best tests

April 2015

Factors that can affect laboratory investigations

Anatomic Pathology Tests

Identifying and managing hereditary haemochromatosis in adults

bpac nz
better medicine

We would like to acknowledge the following people for their guidance and expertise in developing this edition:

Dr Joanne Dixon, Auckland
Dr Cynric Temple-Camp, Palmerston North

Best Tests is published and owned by bpac^{nz} Ltd

ISSN 2324-304X (Print)
ISSN 2324-3058 (Online)

Bpac^{nz} Ltd is an independent organisation that promotes health care interventions which meet patients' needs and are evidence based, cost effective and suitable for the New Zealand context.

Bpac^{nz} Ltd has six shareholders: Procare Health, South Link Health, General Practice NZ, the University of Otago, Pegasus Health and the Royal New Zealand College of General Practitioners

Bpac^{nz} Ltd is currently funded through contracts with PHARMAC and DHB Shared Services.

Contact us:

Mail: P.O. Box 6032, Dunedin
Email: editor@bpac.org.nz
Free-fax: 0800 27 22 69

www.bpac.org.nz

facebook.com/bpacnz

CONTENTS

Factors that can affect **laboratory investigations**

In 2013 we published an article entitled "Best tests?" (Best Tests 18; Feb, 2013). In this article we challenged clinicians to consider whether they could improve the method and manner in which they request laboratory investigations. To put it simply, it is about selecting the right test, at the right time, for the right patient. After making the decision that an investigation is necessary, and selecting the most appropriate test, consideration must be given to what factors are present that may affect the interpretation of results, or even the decision to proceed with the test at that time.

In many scenarios, a diagnosis is based predominantly on the patient's history, with supporting evidence from physical examination and laboratory investigation. Diagnosis is rarely based on the results of laboratory investigations alone, and testing is not always the best clinical course of action. Consideration needs to be given to whether testing will add meaningful information to the overall clinical picture, and then to the factors which may need to be taken into account when interpreting the result. Can the test be done immediately or is the patient required to prepare in some way, e.g. fasting? Is the patient taking any medicines that may influence or invalidate the results, e.g. taking antibiotics prior to faecal antigen testing for *Helicobacter pylori*? Does the test need to be performed at a certain time, e.g. measuring testosterone in the morning? Is the test being done at the right stage of illness, e.g. testing for antibodies after seroconversion has occurred? A test result returned from the laboratory will usually include a reference range or a threshold value based on guidelines. However, this result "on paper" does not always represent the clinical significance of a test, which is only apparent once all other factors for that individual patient have been taken into account.

This article is not intended to be a comprehensive guide, but rather an overview of general concepts to guide clinicians in considering the wide array of factors that can influence laboratory investigations or interpretation of results.

If you are uncertain about how a test may be affected by a specific factor you have identified, discuss this with the testing laboratory first, or include this detail on your investigation request form.

To test or not?

Considerations prior to requesting a laboratory investigation include:

- What is my reason for requesting this test? E.g. am I investigating symptoms and signs? Am I monitoring a disease or effect of a medicine? Is this a screening test?
- Has this test already been done? Does it need to be repeated? If so, when?
- Will the test improve patient (or in some cases, family or partner) care?
- Is this the right test or combination of tests for the clinical situation?
- Is it the right time to do the test?
- How should the sample be taken?
- How should the sample be stored and transported?
- How will the test result be interpreted?
- How will the test result influence patient management?
- What will be the consequences of a false positive result?
- Are there potential harms of doing this test?
- How will the patient be informed of the result?

Biological variation

There are certain variations in laboratory test results that can be expected due to non-modifiable biological factors, such as age, biological rhythms and physiological changes during pregnancy. These factors may be controlled for, e.g. by selecting the most appropriate time in the day, month or year for a test, or may be taken into consideration in the interpretation of results, e.g. different reference ranges or thresholds for clinical significance depending on age, sex or pregnancy status.

Advancing age

The physiological changes associated with ageing, along with increasing co-morbidities and polypharmacy, mean that older people are more likely to have test results that fall outside of the normal reference range. For some tests, laboratories are able to provide an age-adjusted reference range, but for other tests, a result outside of the range in an older patient, needs to be interpreted in the context of their overall clinical picture.

In many cases, assessing the rate and magnitude of change over time offers more information than interpreting the value of an individual result. Often the population range of a test shows much more variation than that for an individual patient, e.g. serum creatinine, liver enzymes. In such cases the patient's own previous results are a useful baseline.

An example of the effect of age, sex and other variables on interpretation of laboratory results is serum alkaline phosphatase (ALP), which may be requested as part of liver function tests. The upper reference limit is markedly increased during puberty as this is the time of maximum bone remodelling. After this period ALP levels fall to a new upper limit through younger adult life, and then rise again, particularly in females around the time of perimenopause, largely reflecting an increase in bone turnover at that time. Marked increases in serum ALP may also occur in women in late pregnancy (due to production of ALP by the placenta), and during other times such as in the weeks after healing of a fracture.

Another example of laboratory values which change with age is lipids. In adults, total cholesterol, LDL and triglyceride levels increase with age, until approximately age 50 to 60 years in males and age 60 to 70 years in females, when they begin to decline in most people; triglyceride levels tend to continue to increase in older females.[1] Many laboratories are no longer reporting reference ranges for lipid levels, as this is considered less clinically useful than treatment targets based on the underlying cause of raised levels and cardiovascular risk.[2]

When interpreting laboratory results in an older patient, ensure that an age-appropriate reference range is used if available. Normal age-related changes, e.g. deterioration in renal function, may explain results outside of the reference range in an older patient, but age alone should not be considered as the only cause of an abnormal result.

Biological rhythms

Many laboratory parameters vary depending on the time of day, week, month or year when they are sampled. Body temperature, hormone production (e.g. cortisol, testosterone), platelet and cardiac function and cognitive function follow a circadian (24 hour) rhythm.[1] To allow for this effect, some laboratory tests are recommended at specific times of the day, e.g. testosterone should be sampled between 7 am – 10 am. This is because peak testosterone levels usually occur in the early morning; evening levels are often substantially (up to 50%) lower, especially in younger males.[2] For most purposes, serum cortisol* should preferably be sampled in the early morning as there is marked diurnal variation, with early morning levels at least 50 – 100% higher than levels in the late afternoon.[2]

Testing vitamin D levels (25-hydroxyvitamin D) is seldom necessary, however, if levels are obtained, they need to be interpreted in the context of the time of year they are sampled. Seasonal variations occur with vitamin D, with the lowest levels usually observed at the end of winter (in countries with defined seasons, such as New Zealand). For example, if a patient has a mild vitamin D deficiency at the end of winter, this is likely to be less clinically significant than a patient with a mild deficiency at the end of summer.

For further information, see "Age-related testosterone decline in males" Best Tests (Jun, 2012) and "Vitamin D supplementation: navigating the debate" BPJ 36 (Jun, 2011).

* Serum cortisol levels have a wide reference range, and therefore this is a relatively inaccurate measure of cortisol excess or deficiency. The dexamethasone suppression test is used to help exclude Cushing's syndrome and the Synacthen stimulation test is used to investigate for primary or secondary hypoadrenalism.[2]

Menstrual cycle

Females who are menstruating have predictable monthly rhythms of FSH, LH, oestrogen and progesterone. In a general practice setting, these hormone levels may be requested to investigate conditions such as oligo/amenorrhoea or sub-fertility. The interpretation and meaningfulness of the results is dependent not only on the value, but on the stage in the cycle when the hormone was measured.

In a normal menstrual cycle LH levels peak mid-cycle to trigger ovulation. FSH levels also peak mid-cycle. Oestradiol (the predominant oestrogen in women who are ovulating) is highest prior to ovulation, and then reduces if fertilisation does not occur. Unless ovulation is being investigated, levels of these hormones are best measured early in the menstrual cycle.[2]

N.B. Investigation of oestradiol levels is not useful in women who are taking oestrogen-containing oral contraceptives as this suppresses the pituitary ovarian axis. LH and FSH are also suppressed in women taking depot progesterone.[2]

Progesterone levels peak in the second phase of the menstrual cycle, after ovulation has occurred, to prepare the endometrium for implantation of an embryo. Progesterone levels are sometimes measured to establish if ovulation has occurred; usually seven days before the expected date of menstruation, i.e. approximately day 21 if the woman has a regular 28 day cycle.

Oestradiol levels decrease and FSH levels increase during menopause, but monitoring these hormone levels is not always reliable in predicting when a woman is entering menopause, as fluctuations occur with varying ovarian activity.

For further information, see "Reproductive hormones: the right test at the right time, for the right patient" Best Tests (Feb, 2013).

Pregnancy

Physiological changes during pregnancy result in alterations in many laboratory parameters, such as blood volume, liver and renal function and hormone levels (Table 1, over page). Reference ranges for different stages of pregnancy are available for some laboratory tests, however, these ranges are often not as well defined as the general reference range. In addition, pregnancy-related changes, such as alterations in binding proteins, can affect assays differently, e.g. free hormone levels can be assay dependent. Therefore caution is recommended in interpreting results based on reference ranges and the laboratory should be contacted if there is any doubt.

When requesting a laboratory investigation in a woman who is pregnant, note the gestational week on the laboratory request form.

Individual variations

A patient's diet, health status and lifestyle factors can all have a pre-analytical influence on laboratory parameters.

Diet and nutritional status

Fasting, calorie restriction, food exclusion diets, malnutrition and dehydration can all affect laboratory results. The significance of some laboratory tests is dependent on controlling dietary factors, e.g. ensuring there is sufficient gluten in the diet for at least several weeks prior to serology investigations for coeliac disease, or fasting prior to assessing the effect of intervention in a patient with previously high triglyceride levels. In other scenarios, assessing dietary factors can help to interpret unexpected laboratory results, e.g. a vegetarian or vegan diet can result in decreased levels of vitamin B12, a low carbohydrate diet can cause increased ketone levels (as part of urinalysis) and a high protein diet can result in increased uric acid levels.

Fasting for 12 hours prior to laboratory testing may be helpful or even necessary, depending on the clinical scenario, to get the most accurate result for the following tests, which are affected by the ingestion of certain foods:[4]

- Glucose; only if indicated – for most patients HbA_{1c} is now recommended as the test of first choice for the diagnosis and monitoring of type 2 diabetes, and does not require fasting
- Triglycerides; for most patients fasting is not required for lipid testing, but may be useful for monitoring in people with high triglyceride levels
- Uric acid; fasting is not usually required in practice to get accurate results but the effect of recent dietary intake may help to explain unexpected results
- Creatinine; a recent meal with high meat content can have a significant influence on serum creatinine, and this should be considered when monitoring eGFR[5]

Table 1: Examples of laboratory values that change with pregnancy[1, 2, 3]

Increases	Decreases
Alpha-fetoprotein (AFP); peaks in third trimester	Haemoglobin; due to haemodilution caused by greater blood volume
Alkaline phosphatase (ALP); up to four fold increase in third trimester	Ferritin; decreases as pregnancy progresses
Blood volume (mean plasma volume); increases by 30–50%	FT4; may decrease slowly in late pregnancy (can be assay dependent)
Lipids; up to 40% increase in cholesterol, triglyceride levels can markedly increase in some women (due to the effect of oestrogen)	Prothrombin and partial thromboplastin times
Creatinine clearance; glomerular filtration rate increases 40-60% (eGFR cannot be reliably calculated)	TSH; decreases first trimester, then returns to normal (due to the effect of hCG)
ESR; increasing to 30–60 mm/h as pregnancy progresses	Sodium; slight decrease due to changes in blood volume and fluid homeostasis
Hormones; oestrogen, testosterone, progesterone, human chorionic gonadotrophin (hCG), prolactin	
Iron binding (transferrin levels); significant increase even in a non-iron deficient woman (due to the effect of oestrogen)	
White blood count; may increase to 15–18 x10^9/L	

Sustained low caloric intake and starvation can result in numerous changes to laboratory parameters such as glucose, thyroid function, electrolytes, liver function, renal function and lipids.[4] Uric acid levels may be increased as a result of ketonaemia (causing reduced clearance).[3]

Malnutrition has varying effects on laboratory results, depending on the nature of the patient's nutritional status. Malnutrition is classically thought of as a deficiency of protein and energy, with or without micronutrient deficiencies. However, malnutrition may be defined as under-nutrition, over-nutrition or deficiency of specific nutrients. Malnutrition should be considered as a cause for results such as decreased ferritin, folate and vitamin B12 levels.

Dehydration can be considered as a cause of sodium and potassium imbalances, and can also affect numerous other indices, such as creatinine and urea, albumin, lipids and haematology indices.

For further information see: "A primary care approach to sodium and potassium imbalance", Best Tests (Sep, 2011) and "Strategies to improve nutrition in elderly people", BPJ Special Edition (May, 2011).

Caffeine

The effect of caffeine on laboratory parameters has not been fully studied. It has a short half life of three to seven hours, but this varies among individuals.[4] Caffeine intake causes transient increases in blood glucose levels and impairs glucose tolerance.[3, 4] It can also affect other specialised investigations such as interpretation of metanephrines when investigating hypertension.

Alcohol

The effect of alcohol consumption on laboratory investigations depends on the duration and extent of use. Acute (transient) effects of alcohol consumption (within two to four hours) include decreased serum glucose and increased plasma

Reference range

A reference range for a laboratory test is a statistically-derived numerical range of results that is obtained by testing a sample of "healthy" individuals. Defining "healthy", however, is not straightforward, and depends on a wide range of factors and assumptions; in many cases to define a range simply using "perfectly healthy" patients would make it unrealistic and unusable.

The range is also commonly assumed to have a Gaussian distribution, in that 68% of values lie within one standard deviation (SD) of the mean value, 95% within two SDs and 99.7% of values within three SDs.[1] However, many ranges do not have a Gaussian distribution, but rather the upper end of the distribution is skewed. In these cases the range can be derived either by log transformation of the data, or simply by identifying the relevant 2.5th and 97.5th percentiles in the population being studied (with attempts to exclude those patients likely to have an underlying pathology by clinical, laboratory and statistical means).

Reference ranges for laboratory results usually include two SDs from the mean value meaning that one in 20 "healthy" individuals will have a test result outside the reference range.[1] Reference ranges vary between laboratories, and can change if new evidence becomes available. The upper and lower limits of the range are not absolute and do not define "normal" and "abnormal", but are points at which the probability of clinical significance tends to increase.

Some "reference ranges" are based on recommendations from international bodies for optimising patient outcomes, rather than on a population statistical distribution. For example, the upper reference limit for TSH in early pregnancy is based on guidance statements from the Endocrine Society and the American Thyroid Association. The recommended limit for serum uric acid in patients taking uric acid lowering treatment (0.36 mmol/L) is based on the European League against Rheumatism (EULAR) guidelines.

Interpretation of a result outside the stated reference range is therefore very dependent on the clinical background of the patient, the pattern of other abnormalities, and the clinical question(s) being asked.

Timing of investigation in relation to stage of illness

The significance of an investigation can be dependent on when the sample was taken in relation to the stage of the disease process. The stage of illness can also influence the selection of the most appropriate investigation. For example, a serology test for syphilis may be falsely negative if the sample is taken too early after exposure, and therefore seroconversion has not yet occurred. Different types of serology test will provide information about active or past infection.

Acute illness can also affect the result of some investigations, e.g. ferritin is an acute phase protein and levels can be increased by inflammation and infection, as well as chronic disease and malignancy.

lactate with a reduction in urinary uric acid excretion due to the inhibition of hepatic gluconeogenesis.[3]

Chronic effects of alcohol consumption on laboratory investigations include:[2]

- Elevated gamma glutamyl transferase (GGT) and mean cell volume (MCV) which are commonly used to test for excessive alcohol consumption
- Elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT) and AST/ALT ratio
- Elevated triglyceride levels
- Elevated uric acid and ferritin levels due to fatty liver and alcoholic hepatitis
- Elevated creatine kinase due to alcoholic myopathy
- Other haematological abnormalities, e.g. anaemia and thrombocytopenia

Alcohol consumption can also contribute to vitamin and mineral deficiencies due to replacement of food with alcohol or as a result of interference of absorption of vitamin and minerals, e.g. decreased folate, vitamin A, vitamin B and calcium levels.

Tobacco smoking

Regular smoking and exposure to nicotine can have both acute and chronic effects on laboratory investigations, although the mechanisms behind these changes are not fully understood. Within one hour of smoking one to five cigarettes, plasma/serum concentrations of fatty acids, adrenaline, glycerol, aldosterone and cortisol are increased.[3] People who are chronic smokers may have persistent increases in leukocyte counts, heavy metals, lipoproteins, tumour markers and haematocrit (PCV), and decreases in the activity of some enzymes (e.g. angiotensin-converting enzyme).[3]

Exercise

The effect of exercise on laboratory parameters is dependent on the health status of the patient, air temperature during exercise and intake of food and water during or following exercise.[4] Extreme exercise or vigorous exercise in a person unaccustomed to this level of activity can result in changes to some laboratory parameters. For example, the most common cause of elevated creatine kinase (CK) levels is

exercise. Intense exercise can cause an elevation in CK levels for several days to a week. Well-muscled people often have CK levels persistently above normal.[2]

Thyroid function is also known to be altered in people undergoing high-intensity exercise. For example, anaerobic exercise increases TSH and FT4 levels, but decreases FT3.[4] Liver function (AST and to a lesser extent ALT) tests can increase after exercise. Transient proteinuria and haematuria are also common after exercise, but usually resolve after a few days. Other analytes that can be increased by exercise include urea, creatinine, lactate dehydrogenase, prothrombin time, and D-dimer levels. Fibrinogen and the activated partial thromboplastin time (APTT) can be reduced.[4] Most of these effects are likely to be transitory (e.g. persistent for a few hours to a few days after exercise), but this depends on individual patient factors.

Medicines

The medicines that a patient is taking can significantly affect some laboratory results, therefore this needs to be taken into consideration when interpreting results. It is good practice to note the relevant medicines that a patient is taking on the laboratory request form, especially if they may potentially influence results, e.g. antihypertensives being taken when investigating secondary causes of hypertension or hormone replacement therapy being taken when requesting endocrine tests.

Medicines can have a direct effect on the sample or laboratory testing process, causing an inaccurate result. For example, when investigating for *H. pylori*, a false-negative result of a faecal antigen test may occur in patients taking a course of antibiotics or proton pump inhibitors (PPIs) as this would decrease the gastric load of *H. pylori*.

Medicines may also cause a biological effect to the patient which would account for an altered result. For example, some antibiotics (e.g. cotrimoxazole and erythromycin), cardiovascular medicines (e.g. amiodarone and propranolol), NSAIDs (e.g. piroxicam) and gastrointestinal medicines (e.g. omeprazole) may account for a raised INR result in a patient taking warfarin who normally has a stable INR.[2] Long term use of metformin or PPIs is a possible explanation for a low vitamin B12 level.[2] Many medicines have an effect on the balance of sodium and potassium in the body, e.g. diuretics may cause hypernatraemia (especially loop diuretics), hyponatraemia (especially thiazides), hyperkalaemia (especially potassium-sparing diuretics) and hypokalaemia (loop and thiazide diuretics).[2]

For further information, see: "A primary care approach to sodium and potassium imbalance", Best Tests (Sep, 2011).

When monitoring the serum concentration or effect of a medicine, the laboratory test needs to be timed depending on the drug's metabolism, e.g. a blood sample for testing lithium levels should be collected 10–14 hours after the last dose and a sample for testing digoxin should be collected at least eight hours after the last dose.[2] When initiating a patient on warfarin, INR levels should be sampled daily in the morning, after an evening dose of warfarin, to calculate necessary dose adjustments.[2]

Analytical variation

Analytical variation occurs due to imperfections in testing methods and equipment, which may cause analyte values to be slightly different each time they are measured. Modern testing methods and laboratory equipment mean that analytical variation is usually less of a factor in differing test results than biological variation. Ideally the variation in measurement (expressed as analytical coefficient of variation, or CVa) should be less than half the individual patient biological variation of the analyte in question (CVi).

For further information on variation with specific analytes, see: **www.westgard.com/biodatabase1.htm**

Collection, storage and transport of samples

If a sample is being collected at the practice, it is important to be familiar with the type of collection container and sample medium that is required by the laboratory for the specific test, as this can affect results, sometimes markedly. For example, a swab for PCR testing for pertussis should be transported in a dry tube or a tube with universal viral transport medium, but not in a tube with charcoal transport medium (which is acceptable for swab culture).

Other examples of collection or transport requirements for optimal test results include:

- Blood samples for coagulation studies, including platelet count, D-dimer, prothrombin time, APTT and fibrinogen, should be transported to laboratory within four hours of collection[6]
- Urine specimens for culture should be stored in a fridge prior to transportation to reduce the rate of multiplication of microorganisms
- Samples for glucose analysis should be separated as soon as possible after collection; this applies even with samples collected in fluoride or oxalate collection tubes, as reduction in glucose concentration still occurs for 60–90 minutes
- Samples for potassium or phosphate should not be left overnight, especially in the fridge, as results can be markedly altered, e.g. late evening collection with delayed transport to the laboratory
- Faecal samples for culture and microscopy should preferably be transported to the laboratory within four hours
- Semen samples for fertility testing should be kept by the patient at body temperature, e.g. by storing in a clothing pocket, and transported to the laboratory within one hour of collection.[2] The same level of urgency is not required for post-vasectomy semen analysis.

Refer to your local laboratory for specimen collection requirements. Some providers have online resources, e.g. **www.labtests.co.nz/referrers/tests/collection-guide**

Haemolysis

Haemolysis is the destruction of red blood cells, resulting in release of haemoglobin and cellular constituents, e.g. potassium, into the plasma. It is a cause of inaccurate blood test results and can occur either *in vitro* during collection, storage or transportation of a blood sample or *in vivo* resulting in haemolytic anaemia if severe.

In vivo and *in vitro* haemolysis have different analytical features, e.g. haptoglobin levels are normal with *in vitro* haemolysis, therefore analysis can reveal if haemolysis occurred during the collection of the blood sample or if it was already present.[2] The possibility of haemolysis should usually be noted on results by the laboratory staff, including the likely degree of interference and reliability of the result. If the haemolysis is severe, a recollection of the sample may be advised.

Some of the analytes that can be affected when *in vitro* haemolysis has occurred include:[2]

- Elevations in potassium, AST (ALT is less affected), lactate dehydrogenase, phosphate
- Reductions in bilirubin, troponin T, insulin

Standardised sample collection and transport processes can help to prevent *in vitro* haemolysis, including:[3]

- Allowing alcohol to dry completely when it is used for skin sterilisation prior to venepuncture
- Not leaving a tourniquet on for longer than two minutes
- Using an appropriately sized needle for collection (20 – 22 gauge needles can be used for most routine collections)
- Not removing the needle from the vein if the vacuum tube is attached
- Not exposing the specimen to extremes in temperature
- Avoiding vigorous mixing or shaking of tubes
- Avoiding delay in sending samples to the laboratory

ACKNOWLEDGEMENT: Thank you to **Dr Cam Kyle**, Chemical Pathologist, Auckland for expert review of this article.

References

1. Lee M, American Society of Health-System Pharmacists. Basic skills in interpreting laboratory data. Bethesda, MD: American Society of Health-System Pharmacists, 2013. Available from: www.123library.org/book_details/?id=108469 (Accessed Mar, 2015).
2. Kyle C (Ed). Pathology handbook: a guide to the interpretation of pathology tests. New South Wales: Sonic Healthcare, 2014.
3. Guder WG, editor. Samples: from the patient to the laboratory: the impact of preanalytical variables on the quality of laboratory results. 3rd, rev ed. Weinheim, New York: Wiley-VCH, 2003.
4. Peck Palmer OM. Effect of age, gender, diet, exercise and ethnicity on laboratory test results. In: Accurate results in the clinical laboratory: a guide to error detection and correction. London ; Waltham, MA: Elsevier, 2013. pp. 9–17.
5. Priess D, Godber I, Lamb E, et al. The influence of a cooked meat meal on estimated glomerular filtration rate. Ann Clin Biochem 2007;44:35–42.
6. Queensland Medical Laboratories. Pathology reference manual. Available from: www.qml.com.au/Portals/0/PDF/RefManV2_WEB_APR09.pdf (Accessed Mar, 2015).

The New Zealand Laboratory Schedule and Test Guidelines: **Anatomic Pathology Tests**

The New Zealand Laboratory Schedule provides clinicians with consistent guidance when considering requesting laboratory tests. It will ensure the uniform availability of tests across District Health Boards (DHBs) in the future. Tests are divided into Tier 1, which all referrers can order, and Tier 2, meaning that the test must be ordered in conjunction with another health professional with a particular area of expertise. In addition, clinical guidance is provided on the use of some tests. In this article, with the assistance of Dr Cynric Temple-Camp (Chair of the Laboratory Schedule Anatomic Pathology subgroup), we focus on the anatomic pathology tests in the Schedule.

For further information on the New Zealand Laboratory Schedule see: **www.dhbsharedservices.health.nz/Site/Laboratory/Laboratory-Schedule-Review-Project.aspx**

Anatomic pathology comprises histology and cytology

The information about anatomic pathology tests in the Laboratory Schedule is divided into two sections:

- Histology
- Cytology – further divided into gynaecological and non-gynaecological sections

As in other specialities, the histology and cytology tests are defined as Tier 1 (all referrers can request) and Tier 2 (specialist guidance is required) in the Laboratory Schedule.

Histology testing

Tier 1 tests for histology include the majority of specimens clinicians send for histological examination. In primary care this predominantly consists of shave, punch, incisional and excision biopsies of superficial soft tissue lesions of the skin. Specimens may therefore include: tissue from the biopsy or excision of basal or squamous cell carcinomas, pigmented naevi, lipomas and sebaceous cysts as clinically indicated.

The Laboratory Schedule also defines a number of activities as Tier 1 tests that are not directly diagnostic but that are

important at the clinical-pathology interface. These include the presentation of pathology at multidisciplinary meetings, referral of material for a second opinion and the increasingly common process of returning tissue specimens to patients.

Tier 2 tests for histological examination are usually requested from a hospital setting and include larger surgical specimens such as breast tissue from mastectomies, and specialist biopsies from lesions in organs such as kidney, lung, bone or brain, often collected intra-operatively.

In some circumstances, usually in a secondary care setting, additional Tier 2 tests are requested by the pathologist providing the initial diagnostic work-up of a specimen. There are many hundreds of immunohistochemical antibody tests available that have a wide variety of applications. The selection of these studies in addition to standard histology can, for example, enable the diagnosis of a tumour's histogenesis and therefore assist in tumour identification. These additional tests may also be important for prognosis and to guide future treatment, e.g. the identification of the endocrine status of a breast tumour by detecting oestrogen, progesterone and HER2 receptors.

Cytology testing

Tier 1 tests for cytology include both gynaecological and non-gynaecological cytology. Tests can be requested by any registered medical practitioner as well as other relevant practitioners, such as midwives and cervical smear takers.

Tier 1 tests for gynaecological cytology include conventional and liquid based cytology (LBC). In the New Zealand context, tests for cervical, vaginal and vulval cytology now predominantly utilise LBC. The schedule also includes HPV PCR testing as a Tier 1 test as defined by the requirements of the National Cervical Screening Programme.

Non-gynaecological examples of Tier 1 tests requested in general practice include the assessment of sputum and urine samples. Aspirates from cysts or other lesions, and material from fine needle aspiration (FNA), sent for routine cytology are also included as Tier 1 tests.

Tier 2 tests for cytology include material from investigations requiring more specialised collection techniques, usually in a secondary care setting, such as bronchial washings, bronchiolar alveolar lavages and those collected during operative procedures.

No specific additional guidance has been developed for anatomic pathology testing

There are no specific referral guidelines for anatomic pathology in the Laboratory Schedule. All tissue and aspirated fluid recovered by a medical procedure should ideally be submitted for examination. However, in practice, there are a number of exceptions to this, although these are of more relevance in a secondary care setting. Tissues that are not usually submitted for testing include tonsils, hernia and hydrocele sacs, femoral heads from patients undergoing hip joint replacement and placentas from women who have normal vaginal deliveries at term. The Laboratory Schedule therefore makes no recommendation to clinicians over which tissues or fluids not to submit, but leaves this as a clinical judgement.

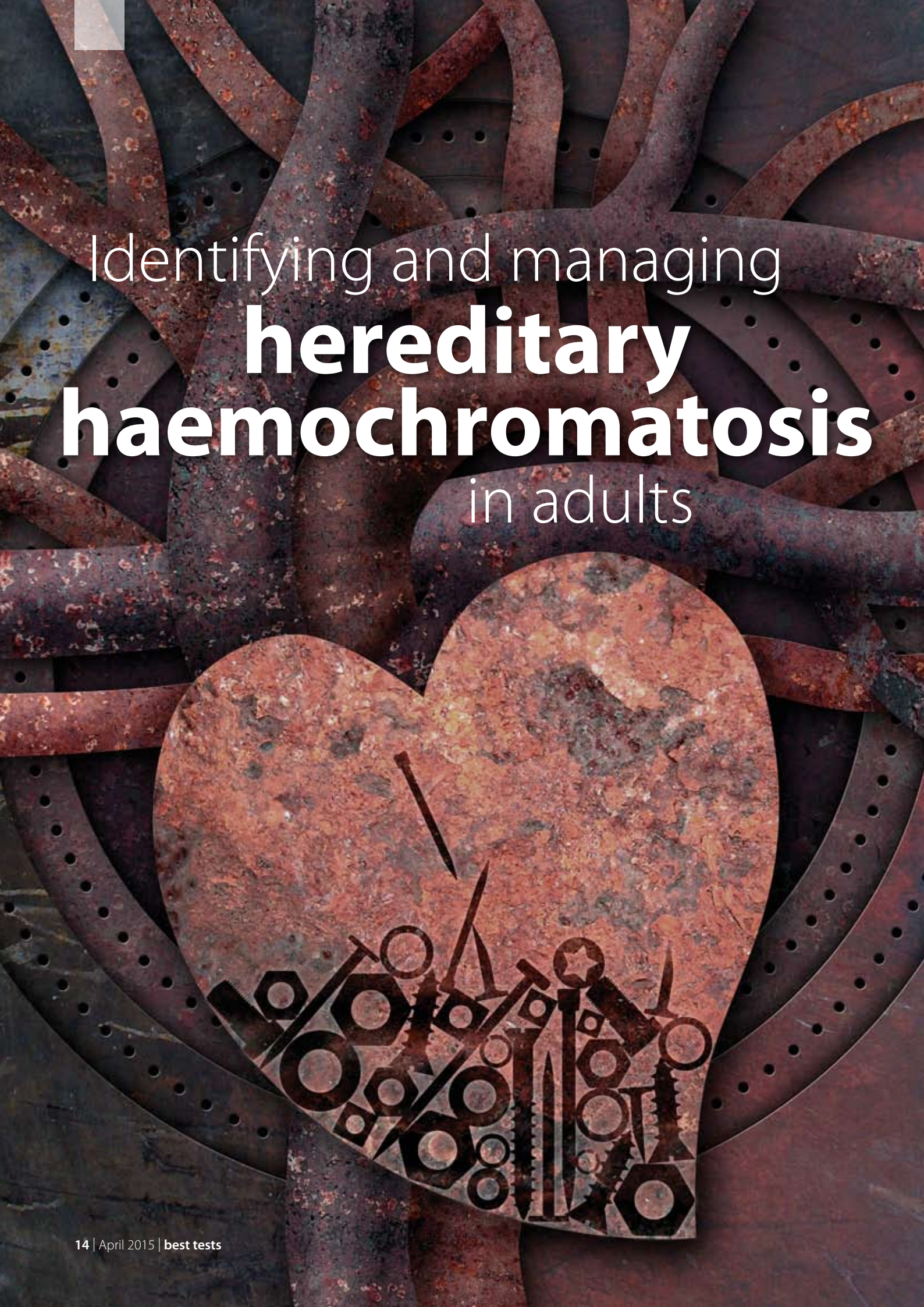
Future planning

In the future, molecular diagnostic testing in anatomic pathology will become increasingly important and the selection of these tests will become a critical part of clinical management. Molecular pathology is a multi-disciplinary field encompassing aspects of anatomic and clinical pathology, molecular biology, biochemistry and genetics. It is a growing field utilising diagnostic tests such as polymerase chain reaction (PCR), fluorescence in situ hybridisation (FISH) and gene mutation testing. There is increasing use of molecular testing in patients with cancer and the results are useful in both diagnosis and selection of treatments. Molecular tests are not performed in all laboratories and are only available at some local, national and international specialised centres. The selection of molecular tests is usually made by a secondary care clinician or pathologist and therefore these tests are likely to be Tier 2, however, this will depend on local availability and whether or not they are funded.

The Laboratory Schedule leaves the introduction of future molecular testing open, however, tests will have to meet the criteria of appropriate clinical relevance as well as cost effectiveness. The Schedule provides a background basis on which current and future evidence-based spending on pathological testing can be developed.

ACKNOWLEDGEMENT: Thank you to **Dr Cynric Temple-Camp**, Anatomical Pathologist, Chair of the Laboratory Schedule Anatomic Pathology subgroup for contribution to this article.

Identifying and managing **hereditary haemochromatosis** in adults



Hereditary haemochromatosis is the most common genetic disease in European populations. It is an autosomal recessive disorder which leads to elevated iron absorption. This in turn can lead to iron deposition in tissue which adversely influences organ function, leading to a range of complications, such as arthralgia, diabetes, heart disease, liver cirrhosis and hepatocellular carcinoma. Patients who have biochemical evidence of abnormal iron metabolism, measured by ferritin levels and transferrin saturation, require genetic testing after excluding non-specific causes of ferritin elevation. Treatment of haemochromatosis aims to reduce body iron stores by regular venesection until target ferritin levels are reached. Venesection reduces the risk of some complications, but not others, and continued monitoring of iron levels and possible clinical consequences is necessary.

Haemochromatosis is usually a hereditary condition, characterised by increased iron absorption leading to iron deposition in tissues and ultimately organ damage. Iron is an essential mineral in the diet. It is a key constituent of haemoglobin and helps regulate a number of biological processes involved in the immune response, oxygen transport and the function of various enzymes in the body, including the hepatic cytochrome enzymes. The average person has around three to four grams of iron in their body.[1] As there is no control mechanism for iron excretion in humans, iron stores in the body are regulated by controlling iron absorption; a key hormone involved is hepcidin, which is released by the liver and acts on duodenal cells to inhibit iron absorption.

In 1996 it was discovered that approximately 80% – 85% of cases of hereditary haemochromatosis are caused by common variants of the HFE gene.[2, 3] Although the molecular mechanism is not clear, these common variants increase the chances of low hepatic production of hepcidin, leading to increased iron absorption. People with this form of haemochromatosis are referred to as having HFE hereditary haemochromatosis (HFE-HH). However, the condition is recessive and people who inherit only one copy of identified genetic risk factors will not develop clinical disease. Iron accumulation in people with HFE-HH is usually not evident until adulthood.

The prevalence of hereditary haemochromatosis in New Zealand is unknown, but a study of over 1000 people in Christchurch (predominantly Caucasian) found that 38.4% of the population had at least one copy of a risk allele; however, only 0.28%, or one in 355, had haemochromatosis requiring treatment.[4]

Who is most at risk?

A patient's ethnicity is a key feature to consider when assessing their risk of hereditary haemochromatosis, and sex is a risk factor for developing clinical complications.

European ethnicity

Caucasian ethnicity is a risk factor for hereditary haemochromatosis, but people of other ethnicities may still develop the condition.

The highest prevalence of genetic risk alleles occurs in people of European descent, with prevalence in Asian and Pacific peoples one-third or less of that seen in Caucasians (see: "Genetic terminology and haemochromatosis", Page 18 for a description of risk alleles).[5] There is no direct data available on the prevalence of risk alleles for haemochromatosis in Māori.

Males are at greater risk of developing iron overload and clinical complications

Females show much lower rates of progression to symptomatic disease resulting from iron deposition.

There is no sex difference in the inheritance of haemochromatosis alleles, but males are more likely to develop iron overload and progress to clinical disease. This is assumed to result from females having greater iron loss than males, due to menstruation. The prevalence of iron overload-related complications (e.g. cirrhosis, hepatocellular carcinoma, biochemical evidence of liver damage, arthropathy) in people who are homozygous for C282Y is reported to be 28% in males and 1% in females (see: "Genetic terminology and haemochromatosis", Page 18 for an explanation of genetic terms).[6]

Who to test and what to look for

Early symptoms of haemochromatosis are non-specific

In the early stages of haemochromatosis, patients may experience vague, non-specific symptoms, such as lethargy or gastrointestinal symptoms. In more advanced haemochromatosis, symptoms arise as a result of iron overload causing damage to specific organs.

Patients may experience:[2, 6, 7]

- Early, non-specific symptoms
  - Lethargy, apathy, malaise
  - Weight loss
  - Gastrointestinal symptoms, abdominal pain
- Symptoms arising from clinical consequences:
  - Arthalgias (from joint effects; hand and knee joints are most commonly involved[8, 9])
  - Loss of libido, erectile dysfunction, amenorrhea (from reproductive system effects)
  - Chest pain, shortness of breath (from cardiac effects)
  - Weight loss, frequent urination and symptoms of diabetes (from effects on the pancreas); consider assessing ferritin and transferrin saturation in adult patients with a new presentation of type 1 diabetes
  - Sensitivity to cold, weight gain (from thyroid effects, e.g. hypothyroidism)

Signs to specifically look for on clinical examination include:[2, 6, 7]

- Liver tenderness, hepatomegaly and other signs of liver disease (e.g. cutaneous signs of chronic liver disease)
- Skin pigmentation or nail changes, porphyria cutanea tarda (discolouration or lesions on light-exposed skin such as the back of the hands), koilonychia (spoon-shaped nails)
- Oedema and signs of congestive heart failure
- Testicular atrophy and gynaecomastia in males
- Loss of body hair
- Early osteoarthritis

Biochemical testing of high iron stores

The key laboratory tests for the evaluation of body iron stores in patients with suspicious signs or symptoms are:

- Ferritin levels: increased hepatic iron stores causes elevated ferritin levels, but elevations may be due to other non-specific causes
- Transferrin saturation: transferrin binds iron in the circulation. Transferrin saturation is calculated by measuring serum iron levels and iron binding capacity.[10]

N.B. A fasting sample may improve the accuracy of results if there is uncertainty about an abnormal result.[2]

Haemochromatosis is likely in patients with elevated ferritin (> 300 micrograms/L in males or > 200 micrograms/L in females) or transferrin saturation (> 45%) levels which cannot be explained by other reasons. Elevated ferritin is a less specific marker for haemochromatosis as there are a number of clinical scenarios which can result in abnormal test results. Clinical guidelines differ as to whether elevations in both ferritin and transferrin saturation are necessary, or one alone is sufficient, for further follow-up for haemochromatosis.[2, 6, 11] Table 1 offers guidance on appropriate follow-up of ferritin and transferrin saturation results. Clinicians should **only request genetic testing in a patient with biochemical evidence of abnormal iron metabolism***.

Other possible reasons a patient may have elevated ferritin include:[6, 12]

- Acute illness or inflammation: the inflammation associated with an infection, or chronic inflammatory conditions, causes increased ferritin. Measuring CRP can help distinguish these patients. Evaluation of iron metabolism should be repeated or delayed until after the acute illness has resolved.
- Alcohol intake: alcohol increases ferritin levels. Patients should be questioned about their alcohol consumption and, if indicated, liver function tests requested.
- Other forms of liver disease: patients with non-alcohol fatty liver disease, hepatitis or alcoholic liver disease can have elevated ferritin levels.

* Or if performed as part of family screening. However, screening of asymptomatic family members is not recommended unless performed under the advice of a clinician with relevant genetic experience or after discussion with a genetic counsellor, see: "Screening of first degree relatives", Page 19.

- Iatrogenic: people who receive blood or iron transfusions may have elevated iron levels following a transfusion
- Cancer: some tumours and tissue necrosis can lead to elevated ferritin[10]
- Excessive dietary intake of iron or vitamin C through supplementation

Although initial biochemical testing is performed to establish a diagnosis, it may also reveal information of prognostic importance about the presence of organ damage due to iron overload, e.g. ferritin levels > 1000 micrograms/L can indicate iron-overload induced cirrhosis.

Patients with biochemical results suggestive of haemochromatosis which cannot be explained by other diagnoses should undergo genetic testing, particularly if they have a family history or symptoms and signs of haemochromatosis.[2] Requests for haemochromatosis gene testing can be made according to local guidelines. Clinicians can contact Genetic Health Services New Zealand for advice, or refer patients to the service, if there are questions that are specifically related to family risk they cannot answer.

Further information and contact details for Genetic Health Services New Zealand is available on their website:

www.genetichealthservice.org.nz

For more about Genetic Health Services New Zealand, see:

www.bpac.org.nz/BT/2014/November/ghsnz.aspx

www.bpac.org.nz/BT/2014/November/genetic-tests.aspx

Incidental discovery of asymptomatic patients

Asymptomatic patients with haemochromatosis may be identified during investigation for other conditions. The finding of abnormally high iron levels in a patient, particularly if associated with abnormal liver function tests, should be considered as suspicious for haemochromatosis.[2] These findings should be followed up by biochemical testing for abnormal iron metabolism, particularly in patients with a first degree relative with hereditary haemochromatosis. A radiologist may also suggest that a patient be assessed for iron overload due to suspicious signs on radiological examinations for other conditions, e.g. chondrocalcinosis often occurs in patients with hereditary haemochromatosis.[9]

Table 1: When to request genetic testing in a patient with biochemical evidence of abnormal iron metabolism[2, 6, 11]

	Ferritin normal	Ferritin elevated
Transferrin saturation < 45%	Haemochromatosis highly unlikely; other reasons for any signs or symptoms should be investigated	Haemochromatosis possible; investigate other reasons for elevated ferritin and if other causes ruled out (and elevated ferritin persists), proceed to genetic testing
Transferrin saturation > 45%	Proceed to genetic testing for haemochromatosis	Proceed to genetic testing for haemochromatosis

Genetic terminology and haemochromatosis

Alleles versus mutations and genetic testing for haemochromatosis:

Mutations are changes in the genetic code which are rare in the population (with a prevalence of <1%)

Alleles are differing versions of a gene which are common in the population. For any gene there are usually at least two relatively common alleles in the population.

Alleles of the HFE gene which contribute to the development of haemochromatosis are common. For example, in a study in Christchurch, 38.4% had at least one risk allele (out of 1064 people).

Most cases of haemochromatosis are due to common risk alleles of the HFE gene and these are assessed in routine genetic testing for haemochromatosis. However, some patients may develop haemochromatosis due to other mutations in genes involved in iron metabolism. Further genetic testing would be required to identify underlying genetic causes of haemochromatosis in these patients.

Haemochromatosis allele terminology

There are two main alleles of interest for the investigation of haemochromatosis: C282Y and H63D. In laboratory test results or clinical correspondence, these alleles may be written in different ways but are all synonymous:

- C282Y allele: may also be referred to as Cys282Tyr or Cys → Tyr 282
- H63D allele: may also be referred to as His63Asp or His → Asp 63

Homozygous and heterozygous: This refers to how many copies of a mutation or allele a person has. Someone who is heterozygous has one copy, someone who is homozygous has two. Most cases of haemochromatosis are due to people being homozygous for the C282Y allele.

Recessive versus dominant: In genetic conditions which are dominant, patients only need to have one copy of an allele or mutation to develop a clinical condition. Hereditary haemochromatosis is recessive, so that a person who has one copy of a risk allele is not at risk of developing complications from iron overload; two copies are necessary.

Penetrance: This term describes how likely it is that someone with a specific genotype will develop a clinical condition. The risk alleles for hereditary haemochromatosis show low penetrance: only 10% of people homozygous for haemochromatosis risk alleles develop the clinical effects of haemochromatosis. For this reason, genetic screening of the general population is not recommended.[2]

In summary, many people in the population have at least one risk allele for haemochromatosis. However, a person would need two copies of common risk alleles to be at risk of developing haemochromatosis (or have other rare dominant mutations). Even with two copies of common risk alleles not all people will develop symptoms or biochemical evidence of abnormal iron metabolism.

Genetic testing for hereditary haemochromatosis

Standard genetic testing for haemochromatosis assesses whether a patient has common risk alleles and how many copies they have.

A diagnosis of HFE hereditary haemochromatosis can be made on the basis of biochemical signs of iron overload (increased ferritin and/or transferrin saturation) and the presence of risk alleles.

Most cases of hereditary haemochromatosis (approximately 80%) are due to a patient inheriting a C282Y allele of the HFE gene from both parents (homozygous for the C282Y HFE allele).[2] This C282Y allele is known as the major risk allele. Another risk allele is H63D; this is more prevalent in the population but less likely to cause haemochromatosis and is referred to as a minor risk allele. Approximately 5% of cases are due to inheriting one copy each of the C282Y and H63D alleles (referred to as "compound heterozygous").[3]

The most important findings from genetic testing for the diagnosis and management of patients are identifying those with:

- **Two C282Y alleles, i.e. homozygous for the major risk allele.** These patients are most likely to develop iron overload and clinical complications
- **One C282Y allele and another minor risk allele** (e.g. a C282Y/H63D genotype). People with this genotype may also develop haemochromatosis although are less likely to do so than people homozygous for two C282Y alleles. Particular attention should be paid to excluding other causes of elevated ferritin before establishing a diagnosis of haemochromatosis in these patients.
- **Variant genotype.** Biochemical evidence of iron overload or even elevated iron stores on liver biopsy but with a genotype other than those above (e.g. patients with a H63D/H63D genotype with elevated ferritin).[2, 13] These patients do not develop sufficient iron deposition to progress to clinical disease and can be reassured that they do not need treatment.[2]

Genetic testing forms part of the diagnostic process and can provide some prognostic information, however, clinical management and treatment is the same for all individuals with hereditary haemochromatosis who develop iron overload, regardless of their underlying genotype.

Screening of first degree relatives

Upon learning they have a condition with a strong genetic component, patients may enquire about genetic testing of family members or relatives. In general, the evaluation of family members should follow the same diagnostic process described here: family members with symptoms or signs that may be suggestive of haemochromatosis should have ferritin and transferrin saturation tests performed to establish whether they have biochemical evidence of abnormal iron metabolism, and if so, be followed up with genetic testing.

Hereditary haemochromatosis is an adult onset disorder, so testing children in affected families is not indicated.

Once children reach adulthood, the family diagnosis can be discussed with them and if patients without symptoms or biochemical evidence of altered iron metabolism wish to undergo genetic testing they should first be referred to a genetic counsellor; Genetic Health Services New Zealand recommends that genetic testing of asymptomatic adult family members of an affected individual should only be undertaken following the recommendation of a clinician with relevant genetic experience or after discussion with a genetic counsellor. It is important to note that people can be genetically at risk, with one of the above combinations of haemochromatosis alleles, but not progress to develop haemochromatosis. Therefore, genetic testing of asymptomatic family members may identify people who are genetically at risk but without biochemical evidence of abnormal iron metabolism or suspicious symptoms and signs. If this is the case, these family members should have serum transferrin and ferritin levels measured annually to monitor the potential development of iron overload.[6]

For further information, see: "Possible patterns of inheritance of haemochromatosis alleles", Page 23.

Non-HFE hereditary haemochromatosis and other diagnoses

Patients with unexplained elevated ferritin or transferrin saturation, or clinical signs suggestive of haemochromatosis but without identified HFE risk alleles after referral to genetic testing services should be followed up, particularly if aged 20 years and under (see "**Other forms of haemochromatosis**"). Referral to a haematologist is recommended.

Some patients, with a family history of the condition, develop haemochromatosis but without identifiable risk alleles or

mutations in the HFE gene, and are classified as having non-HFE hereditary haemochromatosis. These patients are uncommon, making up approximately 5% of all people with hereditary haemochromatosis.[2] Risk alleles or mutations in other genes involved in iron homeostasis may be the underlying cause of their condition. Standard genetic testing for haemochromatosis assesses the presence of common risk alleles and screening for other alleles or mutations would only be performed if there were indications for doing so.

Patients with unexplained biochemical evidence or signs of iron overload may require investigation for rare diagnoses: ineffective erythropoiesis, such as in β-thalassemia, can cause inappropriately high iron levels; patients may show signs such as splenomegaly or jaundice or have biochemical evidence of microcytosis and hypochromia.[1, 14]

Following diagnosis: treatment and prevention of complications

Following a diagnosis, the most important clinical steps are to investigate patients for the presence of haemochromatosis complications and initiate treatment. Patients should be assessed for the presence of complications arising from iron overload and treated, such as diabetes mellitus, joint disease, endocrine disturbances (hypothyroidism and hypogonadism), cardiac disease, porphyria cutanea tarda and osteoporosis.[6]

The key clinical intervention for treating haemochromatosis is venesection (phlebotomy) to reduce iron stores. **Clinical guidelines recommend that all patients with haemochromatosis are offered venesection to normalise ferritin levels.** Some patients with hereditary haemochromatosis may maintain mildly elevated iron stores without progressing to clinical complications, such as liver disease, arthropathy, heart problems or other conditions resulting from iron overload. For these patients venesection may represent a form of overtreatment. However, as there is no reliable method of predicting which patients will develop complications, and venesection is a low-risk procedure, clinical guidelines recommend offering venesection to all patients with elevated ferritin levels.[2] Where venesection is not initiated, patients should be monitored for worsening of biochemical measures of iron overload or the development of clinical complications, and to report if suspicious symptoms develop.[2]

Other forms of haemochromatosis

Haemochromatosis in young people

Younger patients (aged 20 years or less) with biochemical evidence of abnormal iron metabolism or suggestive signs and symptoms may have juvenile haemochromatosis. This is a rare condition caused by mutations in genes other than HFE, and results in more extreme iron overload and a worse clinical prognosis than HFE hereditary haemochromatosis in adults; they are likely to require follow-up with a haematologist.[2]

Iatrogenic haemochromatosis

In a minority of patients elevated iron can occur due to non-hereditary causes, such as excessive iron intake due to supplementation, or from receiving blood transfusions on an ongoing basis resulting in inappropriately high iron levels.

Table 2: Key practice points for venesection in hereditary haemochromatosis[2]

How often should venesection be performed?	How much blood should be removed?	Measures during treatment		Measurement targets
		Analyte	When to measure	
Initially every one to two weeks until ferritin normalises	500 mL (one unit) per session	Haematocrit Haemoglobin	Baseline and before each session	Within 80% of baseline values – delay venesection if values fall below 80%
		Ferritin	Baseline and check after four venesections	50 – 100 micrograms/L

Referral for ultrasound and liver biopsy

Patients with haemochromatosis and ferritin levels > 1000 micrograms/L have been found to have a prevalence of cirrhosis ranging from 20 to 45%.[2] As raised liver enzymes may also indicate cirrhosis, it is recommended that:[2]

- Patients with ferritin > 1000 micrograms/L undergo an ultrasound and liver biopsy
- Patients with ferritin < 1,000 micrograms/L and with altered liver enzymes (AST, ALT) undergo an ultrasound and liver biopsy

It has been reported that a study of 670 patients with two haemochromatosis risk alleles found that ferritin levels > 1000 micrograms/L had a 100% sensitivity and 70% specificity for identifying cirrhosis, and conversely, no patients with cirrhosis had a ferritin level < 1000 micrograms/L. See "**Monitoring clinical complications"**, Page 24, for further information.

Liver biopsy is the gold standard technique for the assessment of liver complications in patients with haemochromatosis, as it allows for the histological assessment of the degree of liver damage and direct measurement of hepatic iron content.[2] Liver biopsy can be prone to sampling error, and ultrasound assessment of the liver allows a wider assessment of the distribution of cirrhotic or fibrotic liver tissue.[15]

Venesection normalises ferritin levels

Venesection to reduce iron stores may be performed under the guidance of a haematologist or a general practitioner. For patients unable to tolerate venesection or where it is contraindicated, referral to a haematologist is recommended; treatment with deferoxamine mesilate (subcutaneous infusion for iron overload) in a hospital setting may be an option.

The aim is to reduce ferritin levels to 50 – 100 micrograms/L by having patients undergo venesection every one to two weeks, removing 500 mL of blood each time, until this treatment target is reached. This amount of blood would typically contain 200 – 250 mg of iron.[2] Targeting treatment below 50 micrograms/L has been found to cause a paradoxical increase in iron absorption.[2] Table 2 shows treatment and monitoring information for patients undergoing venesection.

There is a high degree of individual variability in the rate of iron accumulation after venesection ceases, and little evidence to guide subsequent monitoring and treatment.[2, 6] Current practice is for patients to be monitored for changes in serum ferritin and transferrin saturation to guide when or whether to re-initiate venesection.

Adverse effects and patient experiences of venesection

Patients often experience adverse effects with venesection treatment and these are similar to the adverse effects of blood donation. Most commonly these include tiredness, loss of appetite and pain or discomfort at the needle site.[16] Light-headedness and fainting during or after the procedure is also possible.

In one survey of over 200 patients, 50% reported experiencing adverse effects during most or all venesection treatments. In addition, the time burden of attending regular venesection sessions was rated as influencing their work or daily routine "most of the time" or "all of the time" by 50% of patients. However, there was an overall high level of acceptance of venesection, with 87% of patients regarding treatment as worthwhile and only 16% reporting that they would opt out of venesection if another treatment was available.[16]

Patient guides and resources:

- Leukaemia and Blood Cancer New Zealand: a patient guide to haemochromatosis and venesection record book are available at: **www.leukaemia.org.nz/page/387**
- Since 2011 haemochromatosis patient support has been available from Leukaemia and Blood Cancer New Zealand, which was formerly provided by IRONZ, the New Zealand Haemochromatosis Support & Awareness Group: **www.leukaemia.org.nz**

Treatment improves some symptoms and complications, but not others

Patients undergoing venesection often experience improvement in subjective symptoms of lethargy and abdominal pain, and changes in skin pigmentation. Biochemical measures of liver function and diabetes control (if present) generally improve, and liver fibrosis has been shown to reverse in 30% of cases.[10] However, venesection does not reverse all the characteristic symptoms and sequelae of iron overload: liver cirrhosis, arthropathy, testicular atrophy or thyroid dysfunction, and the symptoms patients experience as a result of these complications, usually do not improve with treatment.[10] Consultation with, or referral to, an appropriate specialist is recommended for the management of complications due to haemochromatosis.

Studies suggest that patients with haemochromatosis who are adequately treated, and do not have liver cirrhosis or diabetes, have the same life expectancy as the general population.[6]

Dietary advice

Given that haemochromatosis involves increased iron absorption in the gut, restricting dietary iron would appear at face value to be an intuitive treatment. However, there is limited evidence to support a change in diet. Patients with haemochromatosis should avoid dietary supplements containing iron, and also avoid supplements with vitamin C.[3, 6, 10] Since people with haemochromatosis are already at risk of liver disease, it is recommended that patients are advised to limit alcohol intake.

A systematic review published in 2013 found that no randomised controlled trials had assessed dietary iron reduction and its effects on haemochromatosis management.[17] However, from available limited evidence the authors estimated that dietary iron reduction could reduce the need for venesection by one to three sessions per year, depending on patient characteristics. Therefore, dietary iron reduction may reduce clinical burden but there is no data on the longer-term effects of dietary iron reduction on prognosis.

Possible patterns of inheritance of haemochromatosis alleles*

		Genetic risk of haemochromatosis for offspring **
Two C282Y heterozygote parents	**Parents** C282Y / – (other allele not associated with haemochromatosis) + C282Y / – **Offspring** C282Y / C282Y C282Y / – – / C282Y – / –	One in four homozygous for C282Y, at risk of hereditary haemochromatosis Two in four heterozygous for C282Y, not at risk of hereditary haemochromatosis but may pass risk to their own offspring One in four without C282Y risk alleles
One C282Y heterozygote parent + one C282Y homozygote parent	**Parents** C282Y / C282Y + C282Y / – **Offspring** C282Y / C282Y C282Y / C282Y C282Y / – C282Y / –	One in two homozygous for C282Y, at risk of hereditary haemochromatosis One in two heterozygous for C282Y, not at risk of hereditary haemochromatosis but may pass risk to their own offspring
One C282Y heterozygote parent + one H63D heterozygote parent	**Parents** C282Y / – + H63D / – **Offspring** C282Y / H63D C282Y / – – / H63D – / –	One in four with "compound heterozygote" genotype C282Y/H63D. At risk of hereditary haemochromatosis, but risk is lower than C282Y/C282Y genotype Two in four heterozygous for C282Y or H63D. Not at risk of haemochromatosis but may pass risk to their own offspring One in four without risk alleles
One heterozygous parent + one parent without risk alleles	**Parents** C282Y / – + – / – **Offspring** C282Y / – C282Y / – – / – – / –	Since condition is recessive no offspring are at risk, although two in four may pass risk to their own offspring

* The purpose of this table is to highlight common scenarios. Other combinations of parental genotypes are possible, e.g, in the rare circumstance that both parents are homozygous for risk alleles, all offspring will be genetically at risk for haemochromatosis.

** These figures represent theoretical mathematical averages. It is possible in clinical practice to find a family where siblings have a distribution of risk alleles which differs from that shown here.

Monitoring clinical complications

The range of complications and other conditions which can arise from iron overload is diverse. Particular attention should be paid to the potential development of liver disease. Clinicians should also ensure that patients with haemochromatosis have up to date hepatitis A and B vaccinations to reduce the risk of liver damage.

Haemochromatosis can result in a wide range of complications due to the deposition of iron in tissues around the body. There are no specific guidelines for monitoring all of these complications. In general, clinicians should be aware that patients with haemochromatosis may present with a variety of symptoms, and have a lower threshold for investigating other conditions. During ongoing management clinicians should be alert for the development of symptoms and signs suggestive of complications related to iron overload.

Patients with haemochromatosis are at increased risk of liver disease

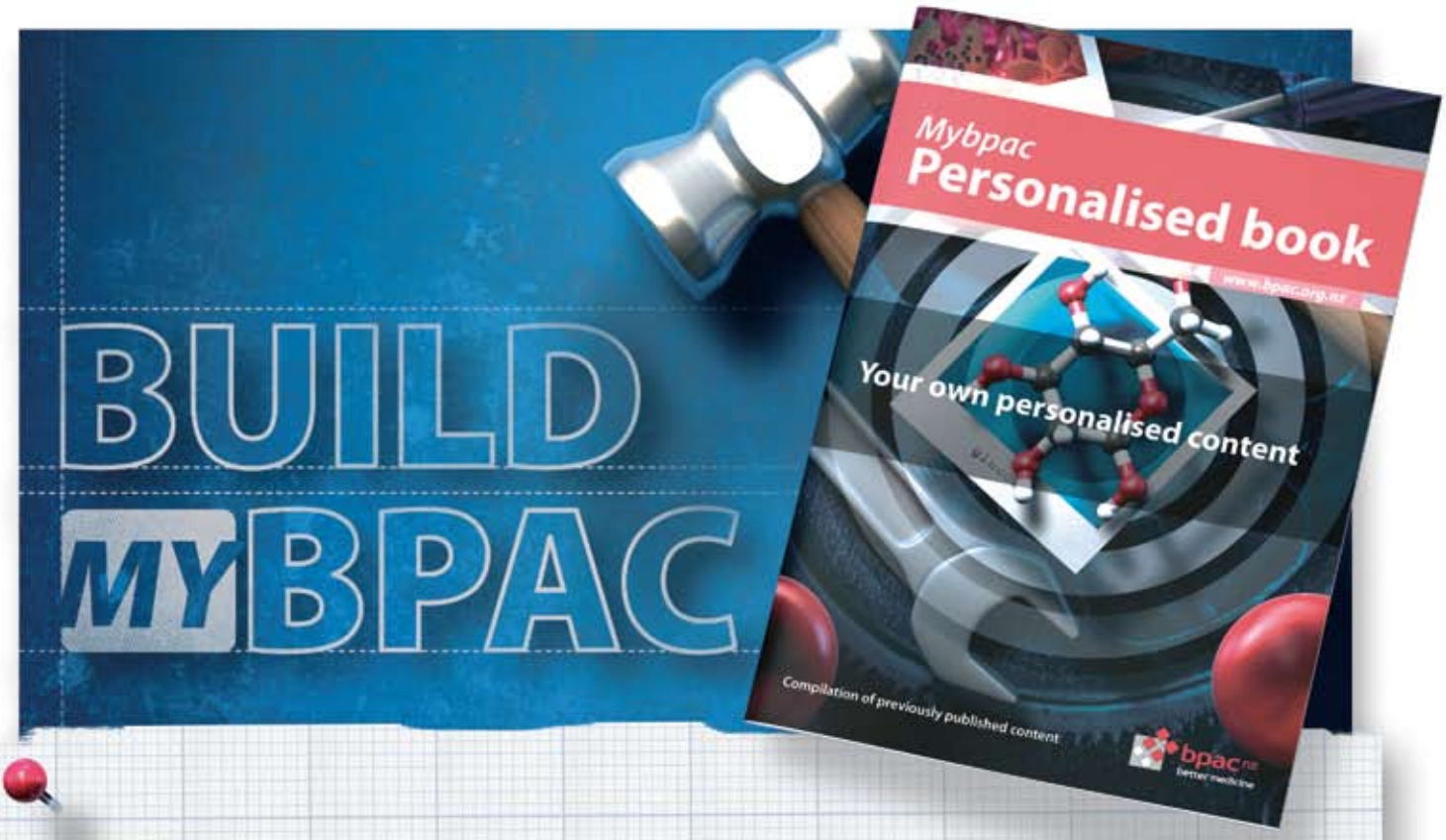
The development of liver disease is one of the most pressing concerns in the management of patients with haemochromatosis. Patients are at greatly increased risk of hepatic fibrosis and cirrhosis, as well as hepatocellular carcinoma. Key points include:

- The absolute risk of liver disease in people with two C282Y risk alleles is approximately 5% for males and 1% for females[3]
- People with haemochromatosis and cirrhosis have a reduced life expectancy[6]
- Hepatocellular carcinoma is reported to account for 30% of deaths in people with haemochromatosis (very rarely without cirrhosis[10]
- Patients with hereditary haemochromatosis and cirrhosis should be screened for the development of hepatocellular carcinoma by ultrasound every six to twelve months,[10] or an alternative screening strategy discussed with a haematologist or oncologist.

ACKNOWLEDGEMENT: Thank you to **Dr Joanne Dixon**, National Clinical Director, Genetic Health Service New Zealand for expert review of this article.

References:

1. Ganz T. Systemic iron homeostasis. Physiol Rev 2013;93:1721–41.
2. Bacon BR, Adams PC, Kowdley KV, et al. Diagnosis and management of hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases. Hepatology 2011;54:328–43.
3. Bokhoven M, Deursen C, Swinkels D. Diagnosis and management of hereditary haemochromatosis. BMJ 2011;342:c7251–c7251.
4. Burt MJ, George PM, Upton JD, et al. The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 1998;43:830–6.
5. McLaren GD, Gordeuk VR. Hereditary hemochromatosis: insights from the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Hematology Am Soc Hematol Educ Program 2009;195–206.
6. European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol 2010;53:3–22.
7. Sood R, Bakashi R, Hegade VS, et al. Diagnosis and management of hereditary haemochromatosis. Br J Gen Pract 2013;63:331–2.
8. Harty LC, Lai D, Connor S, et al. Prevalence and progress of joint symptoms in hereditary hemochromatosis and symptomatic response to venesection. J Clin Rheumatol 2011;17:220–2.
9. Sahinbegovic E, Dallos T, Aigner E, et al. Musculoskeletal disease burden of hereditary hemochromatosis. Arthritis Rheum 2010;62:3792–8.
10. Crownover BK, Covey CJ. Hereditary hemochromatosis. Am Fam Physician 2013;87:183–90.
11. Vanclooster A, Cassiman D, Van Steenbergen W, et al. The quality of hereditary haemochromatosis guidelines: A comparative analysis. Clin Res Hepatol Gastroenterol Published Online First: 23 October 2014.
12. Alkhateeb AA, Connor JR. The significance of ferritin in cancer: anti-oxidation, inflammation and tumorigenesis. Biochim Biophys Acta 2013;1836:245–54.
13. Pedersen P, Milman N. Genetic screening for HFE hemochromatosis in 6,020 Danish men: penetrance of C282Y, H63D, and S65C variants. Ann Hematol 2009;88:775–84.
14. Martin A, Thompson AA. Thalassemias. Pediatr Clin North Am 2013;60:1383–91.
15. Lefton HB, Rosa A, Cohen M. Diagnosis and epidemiology of cirrhosis. Med Clin North Am 2009;93:787–99, vii.
16. Brissot P, Ball S, Rofail D, et al. Hereditary hemochromatosis: patient experiences of the disease and phlebotomy treatment. Transfusion 2011;51:1331–8.
17. Moretti D, van Doorn GM, Swinkels DW, et al. Relevance of dietary iron intake and bioavailability in the management of HFE hemochromatosis: a systematic review. Am J Clin Nutr 2013;98:468–79.



Build My bpac is now available for all website users.

"**Build My bpac**" gives you the ability to create your own personalised booklet using content from our published articles.

This functionality is now available for all registered users, and allows whole articles, or individual sections from articles to be selected for inclusion in your own personal booklet.

You will have the ability to add your own title, insert notes on the sections you save, rearrange content and download the booklet for a desktop reference or printing.

Use **Build My bpac** as a way to create a customised journal related to a specific field of medicine, or as your own study or quick reference guide.

We hope you'll find this new function useful, and would welcome your feedback – so please feel free to contact us at: **contact@bpac.org.nz**

Log in, and start building!

www.bpac.org.nz/Mybpac/MyBook

visit us at **www.bpac.org.nz**

Call us on **03 477 5418** Email us at **contact@bpac.org.nz** Freefax us on **0800 27 22 69**