000
Femoral vein	189.7
Ratio	26.4

It was biochemically proven that the culprit might be the hyperfunctioning superior parathyroid gland which was missed during the surgery. He underwent re-do parathyroidectomy. Intra operative USS of the neck revealed a deep-seated superior parathyroid gland in the right thyroid bed which was excised and parathyroid adenoma was proven histologically.

2. Insulinoma and selective intra-arterial calcium stimulation test

A 29-year-old man presented with three episodes of early morning unresponsiveness associated with excessive sweating over six-month duration. His wife has noticed these episodes after skipping of his dinner and he recovered when she served him a cup of tea. He was not on any medication. He was investigated for recurrent hypoglycaemia. Adrenal insufficiency and hypothyroidism were ruled out with normal 9 am serum cortisol and TSH levels. During his 72-hour observed fast test, he developed hypoglycaemic symptoms at plasma glucose level of 47 mg/dL at around 12 hours of fast, at that point blood was taken for serum insulin and serum C-peptide levels.

Test	Result	Reference range
Plasma glucose	47	mg/dL
Serum insulin	194.39	< 18 pmol/L
Serum C-peptide	1.27	< 0.2 nmol/L

Serum insulin level of >18 pmol/L (3 µIU/L) and serum C-peptide level of >0.2 nmol/L (0.6 ng/L) are diagnostic of endogenous hyperinsulinism when the plasma glucose is <55 mg/dL (3 mmol/L) or at symptoms or signs of hypoglycaemia.[4] Serum or urine sulfonylurea screening was not done due to unavailability of the test. USS abdomen did not reveal any pancreatic lesion. Computed tomography with contrast (CECT) reported as benign calcification in the segment VIII of the liver, probably a calcified granuloma with no CT evidence of insulinoma of the pancreas. The negative CT scan result warranted

further evaluation. Endoscopic ultrasound scan (EUS) of the pancreas revealed 20 mm, hypo-echoic lesion adjacent to the portal confluence and on the left side of the splenic vein. No other lesion in the pancreas from head to tail and uncinate process of the pancreas were found. Negative CECT and equivocal EUS led to further evaluation for the highly suspected insulinoma with selective intra-arterial calcium stimulation in major pancreatic arteries and hepatic venous sampling for insulin and c-peptide. The stimulation was done in the order of proper hepatic (PHA), common hepatic (CHA), gastro duodenal (GDA), distal splenic (DSA), proximal splenic (PSA) and superior mesenteric (MSA) arteries (figure 1) and samples were collected from the hepatic vein at -120, 0, 30, 60, 90, 120 and 180 seconds of the calcium gluconate infusion. The result of insulin (figure 2) and C-peptide (figure 3) concentration in relation to the time of calcium infusion in each major artery are represented graphically. Two-fold step up of insulin concentration at any point, from the basal value suggests the location of the lesion in the territory of the particular arterial supply.[2]

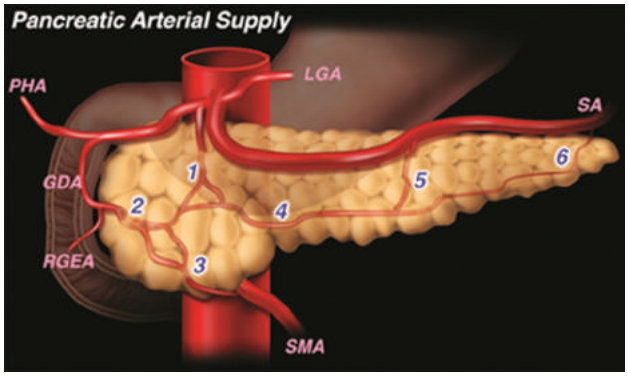

Figure 1: Normal pancreatic arterial supply. The proper hepatic (PHA), gastroduodenal (GDA), left gastric (LGA), right gastroepiploic (RGEA), and superior mesenteric (SMA) arteries give rise to the following arteries: 1 = dorsal pancreatic, 2 = superior pancreaticoduodenal, 3 = inferior pancreaticoduodenal, 4 = transverse pancreatic, 5 = pancreatica magna, 6 = caudal pancreatic. (Adapted

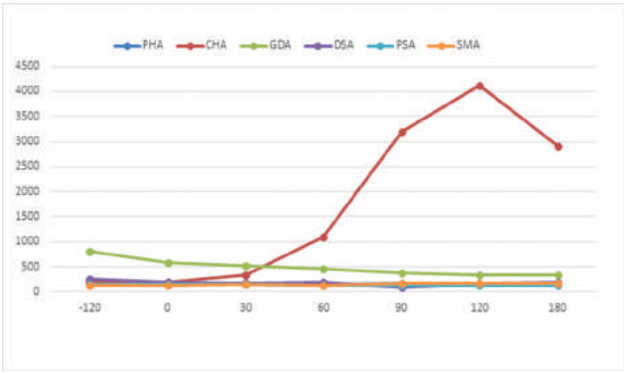

Figure 2: Insulin concentration (pmol/L) vs time (s)

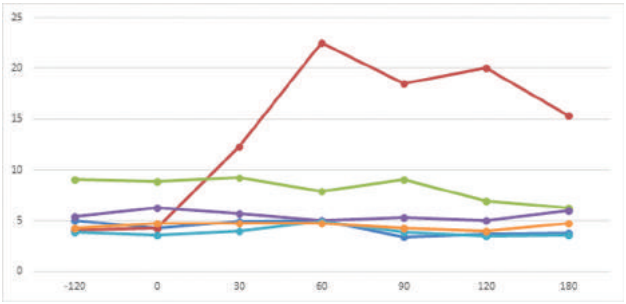

Figure 3: C-peptide concentration (nmol/L) vs time (s)

IACS test is based on the stimulation of insulinoma cells by calcium and secretion of stored insulin in to the circulation. In this patient, it was taken as possibly high insulin secretion seen following the stimulation of the GDA which might be happened at the time of injecting calcium in the CHA, because common hepatic artery is giving away the PHA and the GDA. However, the same response was not seen at proper stimulation of GDA, that may be explained by the biphasic nature in secreting insulin following a physiological stimulus, which is glucose and the stimulus is mediated via calcium dependant pathways and exocytosis of insulin granules is elicited by burst of calcium dependant action potentials.[5] First phase is rapid and transient whereas the second phase is sustained.[5] However, it is not known whether particular concept is applicable for the tumour cells also. In this case, it was considered that bi-phasic nature of secretion of insulin is the reason for the absence of response

for the second stimuli in a short while and it was taken as the tumour might be in the territory of GDA. It gave a path for searching the tumour in the head or neck or its close proximities. During surgery, with bimanual palpation a solitary tumour was found in the proximal body of the pancreas, extending anterior to posterior surface. He was offered spleen-preserving distal pancreatectomy. Histology revealed a well-differentiated, grade 1 neuroendocrine tumour with cytoplasmic positivity with synaptophysin and chromogranin in immunohistochemistry.

3. Cushing disease and bilateral petrosal sinus sampling

A 29-year-old unmarried female presented with weight gain, hirsutism and secondary amenorrhoea. She is a known diabetic for 1-year duration and was on oral hypoglycaemic agents but not on any other long term medication. She denied any history of headache, visual disturbances or galactorrhoea. On examination, her BMI was 27 kg/m^2 and had cushingoid features like plethora, round face, truncal obesity, thin skin and proximal myopathy. Her blood pressure was 160/100 mmHg. A clinical diagnosis of Cushing syndrome was made and her biochemical tests were as follow :

Test	Serum cortisol (nmol/L)	Normal response
Overnight dexamethasone suppression test (ODST)	835	<50 [6,7]
Low dose dexamethasone suppression test (LDDST)		
D_1	812	<50 [6,7]
D_3	712	

Both ODST and LDDST were not suppressed, making biochemical confirmation of the clinical diagnosis of Cushing syndrome. Other screening tests for Cushing syndrome, 24-hour urinary free cortisol and midnight salivary cortisol were not done due to the unavailability of these tests. On the evaluation of anterior pituitary hormones, ACTH was 123 pg/ml (6 – 48). This favoured the diagnosis of ACTH-dependant Cushing syndrome. At this point Cushing disease should be differentiated from ectopic ACTH secretion. She underwent high-dose dexamethasone suppression test (HDDST) and the serum cortisol levels were as follow,

Test	Serum cortisol	Interpretation
D_1	569 nmol/L	
D_3	99 nmol/L	>50% reduction from the baseline favours Cushing disease [6,7]

More than 50% reduction of serum cortisol level following the HDDST favours the diagnosis of Cushing disease. [6,7] On imaging, MRI brain revealed 8 x 8 x 6 mm lesion in the right side of the pituitary, compatible with pituitary micro adenoma. To confirm that lesion identified by MRI is hyperfunctioning, BIPSS was done which revealed that the adenoma of the pituitary is the probable culprit.

Site	ACTH (pg/mL)
Right inferior petrosal sinus (R/IPS)	1057
Left inferior petrosal sinus (L/IPS)	215
Right femoral	87
R/gradient(IPS/femoral)	12.1 (<2)
L/gradient	2.48
R/IPS : L/IPS	4.9 (<1.4)

In BIPSS, central-to-peripheral ACTH ratio 2:1 or higher confirms Cushing disease [1,2,7,8] and interpetrosal sinus ACTH gradient of 1.4 or higher allows the prediction of lateralization.[2,8] In our patient, Cushing disease was confirmed with BIPSS and the culprit would be the right pituitary adenoma. She underwent endoscopic transsphenoidal adenomectomy (TSA) and histology revealed benign pituitary adenoma with the expression of ACTH and GH in immunohistochemistry.

Conclusion

In all three cases, selective venous sampling favoured the precise pre-operative localization of the tumour which led to minimal access surgeries. As a member of the team involved with selective venous sampling, it is important to be present at the time of intervention to receive, collect, label and transport the samples to the laboratory in a timely manner as per the standard operating procedure of the particular analyte and proper storage to avoid pre-analytical errors. Well-organised specimen handling is mandatory, otherwise it would end up with a "wrong side" surgery. As a laboratory professional, preparation of the laboratory in advance and giving proper instructions to relevant parties and laboratory technicians are also important in producing quality and accurate reports.

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Role of sFlt-1 and PlGF in preeclampsia

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Preeclampsia (PE) is a pregnancy complication which has a detrimental effect on both mother and the foetus. Its prevalence is around 2 to 8 % worldwide.[1] It is a multifactorial disorder which is thought to be due to abnormal placentation. However, its pathogenesis is not well established.

Preeclampsia is characterised by new onset of high blood pressure after 20 weeks of pregnancy and proteinuria of 30 mg/mmol, a threshold which is considered as significant proteinuria. The signs and symptoms of preeclampsia include severe headache, blurred vision, severe epigastric pain, sudden swelling of face, hands or feet, oliguria and clonus.

Placental growth factor (PlGF) is a potent angiogenic factor and in normal pregnancy PlGF level increases and it peaks at 26 to 30 weeks.[2] Soluble fms-like tyrosine kinase-1 (sFlt-1) is a protein which is thought to inhibit proteins associated with blood vessel formation. It is responsible for peripheral vasoconstriction causing high maternal blood pressure. It is found that in women who develop PE the levels of sFlt-1 are higher than those seen in normal pregnancy. In contrast to this PlGF is markedly reduced as they are captured and inhibited by sFlt-1. The imbalance of these products plays a major role in the pathogenesis and clinical manifestations of PE[3].

The prompt diagnosis and accurate management of PE are always challenging as diagnostic criteria are still based on non-specific signs and symptoms. New onset hypertension and proteinuria do not always precede the onset of complications. Furthermore, the severity criteria do not correlate well with adverse maternal and foetal outcomes and therefore the course of the disease is unpredictable.[4] There is evidence that majority of adverse outcomes occurred in women who did not fulfil the classical definition of PE or have risk factors.[5] These limitations may lead to diagnosis of PE in an advanced stage of the disease preventing optimal management and potentially resulting in severe maternal and perinatal complications.

The sFlt/PlGF ratio has been shown to be a useful tool to detect true risk of developing PE among women presenting with clinical suspicion of PE. The recent prospective multicentre observational PROGNOSIS study conducted in 14 countries assessed whether the sFlt/PlGF ratio is predictive of absence or presence of PE in women with suspicion of PE between 24 to 36 plus 6 days weeks of pregnancy and it showed that a sFlt/PlGF ratio of 38 can rule out PE in the subsequent week as its negative predictive value (NPV) was 99.3% (95% confidence interval, 97.9 to 99.9 %) and the likelihood of developing PE in next 28 days is only <3%.[6,7] However, the positive predictive value of (PPV) sFlt/PlGF ratio of >38 to rule in PE within the next 4 weeks was 36.7% with a sensitivity and specificity of 66.2 and 83.1% respectively.[6]

In women with sFlt/PlGF ratio between 38 and 85 the likelihood of developing PE in the next 7 days is only 20%.[7] Therefore, if there is no evidence of significant proteinuria or end organ dysfunction it is unnecessary to hospitalise them for suspected PE.

On the other hand the cut-off of the ratio, >85 has shown to be useful in accurate diagnosis of early onset PE with a high specificity of 99.5%.[8] The likelihood

of these women developing PE in the next 7 days is 56%.[7] There is evidence that if the value was >85 delivery occurred within 2 weeks in 86% compared to 15.8% of women with value <85.[9]

Before 34 weeks the sFlt/PlGF ratio does not increase significantly in pregnant women with chronic hypertension or gestational hypertension compared to normotensive pregnant women. However after >34 weeks of gestation a considerable rise in the sFlt/PlGF ratio was seen in hypertensive pregnant women without PE, but only <10% had >110.[10] It is also found that a ratio of >655 is associated with a high risk of delivery within 48 hours.[6] In this category only 29.9% and 5.9% women remained pregnant more than 2 days and 7 days respectively.[10]

The PELICAN study was the first and largest prospective study conducted in seven maternity units in UK and Ireland with the aim of evaluation of PlGF which showed that the PlGF test alone had a very high accuracy for predicting PE needing delivery within 14 days for women presenting with suspected PE between 20 and 34 weeks of gestation. For a cut-off of 100 pg/mL PlGF had sensitivity and specificity of 96% and 56% respectively resulting a PPV of 44% and a NPV of 98%.[11] It revealed that PlGF at 100 pg/mL was a better predictor of PE compared to other commonly used predictive tests as it had a 0.87 of area under the curve compared to 0.76 of the next best predictor.[11]

The PARROT was the first multicentre stepped wedge cluster randomised controlled trial conducted in 11 maternity units in the UK to assess clinical utility of PlGF based testing in PE suspected women aged 18 years and above presented between 20 to 36 plus 6 days weeks of gestation with a singleton foetus. They found a significant reduction in time to diagnose PE compared to routine clinical care. Therefore, maternal severe adverse outcomes declined considerably. However, there was no difference in gestational age at delivery or adverse perinatal outcomes.[12]

In PROGNOSIS study, Elecsys immunoassay (Roche diagnostics) was used to quantify both PlGF and sFlt. The sFlt/PlGF ratio is formed by combining the results from sandwich electrochemiluminiscence immunoassays which are compatible with both the Roche Elecsys and the Cobas e automated analysers. The sFlt-1 assay has a LoD of 10 pg/mL and LoQ of 15 pg/mL with a measuring range of 10 to 85000 pg/mL. The PlGF assay has LoD and LoQ of 3 and 10 pg/mL respectively and the measuring range is 3 to 10000 pg/mL. The turnaround time is around 18 minutes.

In PELICAN study PlGF was measured using the Alere Triage PlGF test (Alere International). It is a fluorescence immunoassay used with the Triage MeterPro point of care analyser. This test quantifies PlGF in plasma samples and it has a LoD of 9 pg/mL with a measurable range of 12 to 3000 pg/mL. The test turnaround time is about 15 minutes.

National Institute for Health and Care Excellence has recently published guidance on in-cooperation of Elecsys immunoassay sFlt/PlGF ratio and Triage PlGF testing to be used with standard clinical assessment to rule out PE for women presenting with suspected PE between 20 and 34 weeks plus 6 days of gestation.[2] However, it does not advise these tests to be used to diagnose PE until further research is available.[2]

It is evident that a growing number of women with PE suggestive symptoms and or signs will not develop any complication of pregnancy, but they are often hospitalised until PE and related adverse outcomes are ruled out. They undergo standard evaluation and monitoring which amounts to a considerable cost derived from hospitalisation, laboratory analysis and foetal well-being tests. Therefore, the introduction of PlGF based tests can save health care costs. The savings are due to the test's ability to classify patients reliably compared to current practice as its ability to reduce false negatives and false positives. Furthermore immediate delivery of the fetus is also

reduced which results in fewer premature babies needing neonatal intensive care unit. It is found that in UK, implementing sFLt/PLGF ratio into current diagnosis algorithm results in cost saving and it was about GBP 344 per patient.[13]

In summary, the sFlt/PlGF ratio has been shown to have a strong predictive and diagnostic value for PE and its implementation saves cost.

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Principles and clinical applications of Liquid Chromatography-Mass Spectrometry

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Introduction

Liquid chromatography-mass spectrometry (LC-MS/MS) is an emerging laboratory technique in Sri Lanka. It combines liquid chromatography (or HPLC) with the mass spectrometry. LC-MS/MS is a powerful analytical technique with a wide range of clinical applications, including therapeutic drug monitoring (TDM), toxicology, endocrinology, paediatrics, microbiology and the field of proteomics.

Mass spectrometry (MS) is a century old analytical technique. Its basic principles were first defined by Nobel winner Sir Joseph John.[1] The coupling of MS with liquid chromatography (LC) (LC-MS) was incomplete for many years primarily due to the relative mismatches of existing MS ion sources with the liquid stream. Several interfaces were trialed but they were cumbersome to use in day-to-day practice.[2]

This situation was improved with new development of the electrospray ion source by Fenn in the 1980s.[1]

LC-MS technique is more applicable than gas chromatography-mass spectrometry (GC-MS) due to the wider range of biological molecules that can be analysed and the greater use of LC separations in clinical laboratories.[2]

Instrumentation

Liquid chromatography-mass spectrometry (LC-MS)

The LC-MS is a combination of LC and MS and is used with separation power of HPLC with detection power of MS.[2]

Liquid chromatography

The LC is a high performance liquid chromatography (HPLC).[2] Separation of components of mixture is carried out by using liquid mobile and solid stationary phases. There are different types of chromatography such as normal phase liquid chromatography, reversed phase chromatography, ion-exchange liquid chromatography, chiral separation and affinity liquid chromatography.[1]

The components of HPLC are;
Pump: It delivers high volume of mobile phase sample injector. It introduces sample volume into the chromatographic system.[1]

Columns: It is stationary phase which consists of silica material in combination with carbon chain. The columns used in HPLC consist of octadecyl (C18), octyl (C8), cyano, amino and phenyl packing's.[1]

Mass spectrometry

The basic principal of MS is to convert the analyte of interest molecules to a charged (ionised) state.[1] MS subsequently detects ions and any fragment ions that are produced during the ionization process. This is based on mass to charge ratio (m/z) of analyte.[2] Sample is introduced through an inlet to ion source in MS. Mass analyzer and detector are placed in a vacuum.[2] (Figure 1)

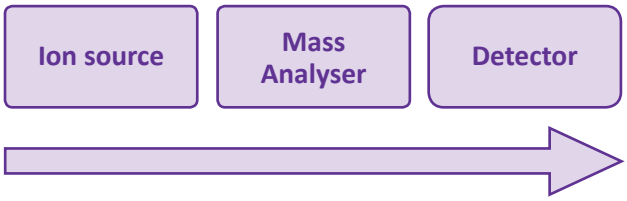

Figure 1: Structure of mass spectrometer

Sample inlet

The sample inlet in LC-MS/MS mediates the transition of the sloid or liquid phase of a biological specimen into its gaseous phase. This transition is essential to proceed with the subsequent steps of mass analysis.[2]

Ion sources

Electro spray ionization (ESI)

ESI charges the liquid first and then turns the liquid into gases.[1] ESI works well with moderately polar molecules and is appropriate for analysis of many metabolites, xenobiotics and peptides.[1] ESI is a gentle technique with good sensitivity. It is susceptible to matrix effect.[1]

Atmospheric pressure chemical ionization (APCI)

APCI converts liquid into gas and charge the gas via corona discharge needle. APCI is better for less polar compounds than ESI. It is compatible with high flow rates.[1]

Mass analysers

Types of mass analysers

Quadrupole

Ion trap

Time-of-flight

The quadrupole analyser consists of a set of four parallel metal rods (Figure 2). Ions will oscillate within metal rods and the amplitude of oscillation is unique for particular m/z ratio. Ions can be induced to undergo fragmentation by collisions with an inert gas such as nitrogen or argon.[1]

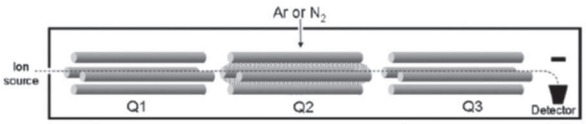

Figure 2: Triple quadrupole analyser

A number of different modes of operation are available for triple quadrupole mass spectrometers, namely precursor, product, neutral loss and multiple reaction monitoring.[2] The triple quadrupole mass spectrometer is an example of tandem MS. In Tandem MS specificity is greatly increased over single mass analysis.[1]

Ion trap

This has three hyperbolic electrodes to trap ions in a three-dimensional space using static and radio frequency voltages. Ion trap mass analyser has high resolution and high sensitivity.[2] It has multiple product ion scan capability.[1]

Time-of-flight

The time-of-flight (TOF) is the most robust mass analyser. It is used for wide range of ions sources and inlet systems.[2] TOF functions without a magnetic field; maintenance and calibration are easy and straight forward.[1]

Detectors

The detector produces the current that is proportional to the number of ions strike it.[1] Commonly used detectors are point ion collector detector and array detector.[2]

Applications

Biochemical screening for genetic disorders

LC-MS/MS is the leading technology used in paediatric laboratories for newborn screening programs due to its versatility, sensitivity, and specificity. It is used to measure acylcarnitines for medium chain acyl CoA dehydrogenase deficiency (MCADD) and other amino acid disorders like

phenylketonuria.[2] Further, it is used to detect maple syrup urine disease, methylmalonicacidaemia and 17α-hydroxyprogesterone level in 21-hydroxylase deficiency. LC-MS/MS is used to screen for disorders of porphyrin, purine and pyrimidine, peroxisomal and bile acid metabolism.[1]

Therapeutic drug monitoring and toxicology

Assays have been developed to detect immunosuppressants such as cyclosporin, tacrolimus, sirolimus, everolimus and mycophenolic acid. Further, antiretrovirals and anticancer drugs detection have also been developed.[2]

Vitamins and related metabolites

LC-MS/MS is used to detect vitamin D related 1,25- and 24,25-dihydroxy metabolites, fat-soluble vitamins such as various forms of vitamin E and vitamin K.[1]

LC-MS/MS detect total homocysteine and methylmalonicacid which are important functional indicators of vitamin B_{12} status.[2]

Steroid hormones

Difficulties with the measurement of low testosterone and dihydrotestosterone levels found in women and children using conventional immunoassays is overcome by using LC-MS/MS.[2] Assays for urine free cortisol, oestrogens and adrenal steroids have also been developed.[1]

Field of proteomics

Key biomarkers for immunity, autoimmunity, cancer detection, and immune system function are some of the proteomics detected by LC-MS/MS.[2]

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Utility of biochemical markers in COVID-19

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Corona Virus Disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus primarily invades type II pneumocytes in the lungs, by binding to its receptor, angiotensin-converting enzyme 2 (ACE2) in the cell surface. Following endocytosis of the virus-ACE2 complex, ACE2 is down regulated.[1] Subsequent unopposed angiotensin II accumulation leads to local activation of the renin–angiotensin–aldosterone system with massive cytokine activation resulting in lung injury.[1,2] ACE2 is highly expressed in cells of lungs, heart, kidneys and the gastrointestinal tract and this is reflected by the clinical presentation and changes in biochemical markers in patients with COVID-19.[1,3]

The number of infected patients is rapidly increasing worldwide because of certain features of the infection such as transmission from person-to-person through respiratory droplets, the high affinity of the spike glycoprotein of the virus for ACE2 in human host cells, infectivity in the latent period, asymptomatic infection, atypical clinical symptoms and insufficient attention in the early stages.[4]

The case identification is performed by SARS-CoV-2 RNA detection in nasopharyngeal swab by real-time PCR. Serological assays to detect IgG/IgM have emerged as tools to trace contacts, activate serological surveillance and identify those who have had contact with virus.[5]

While the diagnostic tests are carried out to detect infected patients, biochemical tests have played a significant role in detecting and managing patients with complications and risk stratification. Some hospitalized patients with COVID-19 have developed acute kidney injury, acute cardiac injury, and liver damage leading to multi organ failure.[3] The severity of multiple organ dysfunction may be reflected by the abnormalities in biochemical parameters measured in blood.

Zhou et al present that cardiac complications, including new or worsening heart failure or arrhythmia, or myocardial infarction were common in patients with pneumonia. Increased high-sensitivity cardiac troponin I (TnI) during hospitalization was found in more than half of those who died.[6]

Greater degrees of TnI elevation is associated with higher mortality rate in hospitalized patients.[7] Interestingly markers of inflammatory response, such as C-reactive protein (CRP), IL-6 and leukocytes were significantly increased among patients with cardiac injury. The activation or enhanced release of these inflammatory cytokines can lead to apoptosis or necrosis of myocardial cells.[7] Guo et al reveal that TnT levels are significantly associated with not only levels of CRP but also with N-terminal pro-B-type natriuretic peptide (NT-proBNP), which reflects the association of myocardial injury with severity of inflammation and ventricular dysfunction.[8]

Identified risk factors for cardiac events were older age, pre-existing cardiovascular disease and severe pneumonia at presentation. [6,7,8]

Contributory factors for cardiac injury include systemic pro-inflammatory cytokine responses that are mediators of atherosclerosis directly contributing to plaque rupture through local inflammation, induction of procoagulant factors and haemodynamic changes, which predispose to ischaemia and thrombosis. In addition, ACE2 is expressed on myocytes and vascular endothelial cells, hence potential possibility of direct cardiac involvement by the virus is described.[9]

Higher AST and ALT in one fifth of patients with COVID-19 and total bilirubin levels in 6% of patients with severe COVID-19 are reported in a metaanalysis.[4] According to a comprehensive study on COVID-19, the available data supports a higher prevalence of abnormal aminotransferase levels in severe COVID-19 disease, but clinically significant liver injury is uncommon, even when data for the most severely ill patients are selected.[10] While the mechanism of elevated liver enzymes is not fully understood, the authors suggest the injury can be because of virally induced cytotoxic T cells and induction of dysregulated innate immune response, myositis, or drug induced hepatotoxicity. The mild derangement of liver enzymes, lack of evidence that later presentation is associated with greater liver enzyme derangement and only microvesicular steatosis, a common finding in sepsis, being identified in postmortem liver-biopsy provide little evidence for direct liver injury caused by COVID-19 viral hepatitis.[10] Other respiratory viruses also produce similar liver enzyme abnormalities related to immune mediated hepatocyte damage.

The incidence of acute kidney injury (AKI) among patients with COVID-19 is as high as 15%.[11] Cheng et al report that AKI is more common among patients with more severe disease.[12] Hence AKI is considered as a negative prognostic factor with respect to survival. The suggested mechanisms for acute kidney injury are systemic inflammatory immune response, in the presence of a cytokine storm, contributing to hypoperfusion-related injury of the renal tubules and direct effect of SARS-CoV-2 on podocytes and tubular epithelial cells where ACE2 is abundant. [13]

Liu et al describe the usefulness of urine biochemical tests in identification and evaluation of the dynamic changes in progression of COVID-19.[14] The positive rates of urine occult blood and protein in COVID-19 patients were higher than those in healthy controls.[14] According to Bonnett et al the frequent presence of granular cylinders and tubular cells was discovered in the urine of patients who died due to COVID-19.[15] The rate of abnormal urea and creatinine values at admission in patients who died (i.e. between 75% and 80%) was high compared to those who survived (i.e. between 20% and 24%).[13]

Utility of lactate dehydrogenase (LDH) has been suggested as another prognostic marker to identify patients with COVID-19 who are likely to develop severe illness. According to Deng et al, more than half of patients with COVID-19 had increased LDH levels in the meta-analysis, and the LDH levels of severe patients were higher than those of non-severe patients.[2]

A cytokine profile resembling a secondary hemophagocytic lymphohistiocytosis is associated with COVID-19 disease severity. The profile is characterized by increased interleukin IL-2, IL-7, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α.[16] Ferritin has been identified as a predictor of fatality in a retrospective, multicenter study of 150 confirmed COVID-19 cases in Wuhan, China. Mean ferritin level of 1297 ng/ml in non-survivors vs 614 ng/ml in survivors is reported ($p<0001$).[17] Henry et al suggest to use markers of virally driven hyperinflammation such as IL-6 and ferritin levels for predicting prognosis in patients with COVID-19 over the period of hospitalization.[18]

Prospective multiethnic studies with more careful designs, larger sample sizes, including wide range of biochemical markers like vitamin D, procalcitonin are needed to further establish the association between biochemical indices and COVID-19 and to develop risk stratification models.

In conclusion, biochemical investigations seem to be candidate markers for prognosis and risk stratification and can be used to predict progression to critical illness in COVID-19.

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Symposium on "Chemical Pathology Testing for Pregnancy and Related Disorders" was held on 27th January 2020 at SLCOG auditorium

A case of carcinoma of unknown primary

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Case Presentation

A 56-year-old, previously healthy lady, presented with a left sided neck lump for 2-month duration. She had a strong family history of carcinoma. On examination, there was a left sided neck lump suggestive of an enlarged lymph node and a small, non-tender, soft lump was detected at her right breast which was not attached to the underlying structures.

Investigations

All the general biochemical investigations were normal.

Tests	Results	Unit	Reference ranges
Creatinine	87.4	µmol/L	71 – 115
AST	21	U/L	0 – 40
ALT	18	U/L	0 – 45
ALP	218.7	U/L	40 – 270
LDH	202	U/L	135 – 214

Tumour markers at the time of diagnosis:

Test	Result	Unit	Reference range
CA125 (At diagnosis)	482	U/mL	<35
CA125 (Pre op)	1161	U/mL	<35
CEA	0.89	ng/mL	<2.5
AFP	3.61	IU/mL	<7.2
CA 19.9	5.18	U/mL	<37

Question 1

What is the most probable diagnosis?

Answer 1

Primary epithelial ovarian neoplasm

Question 2

What are the other causes of elevated CA125?

Answer 2

- Epithelial ovarian cancer, carcinoma of the fallopian tube, endometrial, endocervical adenocarcinoma
- Serous peritoneal carcinoma
- Pancreatic and breast carcinoma
- Non-malignant conditions- endometriosis, cirrhosis, pelvic inflammatory disease and first trimester of pregnancy[1]

Question 3

Discuss further investigations needed to arrive at a diagnosis.

Answer 3

- CT scan chest, abdomen and pelvis
- Whole body PET scan
- Lymph node biopsy and immune histochemistry (IHC)
- Peritoneal washings for cytology
- Other histological investigations relevant to clinical picture

Findings:

- CT scan revealed supra clavicular, mediastinal, para aortic and retro caval lymph node masses with no evidence of a primary lesion.
- PET scan was suggestive of a right ovarian neoplasm.
- Biopsy of the lymph node revealed poorly differentiated adenocarcinoma.
- IHC revealed positivity for Cytokeratin (CK) 7 and negativity for CK 20, TTF1 and CD 20, possible primary sites are female genital tract, pancreas and the stomach.
- Peritoneal washings for cytology did not show malignant cells.
- Bilateral mammography, fine needle aspiration biopsy of the right breast lesion and endoscopic biopsies were normal.

Question 4

What are the possible primary sites?

Answer 4

Female genital tract including ovary, fimbriae and fallopian tube

Question 5

What would be the best management option?

Answer 5

Total abdominal hysterectomy and bilateral salphingo oophorectomy (TAH + BSO)

TAH + BSO done, however the histology was unremarkable. Therefore, it was diagnosed as carcinoma of unknown primary (CUP).

CA125	Value	Reference range
Post-op	1810	<35 U/mL
Post-chemotherapy	7.1	<35 U/mL
Follow up	5.9	<35 U/mL

The patient was started on systemic chemotherapy with the probable diagnosis of the primary site in female reproductive tract.

CA125 normalized after the third chemotherapy cycle and currently the patient remains well.

Question 6

What is CUP?

Answer 6

CUP is a diverse group of cancers for which a standard diagnostic workup fails to identify the site of origin at the time of diagnosis.[2]

Question 7

What is the pathophysiology of CUP?

Answer 7

- Metastasis can occur without the growth of a primary tumour due to the inherent metastatic aggressiveness of cancer cells.
- The primary tumour may be minute, escaping the clinical detection.
- The primary tumour may be disappeared or eliminated by body's defense mechanisms after metastasis has seeded.
- CUP metastases may be having increased survival relative to the primary tumour.[1]

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An elderly patient with renal failure, hypercalcaemia and pancytopaenia

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Case Presentation

A 70-year-old male presented to the medical ward with a history of fever, headache, confusion and reduced urine output for 1-day duration. He had a history of loss of appetite and loss of weight for 2-weeks and backache for 3-months. On examination, he was pale, confused and had flapping tremors. Other system examinations were unremarkable. Two weeks prior he has been treated for dengue fever.

He has been recently diagnosed as having hypertension and chronic kidney disease (Stage III b, baseline serum creatinine 150 µmol/L) with normal HbA_{1c} and lipid profile.

Analyte	Results	Unit	Reference Interval
White Blood Cell Count	1.54	10^9/L	4.00 – 10.00
Haemoglobin	7.2	g/dL	12 – 16
Platelet count	38	10^9/L	150 – 400
Random plasma glucose	187	mg/dL	< 200
ESR	110	mm/1st hour	< 20
CRP	310	mg/L	< 5
ALT	40	U/L	< 50
Serum protein	62	g/L	66 – 83
Serum urea	31	mmol/L	2.8 – 7.2
Serum creatinine	604	µmol/L	74 – 110
Serum sodium	134	mmol/L	135 – 145
Serum potassium	5.4	mmol/L	3.5 – 5.1
Adjusted calcium	3.49	mmol/L	2.15 – 2.54
Serum phosphate	1.87	mmol/L	0.8 – 1.45
Serum magnesium	0.93	mmol/L	0.73 – 1.06
Dengue NS1 Antigen (fever day 2)	Negative		
Arterial blood gas			
pH	7.26		7.35 – 7.45
PCO_2	27	mmHg	35 – 45
HCO_3^-	16	mmol/L	22 – 26
Blood picture	Red cell changes and moderate anaemia are suggestive of anaemia of chronic disease White cell changes and thrombocytopaenia are in keeping with the diagnosis of recent viral infection		
Chest X-ray	No abnormality detected		

Question 1

What are the differential diagnoses?

Answer 1

- Multiple myeloma complicated with acute kidney injury on chronic kidney disease which had been precipitated with an underlying sepsis
- Macrophage Activation Syndrome (MAS) following Dengue fever
- Malignancy with secondary metastasis

Question 2

Further investigations revealed,

Analyte	Results	Unit	Reference Interval
Serum Ferritin	2230	ng/mL	20 – 400
Triglyceride (Fasting)	261.6	mg/dL	< 150
Prostate Specific Antigen (PSA)	1.13	ng/mL	0 – 6.5

- Skeletal survey
  - Few small punched out lytic lesions were noted in the skull
  - Lower thoraco-lumbar spine showed diffuse osteopaenia, wedge fracture at L1 vertebral body
- Serum protein electrophoresis (SPE)
  - No monoclonal band seen. Immunoparesis present. (Figure 1)
- Serum protein immunofixation electrophoresis (SIFx)
  - No prominent band seen in the separation
- Bone marrow biopsy
  - There is diffuse and some focal infiltration of plasma cells accounting for about 60% of marrow nucleated cells

This patient was found to have fever, multi-organ dysfunction syndrome, pancytopaenia, elevated CRP and elevated serum ferritin, which favour macrophage activation syndrome (MAS), as a serious complication of dengue fever. The diagnosis of MAS is based on fulfilling five out of eight diagnostic criteriae used in HLH-2004 trial.[1] Our patient had only 3 criteriae, which make MAS to be unlikely.

1. Fever > 38.5 ^{0}C
2. Splenomegaly
3. Peripheral blood cytopaenia, with at least two of the following: Hb <9 g/dL, platelets <100 x 10^9/L, absolute neutrophil count <1.0 x 10^9/L
4. Hypertriglyceridaemia (fasting >265 mg/dL) and/ or hypofibrinogenemia(<150 mg/dL)
5. Haemophagocytosis in bone marrow, spleen, lymph node or liver
6. Low or absent NK cell activity
7. Serum ferritin >500 ng/mL
8. Elevated soluble CD25- two standard deviations above age-adjusted laboratory specific norms

Elderly male with ESR >100 mm/1st hour and hypercalcaemia, need to consider the possibility of malignancies (with or without bone metastasis) and PSA and CXR done, to look for the evidence of prostate and lung malignancies, which are common at this age.

Question 2

What are the diagnostic criteria for multiple myeloma?

Answer 2

According to 2014 International Myeloma Working Group (IMWG) Criteria.[2]

Clonal bone marrow plasma cells >10% or biopsy-proven bony or extramedullary plasmacytoma and any one or more of the following myeloma defining events.

- Evidence of end organ damage (CRAB)
  - Hypercalcaemia (serum calcium >2.75 mmol/L or >0.25 mmol/L URL)
  - Renal insufficiency (creatinine clearance <40 ml/min or serum creatinine >177 µmol/L)
  - Anaemia (Haemoglobin <10 g/dL or <2 g/dL LRL)
  - Bone lesion (one or more osteolytic lesions on skeletal radiography, CT or PET-CT)
- Biomarkers of malignancy (SLIM)
  - Clonal bone marrow plasma cell >60%
  - Involved : uninvolved serum free light chain ratio >100 (involved FLC must be >100 mg/L)
  - More than 1 focal lesions on MRI studies (>5 mm)

Question 3

What further investigations would you suggest to arrive at definitive diagnosis?

Answer 3

Though, we could not find a myeloma band in the SPE/SIFx, he was found to have plasma cells representing 60% of all nucleated cells in the bone marrow with hypercalcaemia, renal failure, pancytopaenia, lytic lesions in the bones and immunoparesis, which pointed the possibility of light chain multiple myeloma (LCMM).

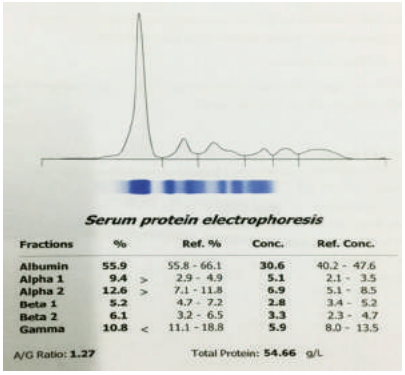

Figure 1: Serum protein electrophoresis

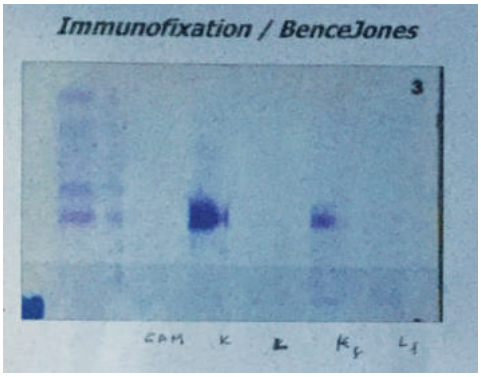

Figure 2: Urine protein immunofixation electrophoresis

Therefore, urine protein electrophoresis (UPE), urine protein immunofixation (UIFx), serum free light chain assay (FLC) and the investigations to prove bone marrow clonality (ƙ/ƛ- light chain restriction with immunohistochemistry or immunofluorescence, or by demonstration of phenotypic clonality by flowcytometry or by immunoglobulin gene rearrangement studies) can be performed.

- Urine protein electrophoresis (UPE)
  - Mild albuminuria with tubular protein loss
- Urine protein immunofixation (UIFx)
  - Monoclonal excretion of kappa light chains noted. (Figure 2)
- Free light chain assay (FLC)
  - Kappa light chains 285.35 mg/L (2.37 – 20.73)
  - Lambda light chains 17.87 mg/L (4.23 – 27.69)
  - Kappa/Lambda ratio 16 (0.22 – 1.74)
- Immunohistochemistry
  - CD_3 – Scattered positivity accounting for about 10 % of cellularity
  - CD_{20} – Scattered positivity accounting for 5 % of cellularity
  - CD_{138} – Diffuse and focal positivity accounting for 60 – 70 % of cellularity

Immunohistochemistry, UIFx and serum FLC ratio were used as a surrogate marker to prove clonality.

Question 4

What are the investigations you would do as prognostic markers?

- Serum beta-2 microglobulin
- Serum albumin
- Serum Lactate dehydrogenase (LDH)
- Interphase FISH

Question 5

What investigations you would request to follow-up this patient?

- Serum free light chain assay (FLC)
- Urine protein electrophoresis (UPEP)

According to the International Myeloma Working Group (IMWG) guidelines, UPEP and serum involved FLC are the measures used to monitor patients with LCMM.[3,4]

Serum assays were more sensitive in indicating the disease and predicting progression free survival and overall survival as compared to urine assays.[5]

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Upcoming Events of CCPSL

- Molecular Pathology Workshop in Kandy.
- Hands-on experience on body fluid analysis for Medical Laboratory Technologists.
- Symposium on “Oncology” at Apeksha Hospital, Maharagama.
- Symposium on “Drugs of abuse” at Colombo North Teaching Hospital.

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Upcoming International Events

- IFCC forum for young scientists on 5th – 6th January 2021 in Seoul, Korea.
- XXIV Worldlab on 6th to 10th January 2021 in Seoul, Korea.
- XXIV IFCC-EFLM Eurolab on 16th to 20th May 2021 in Munich, Germany.
- AACB 58th Annual Scientific Conference 27th to 30th September 2021 in Brisbane, Australia.

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Local and overseas resource persons of 5th AAS of CCPSL