

Dedication of this newsletter

The Staff of **IPath Illawarra Pathology** would like to dedicate this edition of our newsletter to our colleague Yi Zhang Who was tragically killed in the Waterfall train Disaster. Our thoughts go out to her family and friends during this traumatic time.

Pathology Name Change

The Illawarra Health Board has resolved that they endorse the renaming of IAHS Pathology to

IPath Illawarra Pathology.

This name change was initiated to incorporate the change of Illawarra Area Health Service to Illawarra Health, and also for the purpose of trading as a Public Pathology Service.

The Pathology Service will be implementing the name change over the next few months with changes to stationary and other documentation. During this period both names, IAHS Pathology and IPath Illawarra Pathology may be found on relevant stationary.

Autologous Blood Stem Cell transplantation (ABSCT)

Over the last few months the Haematology Laboratory at Wollongong Hospital has commenced routine peripheral blood stem cell quantitation as an adjunct to our expanding program of *Autologous Blood Stem Cell Transplantation* (ABSCT).

ABSCT is a method of intensifying the doses of chemotherapy that may be given to patients with diseases such as myeloma and lymphoma.

This increases the likelihood of curing or controlling the disease in certain patients. To collect stem cells patients are given chemotherapy along with injections of a white cell stimulating drug called G-CSF which mobilises the bone marrow stem cells into the blood. These cells are then collected on a machine by a process called *leucapheresis*. The timing of the collection is critical and is dependent on being able to measure the small numbers of stem cells that circulate in the blood. These are measured by Flow Cytometry (FACS) which is capable of analysing thousands of cells per second. FACS has been available in the laboratory for some years and is used in the routine diagnosis of leukaemia and lymphoma. If the stem cell numbers are adequate then stem cell collection may proceed. Currently, the stem cells are sent to Westmead Hospital for processing and freezing. However, in the next few months facilities will be available in Wollongong for storage of frozen stem cells prior to re-infusing them into the patient as part of the transplant.

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PROTEINURIA

THE NORMAL KIDNEY

Normally, the glomerular filtrate contains about 10 to 30 mg protein per 100 mL of filtrate. Most of the protein present in the filtrate is actively reabsorbed in the proximal renal tubules. About 100 to 150 mg protein per day escapes into the urine.

The kidneys have three mechanisms for preventing blood-borne proteins from being excreted and lost in the urine.

1. **Pore size of the glomerulus** – The pore size of the glomerulus prevents large proteins, such as Immunoglobulin G (150 kDa), from passing through into the glomerular filtrate.
2. **Anion filter** – The glomerular basement membrane contains negatively charged molecules that repel negatively charged blood-borne proteins, such as Albumin (67 kDa) and Transferrin (90 kDa). This mechanism prevents their passage through the glomerulus.
3. **Reabsorption by the renal tubules** – Lower molecular weight proteins, such as alpha-1-microglobulin (33 kDa) and Beta-2-microglobulin (12 kDa), pass freely through the glomerulus but are reabsorbed and metabolized by the cells of the renal tubules.

THE TYPES OF PROTEINURIA

Functional and structural defects in the glomeruli or renal tubules can lead to various and distinct patterns of proteinuria. This proteinuria can be broadly divided into three types: prerenal, renal and postrenal.

1. Prerenal, or Non-Renal, Causes of Proteinuria

Prerenal proteinuria is the result of excessive production of serum proteins. Due to their small size these proteins pass freely through the glomeruli. If their quantity exceeds the renal tubular capacity to reabsorb the proteins then they will appear in urine. Examples of this type of proteinuria include: excessive production of kappa or lambda light chains (Bence-Jones protein), haemoglobinuria (severe intravascular haemolysis) and myoglobinuria (rhabdomyolysis).

2. Renal, or Glomerular, Causes of Proteinuria

Loss of glomerular anionic filter function is characterized by a pattern of selective glomerular proteinuria e.g. microalbuminuria. By contrast, structural damage of the glomerulus, such as in glomerulonephritis and nephrotic syndrome, are characterized by unselective proteinuria. However, even in advanced renal disease very large proteins, such as alpha-2-macroglobulin are not lost through the glomerulus.

3. Post-Renal, or Tubular, Causes of Proteinuria

Reabsorption of proteins by the renal tubular system takes large amounts of energy and is easily disturbed by illness and medication. The most sensitive indicator of tubular disturbances is alpha-1-microglobulin. More severe tubular damage can lead to further structural damage and a disturbed tubular reabsorption of even smaller proteins, such as beta-2-globulin. Involvement of the tubulo-intestitium reflects worsening prognosis. Mixed glomerular and tubular defects are also common in advanced forms of renal disease.

Post-renal defects, with or without haematuria, can also lead to the direct addition of unfiltered blood-borne proteins into the urine, such as alpha-2-macroglobulin.

DIFFERENTIAL DIAGNOSIS OF PROTEINURIA

Protein is normally not detectable in urine, although excretion of up to 150 mg/day is considered within normal limits. Urine dipsticks are commonly used to detect the presence of proteinuria, however, these predominantly detect only the presence or absence of albumin. They are insensitive to many other proteins that may be present in urine. Methods, such as total protein measurement using the dye pyrogallol red, measurement of albumin by immunological methods and urine electrophoresis can give a more accurate assessment of the degree and type of proteinuria. Additionally, 24 hr collection of urine in patients with suspected proteinuria is recommended in order to give a quantitative measure of the amount of protein actually excreted.

Indicator proteins that are useful for the differential diagnosis of Proteinuria are shown in Table 1 below. Urine electrophoresis can be used to identify different patterns of excretion of these proteins.

IPath Illawarra Pathology provides the different quantitative measures for proteinuria.

	Prerenal	Renal	Postrenal
Urine parameter	Non-renal	Glomerulus	tubular
Total Protein	+	+	+
Albumin (microalbumin)			
Kappa or Lambda	+		
Haemoglobin	+		
Myoglobin	+		
Albumin		+	
Transferrin		+	
Immunoglobulin G		+	+
Alpha-1-microglobulin			+
Beta-2-microglobulin			+
Alpha-2-macroglobulin			+

Dr Fernando San Gil
Clinical Chemistry Department
IPath Illawarra Pathology

BREAST CANCER-AN UPDATE ON GENETIC TESTING

In the past few years there has been enhanced public awareness about breast cancer, with emphasis on education and self-help, including breast screening programs and public awareness programs. In addition there have been considerable efforts to detect factors that will help to identify women most likely to develop breast cancer and the genes that contribute to these risks.

Age, gender, family history of prior breast cancer, familial cancer syndromes and atypical ductal hyperplasia are all implicated as the factors associated with increased risk of breast cancer

Only 5-10% of inherited breast cancer is due to germ line mutations in predisposing genes.

BRCA 1 gene is localised to the chromosomal region 17q¹²-q²¹ on the long arm of chromosome 17.

A second breast cancer gene was also discovered on the short arm of chromosome 13 and this is the BRCA 2. In contrast to BRCA1, which is also responsible for a large proportion (more than 75%) of inherited predisposition to ovarian cancers, mutations in BRCA 2 are found in at least one third of cases of familial breast cancer.

It is important for the public and medical personnel involved in care of patients to understand that 90 – 95% of breast cancers are sporadic, and hence the futility of genetic testing in a small proportion of individuals.

To assess cancer risk the two approaches include pedigree analysis done with or without genetic testing and the other is risk based on epidemiological studies.

Hereditary breast cancer occurs typically in a younger age group, with a history of two or more relatives having had cancer through two or more generations, multiple primary tumours, increased incidence of bilateral breast tumours and an autosomal dominant pattern of inheritance.

Testing for increased risk of breast cancer is commercially available for mutation in the genes BRCA 1 and BRCA 2. These are DNA based tests and can be offered to “at-risk” individuals and after adequate counselling.

Different BRCA 1 and BRCA 2 mutations appear and produce different cancer risk, however neither the lifetime risks nor the type (s) of cancer can be predicted. Not all of these mutations can be detected and an increase in hereditary risk can be due to other unidentified gene mutations.

Families with BRCA 1 mutation have an increased risk of developing cancer of the ovary, colon, and prostate in males. BRCA 2 mutations are associated with an increased risk of cancers that include male and female breast, ovary, fallopian tube in females and prostate, colon, oesophagus and pancreas in male.

An affected family member should initially be tested for the presence or absence of a specific mutation. If no mutation is found in the affected member, there is no need to offer genetic testing to the unaffected family

members. However, if a mutation is detected, then it is advisable to test the close unaffected family members for the presence or absence of the same mutation. The unaffected relative is not at increased risk if no mutation is found. However these unaffected relatives can still develop breast cancer later in life.

This test is expensive and there is no proper legislation to protect high-risk individuals from the problems of insurance and employment, as both will be linked to the high-risk status.

Both pre-test and post-test counselling must be provided to the individual and psychosocial and other concerns about the prognosis of the illness and its treatment options should be discussed.

These high-risk individuals are offered increased surveillance and prophylactic surgery. There are approved research protocols for chemoprevention that can be offered to these individuals.

In these individuals cancer can develop in residual breast tissue after prophylactic surgery, and similarly prophylactic oophorectomy does not mean that cancer will not develop in the peritoneal reflection.

Those women who are at risk are advised to undergo clinical breast examination and mammography every 6 – 12 months beginning between ages 25 –35 years, as BRCA 1 tumours have a faster growth rate than sporadic tumours. In addition it is also recommended women with this BRCA 1 mutation have a pelvic examination and transvaginal ultrasound every 6-12 months (these are current NCI guidelines in the USA).

Although genetic testing and risk assessment is still evolving with increased knowledge of the familial cancers, it is important that the public and practising physicians understand our current limitations and the status of the disease. Until more solid proof is not forthcoming concerning the benefits of genetic testing, education and screening are the best methods to prevent breast cancer.

Compiled from references and guidelines from NCI

Area Blood transfusion Policy

The new Illawarra Health Blood transfusion policy and the Transfusion guideline's are now available to view on the Pathology website.

Our website also contains the Pathology collection handbook, Patient collection information and instructions on how to access our Pathology results as well as other useful pathology related information.

Visit us at: <http://internal.iahs.nsw.gov.au/Pathology/>

Increased staffing levels for Shoalhaven District Hospital Pathology Laboratory

As from April **IPath Illawarra Pathology** will be able to offer an improved service to our colleagues and medical practitioners at the Shoalhaven District Hospital. This increased staffing level shall allow us to staff the laboratory 24 hour a day seven (7) days a week. Thereby allowing us to better respond to the demands and need of our customers.

The appropriate Investigation of Genetic Thrombophilia

Thrombophilia refers to a predisposition to thrombosis, which may be related to genetic or acquired factors. Common acquired factors are surgery, immobility and pregnancy. Inherited abnormalities such as Antithrombin III, Protein C and Protein S Deficiency have been recognised for many years but are very rare. In the last 10 years, more common thrombophilic mutations in the factor V gene (Factor V Leiden), prothrombin gene (Prothrombin 20210A), and methyltetrahydrofolate reductase (MTHFR) genes have been described. These genetic factors may give rise to spontaneous VTE or, more commonly, interact with acquired risk factors and increase the risk of thrombosis in these situations. The frequency of these abnormalities in the general population and unselected groups of patients with VTE are shown in Table I. *Controversy remains regarding the cost-effectiveness of screening for these abnormalities in individual patients and the influence that detection of these abnormalities will have on subsequent clinical management.* Higher gene frequencies are found in patients with a positive family history of thrombosis, recurrent thrombosis, younger age and thrombosis occurring in unusual sites, such as the cerebral or mesenteric veins. It is important to emphasise that there is no increased risk of arterial vascular problems associated with these genetic mutations. Therefore there is no role for these tests in the assessment of the vast majority of elderly patients that present with vascular disease e.g TIA/CVA/PVD. Possible exceptions are young patients (age <50) with cerebral events, particularly with no risk factors and an atypical or recurrent presentation. It is reasonable to screen patients with vascular problems for the MTHFR mutation and hyperhomocystinaemia, which are associated with an increased risk of vascular disease.

	Normal Patients	VTE Patients	Relative Risk of VTE
Factor V Leiden			
Heterozygous	5%	20%	5-10 X
Homozygous			80 X
Prothrombin 20210A	2.5%	6%	3 X
MTHFR Mutation (Raised Homocysteine)	7%	15%	3 X
ATIII/ProteinC/Protein S	0.2%	5-10%	2 X

Table I: Frequency and relative risk of VTE due to thrombophilic genetic defects

Patients for whom genetic thrombophilia testing is reasonable

Initial VTE :

It is reasonable to investigate patients who present with an initial episode of VTE with one or more of the following features: *age < 50, a positive family history of VTE, idiopathic thrombosis or thrombosis in an unusual site (e.g cerebral, mesenteric, portal or hepatic vein).* Identification of

patients with *combined genetic defects* or a *single defect in the presence of a life threatening thrombotic episode* may lead to consideration of prolonged or potentially life-long anti-coagulation. Women of child bearing age need to be informed of the risks of foetal loss.

Recurrent VTE:

Patients with recurrent VTE should be screened for genetic abnormalities.

First degree relatives of patients with genetic thrombophilia

First degree relatives should be screened for the presence of the specific defect *as well as* the commonly occurring factor V Leiden and prothrombin G20210A mutations. Co-inheritance of these factors will influence the risk of VTE. The threshold for prophylactic anti-thrombotic therapy may be lowered in these patients, or more intensive prophylaxis given at times of risk. In the event that female carriers of child-bearing age are identified, they should be counselled regarding the risks of the oral contraceptive and be offered alternative forms of contraception. Based on limited evidence a similar management approach is recommended for post-menopausal women with on HRT. In this situation an alternate form of therapy for osteoporosis should be considered.

Women with a personal history or family history of VTE

In the presence of a positive personal or family history of VTE, screening may be considered if the oral contraceptive is to be prescribed or pregnancy is contemplated. In the absence of previous VTE, current data would suggest that only women with ATIII deficiency should receive prophylactic anti-coagulant therapy during pregnancy. However, prophylactic anti-coagulation during pregnancy is justified with other genetic abnormalities if there is a history of prior VTE.

Women with a history of pregnancy loss, severe pre-eclampsia or intra-uterine growth retardation may be screened, although the efficacy of anti-coagulant therapy in these situations is unknown.

Problems in interpretation of tests

If investigation for an underlying genetic disorder is felt to be appropriate, it is important that clinicians are aware of potential problems with the interpretation of certain test results. Abnormal coagulation tests should be repeated and borderline results interpreted with caution. Protein C and Protein S are vitamin K dependent factors and are reduced by vitamin K deficiency, liver failure or therapeutic anticoagulation with warfarin. Protein S levels are normally reduced as part of the acute phase response and in pregnancy. Coagulation assays of Protein C and S may also give falsely low levels in the presence of factor V Leiden, therefore the presence of this mutation should be determined first. Heparin therapy leads to a reduction in Antithrombin III levels.

Available Investigations:

Measurement of activated protein C resistance due to factor V Leiden is usually assessed using an APTT-based clotting assay. Molecular methods of detection have the advantage of identifying the abnormality as a deficiency of factor V Leiden and allowing classification of the underlying genetic deficiency as heterozygous or homozygous. Currently, prothrombin G20210A is only detectable by molecular methods.