Editorial

Welcome to another edition of the Southern IML Pathology Doctor’s newsletter.

We have put together a number of interesting articles on different areas of Pathology for your information and reference.

I would like to introduce Professor Sujatha Fernando to our practice. Prof. Fernando is an experienced Anatomical Pathologist who has worked as the Director of Anatomical Pathology and Cytopathology at South Western Area Pathology services of Liverpool. Sujatha's special interest is Tropical Pathology an area in which she has acquired an international reputation.

At the same time I would also like to introduce Dr. Kyu Naing who trained in general pathology at Southern.IML Pathology and has joined the team of Pathologists in our Wollongong and Nowra Laboratories. Kyu's special interests are dermatopathology and microbiology.

Southern.IML Pathology has made a change to our motto to better reflect our commitment to providing the highest quality pathology service to the Illawarra and South Coast regions. ‘Quality is in our DNA’ I believe this accurately sums up one of our key distinguishing features which have been part of this practice for many years. In 1999 Southern Pathology was winner of an Australian Quality Award for Business Excellence and awarded Illawarra Business of the Year. Our proud history also has seen Southern.IML Pathology become a Registered Training Organisation in 2006 and in 2008 was winner of the Ministers Award for Excellence for Employers of Australian Apprentices.

Quality is integral to our analytical results, but is also reflected in so many other ways in the services we deliver to you and your patients. Our team of Pathologists, Managers, Scientists, Technicians, Collectors, Couriers and Administration Staff are all committed to providing a quality pathology service. Our Laboratories are equipped with modern instrumentation and are fully accredited by NATA.

Your continued support of our local laboratories is greatly valued and is essential for us to support the Medical profession, our local hospitals and community.

Quality is what doctors rely on
Sujatha completed her Master of Science degree at London University and was awarded a mark of distinction, topping the batch of medical graduates. She migrated to Australia and commenced a career in pathology under the direction of Professor Vince McGovern, a world authority on malignant melanoma. From 1984 to 1989, she was the Director of Pathology at Bankstown Hospital, and thereafter, 1989 to 1997 Director of Anatomical Pathology and Cytopathology at South Western Area Pathology Services of Liverpool.

Prof. Fernando has acquired an international reputation in tropical pathology and fine needle aspiration cytology. She has conducted workshops and was invited to present lectures to learned societies. (RCPA, IAP, ASM, ASC, AIMS, ACTM, ADS, ASBD, Histotechnology Group of NSW) in these areas.

A book has been published as a reference text in the field of Parasitic and Tropical Diseases, titled “Tropical Infectious Diseases”, the first in Australia, with capacity to be used as a teaching manual as it focuses on integration of pathology with clinical science.

In the area of teaching at the University of New South Wales, Sujatha improved facilities by as establishing a museum, supervising undergraduate medical students’ research projects, (BSc honours and postgraduate MSc students). Sujatha organised the basic sciences (pathology) component for the Fellowship of the College of Accident and Emergency Medicine and the advanced training programme for Fellowship of the Royal College of Pathologists of Australasia.

Sujatha is also involved in the training of General Practitioner’s in the Central West Skin Clinic in Bathurst and Illawong on several different topics relevant to the general practitioner registrar training program for the Fellowship. She also initiated and implemented an oral soft tissue screening program in conjunction with the Western Rural Oral Health Network for GWAHS.

In recognition of her significant contribution to service, teaching and research in both metropolitan tertiary pathology services and in rural remote NSW, Professor Fernando was appointed as Network Cluster Director of Pathology West in 2009.

After commencing duties as a Network Director some of Sujatha’s achievements, are as follows:

- Implementation of the organisational structure with the reporting lines actioned across 33 laboratories.
- The selection and implementation of a Quality Management System for the service with clean up of over 25,000 unreported tests in the system.
- Establishment of the Patient Quality and Safety committee for the service.
- The definition and implementation of a Critical Results Policy and Procedure.
Tropical infectious diseases are now frequently encountered. This is due to the rapidity and ease of relatively inexpensive air travel, transcontinental trains and ocean liners, which have now become within the reach of many. Most of these diseases are caused by parasites, but less frequently by bacteria, fungi, rickettsiae and viruses.

Two-thirds of world wide travellers visit the tropics and are exposed to these diseases. In addition, with the influx of refugees and extensive use of immunosuppressive therapy, we need to become familiar with these diseases. Surgical pathologists often enlighten a clinical colleague, for example, where a carcinoma has been suspected an amoeboma or hydatid cyst in the liver may be found. A “lymphoma may be toxoplasmic lymphadenitis, coeliac disease” may be giardiasis, to name a few examples.

A book titled, “Tropical Infectious Disease” had been published by Prof. Fernando with her husband as co-author. This article will illustrate a case of Buruli ulcer encountered by Prof. Fernando personally as an anatomical pathologist in Australia.

Buruli (Bairnsdale) Ulcer

Buruli (Bairnsdale) Ulcer is an infectious disease involving skin, caused by Mycobacterium ulcerans. This is the third most common mycobacterial pathogen of humans, after M. tuberculosis and M. leprae. It is prevalent in areas of stagnant or slow flowing water.

Other atypical mycobacteria can involve the skin and other organs eg. lymph nodes, lungs and meninges. M. ulcerans and M. marinum cause well defined clinico-pathologic lesions known as “Buruli ulcer” and “swimming pool” (Fish tank) granuloma

Clinical Presentation

A 51 year old farmer in rural NSW, with a non-healing, rapidly expanding ulcer was referred by his GP to a surgeon. The ulcer was on his left forearm for over 2 months duration. The lesion was attributed to a spider bite whilst on a trip to the south coastal area of Victoria. It started as a small nodule, progressing rapidly over 2 months with ulceration. An excised lesion was referred for histopathological examination. A similar lesion pictured below by courtesy of (a) S. Lucas, MD, St Thomas’ Hospital, London UK (b) the late M. Hutt, MD, St Thomas Hospital, London, UK.
**Gross Pathology**

- A specimen of skin, measuring 70 x 40mm with subcutaneous tissue to a depth of 20mm bearing a central ulcer 50mm in diameter involving the full thickness of the skin and subcutaneous tissue was received.

  ![Skin Ulcer H&E 100](Image)

  ![Coagulative Necrosis and Fat Necrosis H&E 200](Image)

  ![Langhans type giant cells in poorly formed Granuloma H&E 200](Image)

  ![Numerous acid fast Bacilli, Ziel-Neilsen stain H&E 400](Image)

  ![Ziel-Neilsen Stain- Higher Magification](Image)

Atypical Mycobacteria are a heterogenous group of acid fast bacilli which differ from M. tuberculosis in clinical, microbiologic cultural characteristics and sensitivity to antimicrobial agents. Lung, skin, lymph nodes and meninges can be infected.

Skin lesions can present as nodules, ulcers, cellulitis or abscesses. An infection is likely to follow minor skin trauma, arthropod bite or surgical manipulation. Species other than M. ulcerans and M. marinum which are responsible for these infections include M. kanasii, M. scrofulaceum, M. szulgae, M. gordonae and M. avium-intracellulare complex. Disseminated infections due to M.avium-intracellulare complex are reported frequently in AIDS patients.
In 1948 Peter McCallum and his co-workers in Australia provided a detailed description of the disease with six patients from the Bairnsdale area near Melbourne. They were the first to isolate the causative organism as M. ulcerans. In South Australia the disease is still referred to as the Bairnsdale ulcer.

In the 1960s, many cases occurred in Buruli Country (now called Nalasongola District) in Uganda, giving rise to the most widely used name “Buruli ulcer”. Buruli ulcer is a neglected tropical infectious disease. It is reported from Central and West Africa, New Guinea, Southeast Asia, Mexico and Australia (Queensland and Victoria).

Antibacterial drug therapy is effective during the pre-ulcerative stage. Surgical excision of lesions with skin grafting and streptomycinrifampcin combination for 8 weeks is the ultimate treatment of choice.

In a recent medico-legal case in Australia, the diagnosis of this infection in a 59 year old Victorian yacht rigger was delayed due to failure to carry out the appropriate special histochemistry. This should be routine in all non-healing granulomatous inflammations.

**Conclusion**

This case illustrates:
- Importance of awareness of this skin infection in extensive, non-healing, non-tumoural skin lesions.
- Obtaining a complete clinical history including the patient’s lifestyle, travel to endemic areas.
- Performing acid fast stains in any suspicious case with or without granulomatous inflammation.
- The organism is prevalent near inland water and rivers.
Introducing Dr. Kyu Naing

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Dr Kyu Naing graduated from Institute of Medicine I, Yangon, Myanmar (Burma) in 2003 and completed her internship and residency training at Yangon General Hospital. She continued basic physician training at Modbury hospital and Royal Adelaide Hospital, Adelaide. She commenced her general pathology training at Southern IML Pathology, Wollongong in 2007. Her training was predominantly based at Southern IML Pathology, Wollongong.

Dr Naing also gained experience working at different public and private laboratories through rotations to Douglass Hanly Moir Pathology, the Liverpool Hospital and the Wollongong Hospital. During her training, Dr Naing has been involved in a number of publications in reviewed journal articles. She considers her main areas of interest and expertise to be chemical pathology, histopathology with special interest in dermatopathology and microbiology.

Hypokalaemia and Hyperkalaemia

Out-of-range potassium results—particularly hyperkalaemia—pose a major problem in primary care, notably due to sample conditions producing spurious results.

Severe hyperkalaemia is life threatening and requires urgent attention. Distinguishing spurious from true abnormalities is therefore critical.

Much observational work dates back 50 or more years, but the problem of sample deterioration in primary care remains. Further work in this area appears essential to reduce wasted resources in investigating spurious results and reducing the risk of failing to identify true clinical emergencies. Much evidence-based research regarding prognosis for a given potassium level does not extend beyond expert opinion. It is unlikely that absolute values alone dictate risk as predisposition to arrhythmia, rate of fall and other co-existent metabolic abnormalities are likely to be of equal importance.

It is, however, important to note that prevention of hyperkalaemia is possible in many situations and certain predictive factors can be used to determine which patients to test, and how often, assisted by other results obtained from the renal and electrolyte profile.

What is a high serum potassium?
The upper limit of the reference range for potassium in healthy adults is approximately 4.9–5.1 mmol/l in serum and 4.4–4.5 mmol/l in plasma. Male/female and pregnancy-related differences are probably too small to be of clinical relevance.
Due to analytical and biological sample variation, a change of 0.5 mmol/l may be taken as highly likely to represent true change, with lesser differences increasingly likely to represent statistical variation.

The higher values in serum occur due to the addition of potassium released from platelets on coagulation. The use of a single significant figure of 5 mmol/l has been advocated as an upper limit for serum.

What should I do about high serum potassium?

**Identify patients at risk of having true rather than spurious hyperkalaemia or at risk from its effects**

- Those with known chronic kidney disease (CKD)
- Patients on potassium-raising drugs—namely, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, potassium-sparing diuretics, potassium salts or laxatives.
- Patients with obstructive uropathy
- Patients with clinical features such as myopathy, paralysis, arrhythmias, bradycardia
- Those at greater risk from severe hyperkalaemia: elderly (>70 years), serum urea (>8.9 mmol/l)
- Patients with acute illness (e.g., acute renal failure, ketoacidosis)

**Consider spurious hyperkalaemia in the absence of all the above.**

**What should I do if I suspect a high potassium result is spurious?**

We recommend:

Considering artefactual causes if the patient has normal renal indices and serum bicarbonate, notably serum creatinine, 100 micromol/l or eGFR >60 ml/min and none of the factors listed above are present.

Consider the following causes:

- The specimen was refrigerated or exposed to cold in transit
- Long delay between venepuncture and separation
- Difficult venepuncture with prolonged tourniquet time
- In vitro haemolysis: shaking sample, transferring to tube through a needle
- Patients with raised blood cell count
- Contamination from potassium EDTA (blood count tube anticoagulant).

Take the following action:

- Take a second specimen to arrive within 3 hours of venepuncture
- Remove the tourniquet before drawing blood
- Do not allow the specimen to cool below room temperature
- Send a simultaneous heparin specimen for potassium analysis
- If recent blood count not available, send full blood count
- If sequential samples are haemolysed, consider intravascular haemolysis.

The incidence of true clinical hyperkalaemia with “normal” urea and creatinine is described as insignificant, but should be considered before assuming a result to be spurious.

It may occur after an overdose of potassium salts or with potassium-sparing diuretics in association with normal renal function (eGFR >90 ml/min).

**Causes of spurious hyperkalaemia**

The main causes of spurious hyperkalaemia in primary care relate to time and temperature problems associated with transporting samples from primary care to laboratories and are exacerbated by cold and separation delays. Delays in transport can be partially addressed by ensuring adequate storage conditions. The use
of centrifugation on the primary care site could resolve several of these problems, but raises practical and health and safety difficulties for the surgery.

**Related to Venepuncture**

- **Hyperkalaemia and prolonged tourniquet time**

Stasis at venepuncture increases potassium concentrations in serum because of the release of potassium-rich fluid from red blood cells and muscle cells with haemoconcentration. Extreme stasis of 3 minutes’ duration increases the serum potassium by 10–20%. Fist clenching to localize veins can also increase serum potassium. Some guidelines recommend that specimens for electrolytes be taken first when multiple tubes are required at venepuncture to minimize the effect of stasis.

- **Haemolysis during blood sampling**

Red blood cells contain approximately 100 mmol/l potassium. Venepuncture may cause haemolysis, which may not be visible but can be detected spectroscopically. In vitro, this is a function of the storage time and exposure to cold temperatures. Mechanical damage to red blood cells occurs due to cavitation when expelling blood through fine-bore needles or during difficult venepuncture with excessive pressure applied to a syringe. Shaking specimens either to mix with anticoagulant or accidentally results in mechanical haemolysis. This may increase the potassium concentration four-fold. More potassium is released when red blood cells leak haemoglobin to produce visible haemolysis. Serum haemoglobin levels of 0.5 g/l increases a serum potassium of 5 mmol/l by 10%, although the effect is variable between specimens.

Visible haemolysis invalidates serum potassium results. In vitro haemolysis is also more likely in patients with a family history of haemolytic disorder. A correction has been proposed using a measure of the degree of haemolysis, interpreted qualitatively on the report form as normal, critically high or critically low, although this is not routinely used.

**Related to sample storage**

- **Refrigeration of samples**

Failure of the Na-K-ATPase in the cell membrane will increase the serum potassium, whether this occurs in vitro or in vivo. In vitro, cold reduces Na-K-ATPase activity. Trull et al undertook a study confirming old data suggesting that refrigeration of specimens increased serum potassium values and reported that storage of specimens above 20.3 °C minimized this effect. Using insulated boxes for transport was suggested to avoid erroneous results. One report, not described in detail, describes issuing simple guidance to users on needle size (21 gauge) and storage at room temperature, which reduced the proportion of samples with serum potassium >5.0 mmol/l almost exclusively to those that had been left overnight before being centrifuged. This report was based on a laboratory where most samples arrived within 4 hours after being taken from the patient. Potassium has also been reported to be stable in whole blood for 16 h at 18 °C suggesting that further work to provide a systematic answer to the practical aspects of sample storage and delivery could potentially reduce this common primary care problem.

High cell counts: pseudohyperkalaemia

Potassium is released from platelets during blood coagulation. Platelet counts of more than 1,000 x 10^9/l will affect the serum potassium noticeably. It has been estimated that a 100 x 10^9/l increase in platelets increases the serum potassium by 0.15 mmol/l. Heparinised plasma is not subject to this release and will therefore have a more reliable potassium level. The difference between serum and plasma potassium may be more than 1.0 mmol/l in thrombocytosis. Severe leucocytosis (more than 20 x 10^9/l) can result in excess potassium leakage. This applies particularly when samples have been stored in the cold for reasons explained above. It is exaggerated in leukaemic cells, which are more leaky. Conversely, with storage at room temperature, leucocytosis may result in potassium uptake that is sufficient to make a result from a hyperkalaemic patient appear normokalaemic (or even hypokalaemic).
Plasma potassium may still be elevated spuriously with leucocytosis but less so than serum, and it is advisable to have these specimens separated rapidly and kept at room temperature until analysis.

Similar changes may occur with hereditary or acquired stomatocytosis, and other red blood cell disorders, which exaggerate the potassium release during prolonged storage and may require rapid separation within 1 hour, suggesting that venepuncture occurs close to the site of analysis.

**In vivo haemolysis**

Finally, in addition to exaggerating potassium release on storage, congenital or acquired haemolytic diseases, embolism and extensive tissue breakdown (such as the tumour lysis syndrome) will also promote in vivo potassium release.

It is important to note that this represents true hyperkalaemia and treatment is directed towards that of the underlying condition and occasionally of the hyperkalaemia itself, which can be life-threatening.

The main diagnostic problem with intravascular haemolysis is that the serum potassium result is not reported by laboratories when visible haemolysis is present. This policy is appropriate for in vitro haemolysis but raises problems with in vivo haemolysis. Laboratories should be contacted specifically to discuss cases of hyperkalaemia that are suspected to be associated with in vivo haemolysis.

**What should I do if I suspect a high potassium result is true?**

**Assess urgency criteria:**

- **Severity:**
  - mild: 5.5–5.9 mmol/l,
  - moderate: 6.0–6.4 mmol/l,
  - severe: > 6.4 mmol/l

- **Changes in serum K, serum creatinine (and estimated glomerular filtration rate (eGFR) if renal function is not changing acutely). Statistically significant changes are 0.5 mmol/l, 15% and 10%, respectively.** Smaller rises could reflect statistical variation
  - Clinical and electrocardiogram (ECG) (K >6.0 and higher than previous sample) findings
  - Values rising over 6–12 hours by >0.5 mmol/l are high risk at any K level.

Follow action guidance below:

**Serum K > 6.4 mmol/l in any patient**

- Perform ECG
- Admit to hospital
- Consider administering first-aid therapy, depending on urgency

**Serum K 6.0 to 6.4 mmol/L**

- Symptoms and signs or abnormal ECG, and those receiving potassium-active agent, or
- Significant deterioration in renal function/rise in K (change in K >0.4 mmol/l, or change in creatinine >15% / eGFR >10%)

Then =>

Perform ECG

Review Na, bicarbonate (HCO3), urea and creatinine levels

Seek urgent opinion or admit to hospital, depending on urgency factors

Stop the administration of potassium-raising and nephrotoxic drugs where possible

K and renal function stable then =>

Consider ECG unless there is a chronic stable result

Review Na, HCO3, urea and creatinine levels

Establish thresholds for future action

Avoid potassium-raising drugs

Advise to follow potassium-reduced diet

**Serum K 5.5 to 5.9 mmol/L**

- Review Na, HCO3, urea and creatinine levels
- No action normally required unless recent significant change.

Treatment with any medication that may increase the serum potassium should normally only be initiated with specialist advice.
Ovarian cancer markers

On occasion the laboratory receives requests from clinicians for ‘ovarian cancer markers’ or ‘ovarian tumour markers’, but without a context it is difficult to know what is really required. Some of the ovarian tumour markers such as HE4 and AMH are privately billed, which makes it important that clinicians specify exactly what they want tested.

There is no established screening test(s) for ovarian cancer in asymptomatic Australian women. The current tests are not specific for cancer: in more than 99% of screened cases, one or a combination of blood tests will be falsely positive. This is a major problem as we know that the earlier the diagnosis, the better the outcome in most forms of ovarian cancer. Women and their GPs need to be attuned to the possible symptoms and signs that ovarian cancer can produce. These include local pain and swelling; menstrual, bowel or bladder upset; and systemic features such as weight loss, breathlessness and nausea.

Detection of a suspicious ovarian or adnexal mass on imaging or examination improves the accuracy of tumour marker tests. The majority of primary ovarian cancers are of the serous epithelial type. The best current combination of tests for investigating a suspicious ovarian mass is HE4 and CA125. This combination has a sensitivity of 80% and a specificity of 90%. From these tests, the ROMA score (Risk of Ovarian Malignancy Algorithm) can be calculated, with cut-offs that take into account the age of the patient. HE4 is more accurate than CA125 in premenopausal women, as it is less likely to be affected by benign reproductive conditions such as menstruation, ovulation and endometriosis. CA125 is more specific in older women as benign ovarian pathology is less likely.

However, CA125 can be elevated if pleural and peritoneal effusions or secondary metastases to the ovaries are present.

Other ovarian cancer types and their associated markers include:

1) Mucinous type: CEA
2) Germ cell cancers, including hydatidiform moles: hCG
3) Granulosa cell tumours: inhibin B, anti-mullerian hormone (AMH), oestradiol
4) Metastatic cancers: tumour marker depends on primary cancer.

These other markers, except for serum hCG, are usually ordered after obtaining the results of histopathology.

In summary

The best combination of tests to order in the investigation of an ovarian mass is HE4 and CA125 (or ROMA). Most other ovarian tumour markers require a histological diagnosis before testing is undertaken.

Faecal calprotectin (FCP)

Faecal calprotectin (FCP) is a novel biomarker of inflammation in the gut. This marker is best used to identify those patients who would most benefit from early endoscopy when investigating possible inflammatory bowel disease. It is also useful to assess the control of inflammatory bowel disease. In Crohn’s disease, FCP best reflects colonic inflammation compared to ileal inflammation.

With a cut-off of 50 mg/kg, the sensitivity and specificity of the assay are both around 90% for active inflammatory bowel disease. The use of NSAIDs or aspirin and the presence of coeliac disease may produce elevated levels, and in addition disease of the small bowel may be missed by FCP.

What to order: Faecal calprotectin

Specimen: Faecal specimen (brown-top jar)

Transport: Ambient

Cost: $80* There is no Medicare Rebate for this test.
Tests that require appointments
A few tests require that patients make an appointment. This may be for one of several reasons; a dedicated or specially trained collector is needed, specialised facilities or equipment are required, a doctor or pathologist must perform the procedure, the sample has to be tested within a short time of collection, the sample has to be couriered directly to a referral laboratory.

We understand your patients’ frustration when they present at one of our branches and are told that their samples cannot be taken on the day. We hope that it may help to reduce this by providing you with the list below.

- Pap smear
- Platelet antibodies
- Whole blood aggregation
- Urine Drug Screen
- Fine Needle Aspiration Biopsy
- Bone Marrow
- Parentage/relatedness testing
- Short Synacthen stimulation test
- Verifi Non-invasive Prenatal Test (NIPT)
- Lactate/pyruvate
- Neutrophil Alkaline phosphatase
- Arterial blood gases
- Holter Monitor or 24 hour BP Monitor (module has to be booked)
- Anti-D injection
- Skin and nail scrapings for fungi

Tests that may only be done on certain days
- Chromosome studies (karyotyping, cytogenetics)
- Tissue typing for transplant
- Miscellaneous other referred tests

Thin Prep Billing
The Thin Prep method is widely recommended to improve cancer diagnosis, although the Australian Government do not provide a Medicare rebate.

This test incurs a fee of $50.00.