

EDITORIAL

Issue Thirteen, Nov 2002

Thank you to all our readers who provided feedback about our last issue; the new format has been well received. The articles in this issue cover two interesting topics Thrombophilia and Faecal Occult Blood Testing.

PaLMS Chatswood Collection Rooms have reopened at a new location, see below. There is another in our series of Pathology FAQs on page 8.

We welcome your feedback and suggestions and an Educational Needs Survey is enclosed, please make use of it. All those who return the survey will go into a prize draw.

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CHATSWOOD COLLECTION ROOMS RE-OPEN IN NEW LOCATION

PaLMS have re-opened in Chatswood
at a more convenient location for your patients.

Station Medical Clinic
Level 3, The Interchange
430 Victoria Ave Chatswood

Phone: 02 9413 9980

Hours: Monday to Friday 8am to 1pm & 1.30pm to 6pm
Saturday 8.30am to 12 midday

Thrombophilia

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Venous thromboembolism (VTE) is a common and potentially life-threatening condition, which can be triggered by a range of environmental or pathological factors. The role of surgery, prolonged immobility or pregnancy as precipitants of VTE have long been recognized. In recent years, the importance of underlying factors in VTE has become clearer, as many new genetic variants have been identified. The term “thrombophilia” is used to describe both inherited and acquired factors, which lead to an increased risk of VTE (Table 1). These thrombophilic factors are important in venous rather than arterial thrombosis and increase risk exponentially when present in combination. Screening for thrombophilic risk factors is particularly important in cases of familial or spontaneous thrombosis.

Table 1.

Risk Factors for Venous Thromboembolism

(Adapted from Rosendaal et al, *Thromb Haemostasis* 2001;86:11)

Acquired	Inherited	Mixed
Age	Antithrombin deficiency	Hyperhomocysteinaemia
Immobilisation	Protein C deficiency	High levels of FVIII
Surgery (esp orthopaedic)	Protein S deficiency	APC-resistance (non-FVL)
Malignancy	Factor V Leiden (FVL)	High levels of FIX
Hormonal therapy	Prothrombin 20210A	High levels of FXI
Antiphospholipid antibodies		High levels of TAFI
Myeloproliferative disorders		
Previous thrombosis		

FVIII = Factor VIII, etc., TAFI = thrombin activable fibrinolysis inhibitor

Current Thrombophilia Screen Assays Available

1. Deficiencies of natural anticoagulants:

Antithrombin III (**ATIII**) complexes with heparin to neutralise thrombin and activated FX and so low levels are a risk for thrombus formation. At PaLMS, a functional assay is performed, which identifies ATIII deficiency caused by genetic defects or consumptive coagulopathies such as DIC.

Protein C (**PC**) is a vitamin K-dependent protein and is the precursor to activated Protein C (APC) which selectively inhibits FVa and FVIIIa. Protein C deficiency due to both inherited and acquired factors (DIC, warfarin, oestrogen therapy) is identified by a functional assay.

Protein S (**PS**) is another vitamin K-dependent protein, which acts as a co-factor in the

(Continued on page 3)

(Continued from page 2)

PC anticoagulant pathway. At PaLMS, a functional screening test for free-PS is performed. This identifies all genetic abnormalities as well as acquired conditions such as DIC, pregnancy, and hormonal therapy. Patients with low free-PS levels are further assayed immunologically for total PS antigen.

2. Activated Protein C Resistance (**APC-R**) is quantitated in the laboratory by accelerated clotting times, representing a resistance to the anticoagulant activity of APC. Because it is often due to a single point mutation in the FV gene, PaLMS performs DNA analysis for **FV Leiden** when indicated or when specifically requested. Unlike the clotting assay, DNA testing can distinguish between heterozygous and homozygous carriers of the FV Leiden mutation. Our laboratory has established an instrument/reagent specific reference range for APCR in order to identify non-FV Leiden APC resistance.
- 3) Elevated coagulation Factor levels are reported to alter the homeostatic balance in favour of thrombosis, possibly by increased thrombin generation.
Prothrombin G20210A mutation is associated with increased levels of FII (prothrombin) and is a mild risk factor for venous thromboembolism. However, laboratory estimation of FII levels is not specific, and DNA analysis is needed to identify carriers of the 20210A mutation. PaLMS has developed a duplex PCR technique to detect FV Leiden and the prothrombin 20210A mutation simultaneously.

A **Factor VIII** level above normal (>150%) is a risk factor for both arterial and venous thrombosis. Levels of FVIII increase with age and as an acute phase reactant, so repeated assays may be required. It remains uncertain whether increased FVIII levels are genetic or a marker of chronic endothelial damage. FVIII activity is quantitated using an automated clotting assay (FVIII:c). Similarly, elevated levels of **fibrinogen** have been associated with thrombosis.

4. Metabolic disorders:
Elevated **homocysteine** levels are associated with both venous and arterial thrombosis. Homocysteine is regulated by two enzymes, methylene tetrahydrofolate reductase (MTHFR) and cystathionine beta synthase. Screening for the MTHFR thermolabile variant by DNA analysis is not recommended, as homozygosity is a poor predictor of thrombosis. Direct measurement of fasting homocysteine is preferable, in order to detect both genetic variants and the common acquired causes of hyperhomocysteinaemia (renal impairment, B12/folate deficiency, drug effects). Elevated homocysteine levels can often be corrected by simple vitamin therapy.
5. Pathogenic antibodies:
Antiphospholipid antibodies are associated with both arterial and venous thrombosis and with recurrent foetal loss. This heterogenous group of antibodies is directed against proteins complexed to phospholipid – in particular, β 2-GP1 and prothrombin. The laboratory investigation of APA includes clot-based tests for lupus anticoagulant (**LAC**) and ELISA-based testing for anti-cardiolipin antibodies (**ACA**). The standardisation of laboratory methods for the demonstration of LAC is recognised as difficult. PaLMS uses 3 phospholipid-dependent clotting tests of differing methodologies and we have established our own reference ranges using over 100 healthy volunteers. Thrombosis may be associated with *weakly* demonstrated LAC also; follow-up assays 2-3 months later will often show an increase in APA titre.

(Continued on page 4)

(Continued from page 3)

Samples Required

1. Citrated plasma for ATIII, PC, PS, APCR and LAC
2. EDTA plasma for plasma homocysteine
3. Serum for ACA
4. EDTA for DNA analysis (separate specimen) – Prothrombin mutation and FVL

Anticoagulation status:

Warfarin- it is recommended **not** to test for PC and PS in warfarinised patients. All other thrombophilic testing is either unaffected by Warfarin or the Warfarin effect is countered within the test system.

Heparin – ATIII may be reduced during the first 2 days of heparin administration. All other thrombophilic testing is either unaffected by heparin or the heparin effect is neutralised within the test system.

Who should be screened?

Complete screening for thrombophilia is expensive and may not be appropriate in patients with a clear precipitating cause of thrombosis, or in elderly patients where management will not be altered. Thrombophilia screening is definitely recommended in young patients (<50 years of age) with idiopathic thromboembolism, or those with a positive family history.

Current Health Insurance Commission regulations allow for rebatable testing in the following situations :-

- i) The patient has a personal history of venous thromboembolism (NOT arterial disease).
- ii) The patient has a first-degree relative with a known defect (screening for known defect only).
- iii) The patient requires confirmatory testing for a demonstrated abnormality or indeterminate result.

These criteria clearly do not cover all cases where screening is clinically or medico-legally appropriate. Examples include women with recurrent second or third trimester pregnancy loss, or thrombophilic families where a defect has not yet been defined. Note that tests are **ONLY** rebatable if clinical details are included on the request form.

Clinical relevance of thrombophilia

The identification of thrombophilic risk factors is a rapidly evolving field, and it is certain that additional factors will emerge. Finding one of these factors implies an increased risk of thrombosis, but the actual risk may be very small; e.g. the majority of FV Leiden heterozygotes will **never** develop venous thrombosis. Estimates of increased relative risk have been established for the common thrombophilic factors.

(Continued on page 5)

(Continued from page 4)

Table 2.
Impact of inherited thrombophilia
(adapted from Bertina R et al *Thromb Haemost* 1999;82:601-609).

	Population Prevalence	Relative Risk of VTE
Antithrombin III Deficiency	0.01%	5 x
Protein C Deficiency	0.1%	6.5 x
Protein S Deficiency	< 0.1%	? 1.7 x
Factor V Leiden (heterozygous)	5%	5-8 x
Prothrombin Mutation	2%	3 x
↑ Homocysteine	>5%	2-3 x
↑ Factor VIII	11%	5 x
OCP		3.8 x

Estimated prevalences from a European population and the relative risk of VTE are listed for each factor. The risk of the oral contraceptive (OCP) is included for comparison.

The following principles are important when applying these ratios to an individual patient:-

- the baseline risk of venous thrombosis varies enormously, from less than 1/10,000/year in healthy young adults, to greater than 1/100/year in patients over 70.
- the risk of **any** thrombophilic factor is greater in patients with a family or past history of VTE, compared to those identified by population screening. This appears to reflect coinheritance of multiple defects.
- the effect of a second factor on thrombosis risk is not additive, but multiplicative (e.g. FV Leiden (5 fold) plus OCP (4 fold) = 35-50 fold increased risk). Many individuals with apparent "thrombophilia" are found to have multiple risk factors.

Future developments

The number of factors associated with thrombophilia is growing rapidly, with new genetic polymorphisms identified from families with recurrent thrombosis. Other variants (e.g. Factor XIII Val34Leu) are proposed to reduce the risk of VTE. Not all of the factors identified by case-control studies are found to be useful in large-scale, prospective studies (viz. homozygosity for variant MTHFR). Hence, some of the factors listed in Table 1 (elevated levels of Factors IX, XI and TAFI) are not yet recommended for routine screening. Given the increasing complexity and costs of thrombophilia screening, efforts are underway to develop "global" functional assays and multiplex PCR methods, to enable simultaneous screening for multiple defects.

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Faecal Occult Blood Tests

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Background

Faecal occult blood (FOB) testing is a screening technique for early detection of colorectal cancer. The purpose of FOB testing is to identify asymptomatic individuals for whom further endoscopic or barium enema investigation is appropriate, in order to exclude or diagnose colorectal cancer or adenomatous polyps at an earlier stage, which allows surgical intervention and further management to prevent tumour progression.¹

What is the faecal occult blood test?

All three types of FOB tests are based on the principle of detecting the presence of blood in the stool as evidence of a bleeding gastrointestinal lesion. The different types of FOB tests detect different components of blood and therefore differ in test sensitivity and specificity.²

1. **Traditional guaiac tests** (eg. Haemoccult, Haemoccult II, Haemoccult Sensa) detect the peroxidase activity of haem. The test reacts similarly to, and cannot distinguish between, intact haemoglobin, haem and any dietary peroxidase sources (such as red meat, raw fruit and vegetables and iron supplements). Therefore, while the test is sensitive for both upper and lower gastrointestinal bleeds, it is less specific for colorectal cancer. The test requires dietary preparation, with exclusion of the above interfering foods for three days prior to sample collection.
2. **Newer immunochemical tests** (eg. Quicktest, MonoHaem, HemeSelect) incorporate an immobilised monoclonal antibody that specifically binds intact human haemoglobin only. The test does not react to dietary peroxidases. It is also less likely to react to upper GIT bleeding, unless heavy blood loss occurs, since haemoglobin from the upper GIT would undergo digestion to haem products that would not be recognised by the specific antibody. This method therefore has the highest specificity for colorectal cancer detection and does not require dietary preparation prior to testing.
3. **The haem-porphyrin assays** use fluorimetry, an acid reduction step and solvent extraction to detect haem and haem-derived porphyrins. This method is relatively labour-intensive, requires dietary preparation and is not as specific as the newer immunochemical tests.

The incidence of colorectal cancers

Colorectal cancer is one of the three most commonly diagnosed cancers in Australia. In 1995, there were 10,615 colorectal cancer cases and 4,508 deaths within Australia, with similar frequencies to the USA, where an estimated 134,000 new cases and 55,000 deaths occurred in the same year.^{3,4} The risk of developing colorectal cancer before 75 years of age is approximately 1 in 18 for men and 1 in 26 for women, with exponential increases in risk after the age of 50 years.³

Study evidence of FOBT effectiveness

The first study to raise widespread awareness of the value of FOBT was the Minnesota Colon Cancer Control Study which indicated that annual testing could reduce colorectal cancer-related mortality by 33%.⁵ This study used rehydrated Haemoccult II with test specificity of only 90%, resulting in a large number of colonoscopy investigations. In the UK, a similar study using Haemoccult without rehydration, reported a 15% reduction in colorectal cancer mortality due to screening.⁶ Several studies have since shown that immunochemical FOBT methods had better specificity whilst maintaining high sensitivity.⁷ In a study of 10,702 patients who were prospectively followed for 2 years, Allison *et al*⁸ found the HemeSelect, an immunochemical test, provided better diagnostic value than the guaiac tests, Haemoccult II and Haemoccult Sensa (see Table 1). Castiglione *et al*⁹ found HemeSelect to have both a higher sensitivity and specificity than Haemoccult II

(Continued on page 7)

(Continued from page 6)

in a population screening study of 24,282 subjects aged 40-70 years.

Table 1: FOBT test performances (Allison *et al*)

Recommendations for FOBT

PaLMS follows the recommendations outlined in the National Health and Medical Research Council's "Guidelines for the prevention, and management of colorectal cancer".

Test	Sensitivity	Specificity
Haemoccult II	37%	98%
Haemoccult Sensa	79%	87%
HemeSelect	69%	95%

the recommendations the National Medical Research Council's "Guidelines for early detection of colorectal cancer".

FOB testing is recommended at least every two years from the age of 50 years for asymptomatic persons at or slightly above average risk. This group comprises approximately 98% of the population. **FOB testing and screening alone is insufficient for individuals with higher colorectal cancer risks.** For example, individuals at moderate risk for colorectal cancer should be offered colonoscopy or sigmoidoscopy plus barium enema investigations every 5 years from the age of 50 years or at an age 10 years younger than the age of colorectal cancer onset in the family. FOB testing could be considered, however, in the intervening years for this higher risk group.

FOB testing has little clinical value in the presence of any symptoms suggestive of colorectal cancer.

Faecal occult blood testing procedure

PaLMS currently uses "Quicktest FOB Test", an immunochemical card test for faecal occult blood testing. This test specifically detects human haemoglobin and has the further advantage of an internal control that confirms an adequate faecal sample has been applied.

Stool collection from three consecutive bowel motions is recommended to enhance test sensitivity. Faecal material is sampled with a stick, provided as part of the kit, attached to the inside of the collection tube cap. The stick is then inserted into the tube which contains an extraction buffer. After mixing, the supernatant is ready for immediate application to the test card, or may be stored at 4°C for up to a week.

A positive result is demonstrated by a coloured line in both the test (T) and control (C) regions, whilst a negative result is demonstrated by a line in the C region only. The absence of a line in the C region invalidates the test and indicates an unsatisfactory collection or test has occurred. In such cases, a repeat test and/or sample collection is required.

References

PaLMS recommends collection of samples from three consecutive bowel motions in three separate small, sterile containers, refrigerated storage for up to 3 days during collection period, with immediate transport to the testing laboratory at the completion of the collection period.

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(Continued on page 8)

(Continued from page 7)

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Pathology F A Q

Q: *Is an ESR or a CRP necessary when requesting pathology for my patient's yearly checkup?*

A: Neither CRP nor ESR should be used as screening tests in asymptomatic people .

Q: *If my patient is symptomatic which is better CRP or ESR?*

A: CRP is the best method for measuring the acute phase response. The CRP is also more sensitive, specific and quicker than the ESR. The ESR may be more useful in some chronic diseases such as SLE. An elevated CRP or ESR does not indicate the cause of the abnormality.

More information on the use of CRP or ESR in Acute Phase Response can be found in Issue Twelve of PaLMS Info Link. Contact PaLMS Administration 9926 8086 if you require a copy.

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