

## REVIEW

### Clinical usefulness of tumour markers

LC LAI (Chairman), \*SK CHEONG, \*\*KL GOH, \*CF LEONG, \*\*\*CS LOH , \*\*\*\*JB LOPEZ, \*H NAWAWI, \*\*V SIVANESARATNAM and \*\*\*\*\*R SUBRAMANIAM.

Clinical Practice Guidelines Committee on Tumour Markers of the Academy of Medicine of Malaysia. Apart from the chairman authors are listed in alphabetical order.

*Faculty of Medicine, International Medical University, \*Faculty of Medicine, Universiti Kebangsaan Malaysia, \*\*Faculty of Medicine, Universiti Malaya, \*\*\*Gleneagles Intan Medical Centre, Jalan Ampang, Kuala Lumpur, \*\*\*\*Institute for Medical Research, Jalan Pahang, Kuala Lumpur, and \*\*\*\*\*Fetal Medicine & Gynaecology Centre, Wisma WIM, Taman Tun Dr. Ismail, Kuala Lumpur.*

#### Abstract

Tumour markers are substances related to the presence or progress of a tumour. An ideal tumour marker is (1) detectable only when malignancy is present, (2) specific for the type and site of malignancy, (3) correlates with the amount of malignant tissue present and (4) responds rapidly to a change in tumour size. At present, no tumour marker fulfills all of the above criteria. The first part of the review discusses the clinical usefulness of the commonly requested serum tumour markers, namely, prostate-specific antigen (PSA), CA 19-9, carcinoembryonic antigen (CEA), CA 125, CA 15-3, human chorionic gonadotrophin (hCG) and  $\alpha$ -foetoprotein (AFP). It is hoped that this review article will decrease the abuse and misuse of these commonly requested serum tumour markers. The second part of the review discusses the clinical usefulness of catecholamines and their metabolites, calcitonin, thyroglobulin, parathyroid hormone, prolactin, adrenocorticotrophic hormone, oestrogen and progesterone receptors, p53, HER-2/c-erbB2, BRCA1 and BRCA2.

*Key words:* tumour markers, prostate cancer, pancreatic cancer, colorectal cancer, breast cancer, ovarian cancer, liver cancer, choriocarcinoma, germ cell tumours

#### INTRODUCTION

Tumour markers are substances related to the presence or progress of a tumour. There are four main groups of tumour markers:

##### 1. Structural molecules

Structural molecules are commonly found on the cell surface. They are common to many epithelial cells, Hence, they are of little value in identifying tumour type. Examples of tumour markers which are structural molecules are carcinoembryonic antigen (CEA), mucins like CA 19-9, CA 15-3 and CA 125,  $\beta$ 2-microglobulin and cytokeratins like CYFRA 21-1 and tissue polypeptide antigen.

##### 2. Secretion products and enzymes

Examples of secretion products and enzymes include alpha-foetoprotein (AFP), human chorionic gonadotrophin (hCG), paraprotein

and Bence-Jones protein, prostate-specific antigen (PSA), neuron-specific enolase (NSE) and placental-like alkaline phosphatase.

##### 3. Non-specific markers of cell turnover

These include neopterin and thymidine kinase.

##### 4. Cellular markers

Examples of cellular markers are Philadelphia chromosome, oncogenes, tumour suppressor genes, oestrogen receptor, progesterone receptor, epidermal growth factor receptor and c-erbB-2.

An ideal tumour marker is:

- detectable only when malignancy is present.
  - specific for the type and site of malignancy.
  - correlates with the amount of malignant tissue present.
  - responds rapidly to a change in tumour size.
- At present, no tumour marker fulfills all of the above criteria.

Tumour markers are used for one of more of the following uses:

- Screening )
- Diagnosis ) *of limited value*
- Prognosis )
- Monitoring response to treatment) *valuable functions*
- Early detection of recurrence }

In general, there are only a few markers which are of use in screening and diagnosis of tumours or in determining prognosis. If a tumour marker has been found to be raised in the serum of a patient who has had a tumour diagnosed histologically then the tumour marker may be useful in monitoring response to therapy and in the early detection of recurrence. Probably only hCG when used as a marker for choriocarcinoma is used for all of the above.

In the USA, the only tumour marker which has been approved for screening is PSA for prostate cancer.

Tumour markers are often used inappropriately in clinical practice and are often part of cancer screening and wellness profiles. This review article first discusses the clinical situations in which the commonly requested serum tumour markers (PSA, CA 19-9, CEA, CA 125, CA 15-3, AFP and hCG) are useful and, hopefully, will reduce the current widespread misuse of these tumour markers. We have produced clinical practice guidelines on the clinical utilisation of these seven commonly requested serum tumour markers (<http://www.acadmed.org>). In addition, this review article also discusses the clinical usefulness of some of the endocrine and newer tumour markers, namely, catecholamines and their metabolites, calcitonin, thyroglobulin, parathyroid hormone, prolactin, adrenocorticotrophic hormone, oestrogen and progesterone receptors, p53, HER-2/c-erbB2, BRCA1 and BRCA2.

## PSA

Prostate cancer (CaP) is currently the most common cancer in men in many Western countries and is the second or third most common cause of death due to cancer. Serum total and prostatic acid phosphatase had, for many years, been used as a serum marker for this cancer but these tests lacked sensitivity, often only becoming abnormal in the presence of metastasis and have been largely replaced by serum PSA.<sup>1</sup>

Prostate specific antigen (PSA) was first identified as a tissue specific antigen in the prostate<sup>2</sup> and later found to be immunologically similar to a seminal plasma protein identified earlier.<sup>3</sup> The development of a serum assay<sup>4</sup> quickly led to its evaluation and use as a marker for prostate cancer in the few years that followed.<sup>5</sup>

## Biochemistry of PSA

PSA is a neutral protease consisting of a 34-kilodalton single-chain glycoprotein of 240 amino acid residues with a carbohydrate side chain. It is related to the kallikrein family. It is found in large quantities in prostatic epithelial cells and seminal fluid. In the serum, three biochemical forms of PSA exist. The smallest proportion exists in the free form as found in the ejaculate. The largest proportion of PSA in the serum is bound to  $\alpha$ 1-antichymotrypsin, a protein that inactivates its proteolytic activity. The third form of serum PSA is bound to  $\alpha$ 2-macroglobulin. This third form is not detected by commercially available PSA immunoassays as all its epitopes are concealed by the binding protein.<sup>6</sup> The serum half-life of PSA has been estimated to be 2 to 3 days.<sup>5,7</sup>

## Standardisation of PSA assay

Numerous commercial immunoassays for PSA are available, even within Malaysia, and variations among them are inevitable. International standards for free and total PSA for the calibration of different assays have been produced<sup>8-10</sup>. Until all laboratories adopt calibration of their PSA assays to these international standards clinicians should be wary of inter-laboratory variation.

## Factors that alter PSA concentration

In general, PSA levels correlate with age<sup>11,12</sup> and this has been attributed to a higher incidence of benign prostatic hyperplasia (BPH) in the elderly male population. However, this observation is not seen across all Asian populations. The Koreans, for example, have reported a poorer correlation of PSA levels with age<sup>13</sup> contrary to the findings in Taiwanese.<sup>14,15</sup> The relation between PSA levels and BPH has also been the subject of extensive study since Stamey's initial report.<sup>5</sup> Attempts to correlate PSA levels with volume of hyperplastic tissue have not been conclusive<sup>16-18</sup> probably due to a large variation of epithelial contribution to the total mass of hyperplastic tissue.<sup>19</sup> In general, PSA levels appear to fall substantially following surgical resection for BPH<sup>5</sup> although less invasive ablation

techniques produce a moderate fall.<sup>20</sup> Finasteride causes a 50% decrease in PSA levels<sup>21</sup> but herbal products such as saw palmetto (*Serenoa repens*) in its pure form<sup>22</sup> and Permixon<sup>23</sup> appeared not to decrease PSA levels significantly. All lower tract endoscopic manipulation can result in a rise in serum PSA concentration. A 53-fold increase after transurethral resection of the prostate (TURP) for benign prostatic hyperplasia (BPH) has been reported whereas the rise (1.25 fold) was modest after TURP of the cancerous prostate.<sup>17,18</sup> Transrectal and transperineal prostate biopsy have also been reported to increase PSA levels and the decline to baseline did not follow the half-life prediction.<sup>24</sup> However, the PSA levels in the majority of cases reached baseline by one month although 7% still had elevated levels. PSA levels should not be measured after acute urinary retention as marked elevations have been reported.<sup>25</sup> The effect of digital rectal examination (DRE) has been widely studied and most workers agree that the rise of PSA after DRE is insignificant.<sup>24,26</sup> Prostatic massage also causes a transient PSA elevation<sup>5</sup>. Similarly, prolonged cycling can cause a rise in PSA,<sup>27</sup> probably from the pressure on the perineum by the bicycle seat although the rise was more significant in subjects with an initially raised PSA.<sup>28</sup> PSA levels are also affected by certain physical activities. PSA rises transiently after sexual activity, peaking approximately 1 hour after ejaculation and returning to baseline within approximately 24 hours.<sup>29</sup> In non-malignant prostate disease, PSA has been reported to be markedly raised in acute infection affecting the prostate and levels up to 100 ng/ml can be encountered.<sup>30</sup>

#### PSA levels in prostate cancer

Both benign and malignant tissues produce PSA. Most clinicians have adopted the reference range of 0 to 4 ng/ml first reported by Myrtle et al (1986)<sup>31</sup> using the Tandem-R PSA assay. In screening programmes, approximately 30% of Western men who are asymptomatic with PSA levels above 4 ng/ml will have prostate cancer.<sup>11,32</sup> With the additional finding of an abnormal digital rectal examination, an incidental PSA of >4 ng/ml is associated with a cancer predictive value close to 50%.<sup>33</sup> Similar studies have not been conducted in the Malaysian male population but personal experience and local reports<sup>34-36</sup> suggest a lower incidence of cancer in the local population using this cut-off. Serum PSA levels have also been shown to have a role in cancer staging. In general, PSA levels correlate with

both clinical and pathological stages.<sup>31</sup> In localised cancers, incidence of capsular penetration on histological examination is higher in patients presenting with PSA levels of greater than 10 ng/ml.<sup>37,38</sup> Prostatic intraepithelial neoplasia (PIN), particularly high grade PIN, is a histological change regarded as a prelude to invasive carcinoma. PSA elevation associated with histological evidence of PIN appeared to be intermediate between what was observed in BPH and in cancer. It was postulated that the observed PSA elevation was probably due to associated occult invasive carcinoma.<sup>39</sup> Thus, the finding of high grade PIN in a man with elevated PSA implies a probable existence of an occult invasive cancer.

#### Improving PSA performance as a tumour marker

In spite of its increasing popularity, the limitations of PSA are all too obvious. Using a cut-off of 4.0 ng/ml, approximately a quarter of all newly diagnosed cancers in the West present with normal PSA levels. Conversely, some two thirds of men with abnormal PSA levels will be shown to have non-cancerous pathology on ultrasound-guided biopsy. The incidence of failure to demonstrate cancer is higher in our local population based on local reports.<sup>34-36</sup>

Various surrogates of PSA assays have been suggested to increase PSA performance. Age-specific PSA cut-off levels were suggested by Oesterling et al. (1993)<sup>12</sup> and Dalkin et al. (1993).<sup>40</sup> Generally, PSA increases with age. This observation is probably not an intrinsic phenomenon of ageing but more likely due to the fact that incidence of prostate pathology (including cancer) increases with age. A lower PSA cut-off in the younger age group will increase sensitivity and a higher cut-off in the elderly age group improves specificity at the expense of lower cancer detection rate. Although some would argue that this is not entirely undesirable in this age group, Brawer (1995)<sup>41</sup> concluded that in the setting of a screening program for prostate cancer, the overall number of years of life saved, using age-specific cut-off levels, would be reduced.

Men with large prostate glands tend to have higher PSA levels and PSA density was initially enthusiastically proposed and shown to be more useful than plain PSA levels<sup>42-44</sup> but subsequent studies failed to reproduce this advantage.<sup>45,46</sup> Sampling was thought to be an important source of error in many of these studies as detection of cancer in a big gland was bound to be less likely

by random sampling unless the number of sampling sites increased corresponding to the size of the gland.

Using archival frozen serum specimen, Carter et al. (1992)<sup>47</sup> demonstrated that the rate of PSA increase (PSA velocity) was higher in cancer patients compared with controls and that this higher velocity was observable 10 years prior to clinical presentation. A PSA velocity in excess of 0.75 ng/ml/year was predictive of cancer. However, methods for calculation of PSA velocity have not been standardised and biological variation between measurements often exceeds this value making its calculation and use impractical.

After the characterisation of different molecular forms of PSA<sup>6,48</sup> and finding that there is a larger proportion of free PSA in BPH than in prostate cancer, there has been considerable interest in the exploitation of the free to total PSA ratio to improve the performance of PSA in cancer detection. In a multicentre trial, Catalona et al. (1997)<sup>49</sup> reported the use of free-to-total PSA ratio in 622 men with total PSA levels of 4.0 to 10.0 ng/ml with no abnormal digital rectal examination findings and concluded that by adopting a cut-off of 25% free-to-total PSA ratio, cancer detection can be maintained at 95% whilst avoiding negative biopsies in 20% of these patients. However, discrepancies between different assays,<sup>50</sup> the lack of an international standard, the lack of consensus on the optimal cut-off ratio and the group of patients on whom this measurement should be used continue to make this measurement impractical. Furthermore, storage conditions have been shown to affect stability especially of the free fraction of PSA and, thus, free to total PSA ratio determination.<sup>51</sup> Results from batch processing at centralised laboratories should, therefore, be interpreted with caution.

#### **PSA in the monitoring of prostate cancer treatment**

Despite the widespread interest in the diagnostic performance of PSA, most assays received approval in the USA only for monitoring of prostate cancer after treatment. PSA generally falls after institution of hormonal withdrawal and this fall has been accredited experimentally to a decrease in the number of viable prostate epithelial cells (malignant and benign) and decreased expression of PSA mRNA in these viable cells (Young et al., 1991; Henttu et al., 1992).<sup>52,53</sup> The absolute levels, nadir and rate of fall of PSA after androgen withdrawal have

been correlated with disease prognosis (Cooper et al., 1990; Siddall et al., 1986; Mecz et al., 1989).<sup>54-56</sup> Following radical prostatectomy for organ-confined prostate cancer, PSA levels should become undetectable within 2 to 4 weeks and rising PSA levels invariably denote disease recurrence. However, the time interval between PSA recurrence and eventual disease can be considerable. For example, the median actuarial time to metastases was 8 years from the time of PSA level elevation in the Johns Hopkins series (Pound et al., 1999).<sup>57</sup> Similarly, PSA levels fall after definitive radiotherapy but the actual nadir that predicts absence of clinical progression remains a matter for debate.

#### **PSA and prostate cancer screening**

In the Western World, it has been estimated that prostate cancer will be diagnosed in 10% of men during their lifetime, and 3 to 4 % will eventually die from the disease. Thus, considerable interest exists in population screening for prostate cancer using PSA measurement. There are compelling arguments against screening. Besides cost, there is the concern of overdiagnosis of clinically insignificant cancers. Large scale randomised controlled studies are underway to resolve this issue but it will be many years before a clear picture emerges. As a public health exercise, full population screening for prostate cancer in Malaysia is very difficult to justify as the disease is not as prevalent and the cost implications on the health care system will be enormous.

#### **Recommendations**

In spite of its many limitations the introduction of PSA assays has transformed the management of prostate cancer management. The American Cancer Society and the American Urological Association have recommended that American men over the age of 50 years should undergo a yearly PSA assay and digital rectal examination. As a public health exercise, this is both difficult to justify in Malaysia on grounds of epidemiology and cost and it is logistically impossible. However, PSA measurement can and should be used judiciously in populations at risk but the merits and limitations of this assay should be explained to the patients.

#### **CA 19-9**

##### **Introduction**

CA19-9 is a mucin which reacts with monoclonal antibody 111 6 NS 19-9. It is believed to be involved in cell adhesion and was originally

discovered in human colorectal carcinoma cell lines.

#### **Conditions where CA 19-9 levels may be elevated**

CA 19-9 may be elevated in pancreatic carcinomas (70-100% of cases), hepatocellular carcinoma (22-51%), gastric carcinoma (42%) and colorectal carcinoma (20%). It may also be elevated in benign conditions such as acute and chronic pancreatitis, cirrhosis, acute cholangitis and cystic fibrosis.<sup>58,59</sup> The elevation of CA 19-9 in benign pancreatic disease is lower than with carcinoma of pancreas and usually does not exceed 120 U/ml. Its greatest usefulness is in pancreatic cancer.

#### **Clinical usefulness for pancreatic cancer**

**Screening:** CA 19-9 should not be used for screening for pancreatic cancer. The sensitivity of CA 19-9 in early (small) pancreatic cancers is low.<sup>60</sup>

**Diagnosis:** Elevated CA 19-9 is of limited use in the diagnosis of pancreatic cancer. In advanced pancreatic cancer, CA 19-9 would be elevated but clinical symptoms and other modalities (imaging) are probably more useful. Nonetheless, it is useful in the differentiation from chronic focal pancreatitis when markedly elevated. It may be useful in guiding resectability of the tumour. Very high levels usually predict presence of unresectable tumours.<sup>59</sup>

**Monitoring response to treatment:** Useful for monitoring progress and response to therapy of patients being treated for pancreatic carcinoma.<sup>61,62,63</sup>

**Early detection of recurrence:** Useful in detecting recurrence early following pancreatectomy, when levels begin to rise.<sup>64</sup>

### **CARCINOEMBRYONIC ANTIGEN (CEA)**

#### **Introduction**

CEA is a 200 kDa glycoprotein and was first described in 1965 by Gold and Freedman<sup>65</sup> when they identified an antigen that was present in both foetal colon and colon adenocarcinoma but that was not found in normal human colonic tissue. As the protein is found in only cancer and embryonic tissue it was given the name carcinoembryonic antigen. CEA is a 200 kDa glycoprotein. Subsequent work has shown that it is also present in certain healthy tissues in low levels. Physiologically, it appears to play a role in cell adhesion.

#### **Conditions where CEA levels may be elevated**

It can be elevated in almost any advanced adenocarcinoma but particularly colorectal carcinoma when distant metastases are present.<sup>66</sup> It may also be elevated in breast cancer, gastric and lung cancer. It is almost never elevated in early malignances. It may be elevated in several benign conditions including hepatitis, cirrhosis, alcoholic liver disease, inflammatory bowel disease, both Crohn's disease and ulcerative colitis, pancreatitis, bronchitis, emphysema and renal disease. It may also be increased in healthy individuals who smoke.<sup>67</sup> Its greatest usefulness is in colorectal cancer.

#### **Clinical usefulness in colorectal cancer**

**Screening:** A sensitivity of 36% and specificity of 87% have been calculated for early colonic cancer (Dukes A and B).<sup>68</sup> The low sensitivity limits its value in colorectal cancer screening.

**Diagnosis:** In symptomatic patients, sensitivity is higher but other investigations (colonoscopy) would obviously supercede testing for CEA. As the tumour marker can be raised in many different conditions it is not of much use in the diagnosis of colorectal cancer.

**Prognosis:** High preoperative levels of CEA predict a worse prognosis.<sup>69</sup> High CEA will help identify patients with aggressive disease who may benefit from adjuvant chemotherapy pre-operatively. High CEA levels post-operatively also predicts a poor prognosis and early recurrent disease.<sup>70</sup> In patients with liver metastases, a high post liver resection CEA predicts further recurrences in the liver.

**Monitoring response to treatment:** CEA measurements are recommended for this indication.

**Early detection of recurrence:** Longitudinal CEA measurements detect recurrent cancer early with a sensitivity of 80% and specificity of 70%.<sup>68</sup> An elevated CEA can help diagnose hepatic metastases with high accuracy.<sup>71</sup> It is less helpful in predicting loco-regional recurrences. In asymptomatic patients, CEA is the most frequent indicator of recurrences.<sup>71</sup>

### **CA 125**

#### **Introduction**

The OC 125 monoclonal antibody was first published in 1981<sup>72</sup> and the immunoradiometric assay of this tumour marker CA125 was first introduced in 1983.<sup>73</sup> This tumour marker is a mucin-like molecule produced by the mesothelial cells of the peritoneum and other tissues of

Mullerian origin. It is shed in body fluids and makes its way to the bloodstream. It is not found in the normal ovary but expressed in more than 80% of patients with epithelial ovarian cancer. The function of this antigen is unknown. The cut off value is 35 U/ml and is elevated in 1% of healthy blood donors, 6% of patients with benign disease, 28% of non-gynaecological malignancy and 82% of proven epithelial ovarian cancer in clinical studies.<sup>73,74</sup>

Recently, the CA 125 II assay with enhanced resolution (especially for low values) and with reduced variability (approximately 50%) has been introduced and found to correlate well with the original CA125 assay in clinical use.<sup>74,75</sup>

**Conditions where raised levels of tumour marker is found**

This marker is raised in both benign and malignant conditions other than ovarian cancer as shown in Table 1.<sup>76</sup>

**Clinical usefulness**

**Screening:** CA 125 should not be used to screen for ovarian cancer since its level may be raised in many benign and malignant conditions.<sup>76</sup>

**Diagnosis:** CA 125 should not be used to diagnose ovarian cancer since its level may be raised in many benign and malignant conditions.<sup>76</sup> Measurement of CA 125 levels has

a limited role in the presence of acute or chronic symptoms (e.g. pelvic pain) when the clinical findings are normal. This can be a problem in premenopausal women when the results are abnormal raising anxiety levels in the patient about the possibility of malignancy. A normal value does not exclude ovarian cancer as it could be a false negative result.

**Prognosis:** In patients with invasive ovarian cancer where the CA 125 level is elevated pre-operatively it is valuable in assessing progressive disease and tumour response to chemotherapy. It appears to be an independent predictor of survival. If the CA 125 reactive determinant is not expressed pre-operatively but present during or after therapy this is a poor prognostic sign.

**Monitoring response to treatment:** When pelvic pathology is noted clinically, and combined with transvaginal ultrasound and colour Doppler imaging, the CA 125 level could aid in coming to a consensus on the best mode of management.<sup>77,78</sup> However, it should be noted that CA 125 levels could be normal in borderline malignancy as well as in mucinous cystadenocarcinoma. The CA125 assay alone should not be used as the sole criteria for surgery as it could be a false positive result.

A raised CA 125 level after surgery is indicative of residual disease. In such instances second-look procedures could be avoided and

**TABLE 1. Conditions in which a raised CA 125 level may be found.**

<u>Gynaecological</u>	<u>Non-gynaecological</u>
<p><b>Benign:</b> Endometriosis Acute pelvic inflammatory disease Adenomyosis Benign ovarian neoplasm Functional ovarian cyst Ovarian fibroma with ascites Menstruation Ovarian hyperstimulation Uterine myomata Unexplained infertility</p> <p><b>Malignant:</b> Ovarian carcinoma Primary peritoneal carcinoma Endometrial carcinoma</p>	<p><b>Benign:</b> Acute hepatitis Acute pancreatitis Chronic liver disease Cirrhosis Colitis Congestive heart failure Poorly controlled diabetes Diverticulitis Non-malignant ascites Mesothelioma Pericarditis Pneumonia Polyarteritis nodosa Systemic lupus erythematosus Renal disease Sarcoidosis</p> <p><b>Malignant:</b> Carcinoma of the pancreas Hepatocellular carcinoma</p>

definitive treatment like aggressive chemotherapy, second cyto-reductive surgery or palliative therapy can be considered. However, a normal CA 125 level does not exclude small volume residual disease.

**Early detection of recurrence:** Elevation of CA 125 may precede clinical or radiographic evidence of recurrence by 3 months, particularly in paracaval or retrocaval lymph nodes. After chemotherapy is completed CA 125 can be measured at regular intervals in order to detect recurrence early. Here again, a normal CA 125 assay does not exclude small-volume residual disease or a tumour that does not express OC125 reactive determinant.

### CA 15-3

#### Introduction

CA 15-3 is a mucin-like membrane glycoprotein.

#### Conditions where CA 15-3 levels may be raised

Elevations have been observed in healthy subjects (5–6%) and more often in individuals with benign diseases, especially those of hepatic origin, in which false positive elevations have been observed in 30% of patients.<sup>79,80</sup> Apart from breast cancer, elevation of CA 15-3 is also observed in ovarian, lung and liver cancers. However, its use other than in breast cancer is not yet defined.

#### Clinical Usefulness

**Screening for breast cancer:** Since CA 15-3 may be elevated in normal individuals and benign conditions and there is a low incidence of CA 15-3 elevation in early stage breast cancer it should not be used for screening.<sup>81</sup>

**Diagnosis of breast cancer:** CA 15-3 should not be used for the diagnosis of breast cancer for the same reasons that it should not be used for screening. Tumour marker sensitivity in patients with early breast cancer is only 15-35%. Low levels of CA 15-3 does not exclude the presence of either primary or metastatic breast cancer.<sup>82</sup>

**Prognosis:** Serum levels of CA 15-3 are related to tumour stage, with significantly higher values in patients with nodal involvement than without nodal involvement and in patients with larger than smaller breast tumours.<sup>82</sup> However, it is still not clear whether CA 15-3 is an independent prognostic indicator.<sup>82</sup> There is little evidence of a relationship between tumour marker levels and likelihood of responding to either chemotherapy or hormone therapy in patients

with breast cancer.

**Monitoring response to therapy:** Tumour marker sensitivity in patients with advanced breast cancer is significantly higher than in loco-regional disease.<sup>83,84</sup> In patients with breast cancer where the serum CA 15-3 level is elevated, the tumour marker may be used to monitor response to therapy. Patients with disease regression usually show decreasing levels while patients with progressive disease generally have increasing levels. However, whether this monitoring leads to enhanced survival or better quality of life remains to be determined.

**Early detection of recurrence:** Serial CA 15-3 determinations are useful in the early diagnosis of recurrence in patients with breast cancer and no evidence of disease after treatment. CA 15-3 has been shown to detect 40-60% of relapses before clinical or radiological evidence of disease with a lead-time of between 2 and 18 months.<sup>85</sup> CA 15-3 is not useful in detecting loco-regional recurrence. Clinical examination is the best detection method for recurrence for these sites. In contrast, CA 15-3 serum levels are raised in 50-70% of patients with distant metastases.<sup>85</sup> The benefit of early detection of recurrent disease remains to be determined. There is no good evidence that treatment of an asymptomatic breast cancer patient with only a raised CA 15-3 level will lead to improvement in disease-free survival and overall survival.

### ALPHA-FOETOPROTEIN (AFP) (as a marker of hepatocellular carcinoma)

#### Introduction

AFP is a glycoprotein that is structurally related to albumin with a molecular weight of 69 kD. In normal physiology, AFP is made by human yolk sac cells and in later embryonic growth by foetal liver which then switches to albumin synthesis as it matures.<sup>86</sup>

AFP levels below 10 ng/ml are found in healthy men and non-pregnant women. As a tumour marker, AFP has application in primary liver carcinoma in adults and hepatoblastoma in children and in germ cell tumours. However, raised levels are also seen in gastric, colorectal, biliary, pancreatic and lung cancers.<sup>82</sup> Transient increases and fluctuations in serum AFP may occur in liver regeneration, hepatitis, chronic liver disease and cirrhosis, especially during exacerbations of hepatitis. It is also raised in pregnancy and in neural tube defects.

Hepatocellular carcinoma (HCC) is a malignant disease that is difficult to detect in its

early stages and has very poor prognosis. Although relatively uncommon among Caucasians it is one of the major malignancies in many countries, particularly in sub-Saharan Africa and the Far East.<sup>87</sup> Liver cancer is the fifth most common cancer in the world. The role of chronic infection with the hepatitis B (HBV) and hepatitis C (HCV) viruses in the aetiology of liver cancer is well established.<sup>88</sup>

AFP continues to be the most established tumour marker in HCC. Recent emphasis in diagnosing HCC has been on the detection of small asymptomatic carcinoma (defined as tumour <3 cm in size) at a potentially curable or resectable stage. Two major approaches have been used: serum testing for AFP and liver imaging with ultrasonography (US).<sup>89,90</sup>

### Clinical usefulness

**Screening:** Chronic carriers of the hepatitis B surface antigen (HBsAg), subjects with chronic HCV infection, patients with cirrhosis and patients with rare metabolic diseases are candidates for screening.<sup>82,91-94</sup> Several trials which have used cirrhotics of various aetiologies<sup>95</sup> and patients who are HBsAg positive<sup>96</sup> have established the benefits of screening of high risk patients with AFP and US. McMahon et al.<sup>96</sup> who studied HBsAg-positive Alaskan native male and non-pregnant female carriers concluded that screening of HBsAg carriers with semi-annual AFP was effective in detecting most HCC tumours at a resectable stage and significantly prolonged survival rates when compared with historical controls in this population.

High risk subjects should be screened for HCC by the use of AFP and US once every 6 months. Screening with AFP is not recommended in populations who are not at high risk of developing HCC.

**Diagnosis:** While most symptomatic HCC are associated with AFP >1000 ng/ml, two-thirds of patients with small asymptomatic tumours will have an AFP level <200 ng/ml. AFP levels of healthy non-pregnant adults are usually <10 ng/ml and values ranging from 10 to 500 ng/ml have been used in the literature as diagnostic cut-off levels to classify HCC patients.<sup>89,90</sup> It should be noted that in some benign conditions, such as benign chronic liver disease especially during exacerbations of hepatitis, AFP elevations may be transient.<sup>89</sup> In malignancy, however, concentrations remain high or even increase. The assay of AFP every 2-3 weeks may therefore eliminate falsely-raised values.<sup>82</sup>

As there are many causes of a raised serum AFP level, a raised serum AFP level should not be used on its own to diagnose HCC.<sup>82</sup>

**Monitoring response to treatment:** After successful surgical resection of HCC, serum AFP levels fall to within the reference range with a half-life of 5-6 days. A fall of AFP to within the reference range (<10 ng/ml) indicates complete, or nearly complete, pathological remission. If resection appears complete but the AFP level does not decrease to the reference range, residual tumour is invariably present.<sup>97</sup> However, the attainment of the reference range does not necessarily imply complete removal of the entire tumour. Micrometastases that do not secrete sufficient AFP to exceed the reference range may still be present.<sup>98</sup>

Serial measurement of AFP may be used to monitor response to chemotherapy and may be better than imaging techniques such as CT.<sup>98,99</sup>

**Early detection of recurrence:** AFP may be used to detect early recurrence. If there is tumour recurrence AFP levels start to rise, often before clinical evidence of disease.<sup>99</sup>

### HUMAN CHORIONIC GONADOTROPHIN (hCG) (as a marker of choriocarcinoma)

#### Introduction

hCG is a sialoglycoprotein with a molecular weight of about 36,500 Da.<sup>100</sup> It is initially secreted by the trophoblastic cells of the placenta, shortly after implantation of the fertilised ovum into the uterine wall.<sup>101-104</sup> The physiological source of hCG is the placenta. It contains  $\alpha$  and  $\beta$  subunits,  $\alpha$  being identical to the  $\alpha$  subunit of LH, FSH and TSH. The amino acid residues specific for the  $\beta$  subunit of hCG confer its immunospecificity.<sup>105,106</sup>

#### Conditions where elevated levels are found

Elevated levels are found in pregnancy and pregnancy-related disorders such as ectopic pregnancy, multiple gestation and molar pregnancy.<sup>107-109</sup> The source of hCG in tumours is trophoblast-like cells.<sup>102</sup> Elevated levels are found in germ cell tumours such as choriocarcinoma (always), teratoma (frequently - 40-60%), seminoma (sometimes - 5-10%) and dysgerminoma (sometimes - 3%).<sup>100</sup>

#### Clinical usefulness

**Screening:** The risk of choriocarcinoma ranges from about 0.003% following normal-term deliveries to 3% following hydatidiform moles, the prevalence of which ranges from 1 in 200

deliveries in South East Asia (SEA) to 1 in 2000 deliveries in Europe and North America.<sup>100</sup> As about 50% of cases of choriocarcinoma follow a molar pregnancy, hCG can be used to screen this high risk group of patients, especially in SEA.<sup>100</sup> However, around 10-15% of all patients with hydatidiform moles have persistently increased or rising hCG levels following evacuation and require further treatment, although not all have choriocarcinoma.

**Diagnosis:** Serum and urine hCG can be used to make the diagnosis of choriocarcinoma.<sup>103</sup> As the tumour arises from placental trophoblasts, it usually produces hCG in quantities that reflect the bulk of the tumour.

**Prognosis:** Serum  $\beta$ hCG is one of the several factors taken into account for prognostication of choriocarcinoma which, in turn, determines the chemotherapy regimen for individual patients. The prognosis is poor if serum  $\beta$ hCG level is >40,000U/L at the time treatment is started.<sup>100</sup>

**Monitoring response to treatment:** Serum hCG is useful in monitoring response to treatment.<sup>100</sup> Contraception should be advocated during the entire follow-up interval to avoid misinterpretation of elevated hCG by pregnancy. Monthly determinations of  $\beta$ hCG are recommended until the level is normal for 12 months.

**Early detection of recurrence:** Serum hCG is also useful in detecting recurrence early.<sup>100</sup>

#### AFP and hCG (as markers of germ cell tumours)

AFP, hCG and its  $\beta$ subunit.  $\beta$ hCG and, lactate dehydrogenase (LDH) are well-established tumour markers integral to the successful management of patients with testicular and other germ cell tumours.<sup>82,110</sup> Germ cell tumours (GCT) are classified as seminomas (40%), non-seminomatous tumours (NSGCT, 40%), or "mixed" germ cell tumours (20%) which contains elements of both seminomatous and non-seminomatous tumours.<sup>82</sup> The availability of effective and curative treatment and the fact that changes in marker concentrations reliably reflect and predict clinical response to the extent that serology may predominate over histology in treatment decisions, makes pre- and post-treatment determinations of tumour markers mandatory for the successful management of patients with testicular and other GCTs.<sup>110</sup>

#### Clinical usefulness of AFP and hCG in germ cell tumours

**Screening:** AFP and hCG should not be used to screen for GCTs.<sup>82,110</sup>

**Diagnosis:** Some 20-60% of patients with germ cell tumours will have raised levels, depending on tumour stage.<sup>111</sup> However, levels within the reference range do not exclude malignancy as 25% of non-seminomatous germ cell tumours (usually teratoma) do not release hCG and AFP into the circulation and only a small proportion of the patients with seminoma (5-10%) or dysgerminoma (3%) have increased hCG levels.<sup>100</sup> Increased hCG levels must be interpreted with clinical and other investigative findings as hCG is also increased in pregnancy and other non-germ cell tumours.<sup>82</sup>

Serum hCG and AFP measurements may be helpful in the diagnosis of patients suspected of having GCTs.<sup>82,110</sup> Tumour marker measurements, together with testicular ultrasound, may be used to help in the differential diagnosis of a painless swelling of one testis.<sup>82</sup> Cerebrospinal fluid (CSF) hCG measurement may improve diagnostic efficiency if metastasis to the brain is suspected.<sup>82</sup>

**Staging:** Sixty percent of patients with NSGCT have metastatic disease at diagnosis. Elevations of AFP may be encountered in 80% of metastatic and 57% of Stage I NSGCT. AFP is not raised in pure seminomas unless the liver is involved and in other circumstances where the histology is mixed GCT. Elevated serum AFP levels indicate the presence of yolk sac elements, i.e., mixed GCTs and occur in all stages of the disease.<sup>82</sup>

Increased serum hCG concentrations occur in both seminoma and NSGCT, with a sensitivity of 40-60% in patients with metastatic NSGCT and 15-20% in those with metastatic seminoma. Trophoblastically differentiated teratomas usually produce hCG while differentiated teratomas and yolk sac tumours rarely do.<sup>82</sup>

Prior to 1997, clinical and pathological staging of germ cell tumours was dependent only on the extent of disease, according to the TNM system, requiring orchidectomy for the staging of the primary tumour and radiographic assessment of chest, abdomen and pelvis to determine nodal and metastatic classification. Pre-treatment concentrations of AFP, hCG and LDH are now universally included in the international germ cell tumour staging system (TNM + S0/1/2/3-category) and should be determined before and immediately after orchidectomy. If markers are raised, serial determinations should be made to

allow for the calculation of half-life and to assess whether markers fall to within reference limits. Staging errors may be reduced from 50% to <15% in Stages I and II by AFP and hCG determinations.<sup>82</sup> In addition, cerebrospinal fluid determinations of AFP and hCG may be helpful in the diagnosis and monitoring of intracranial GCT.<sup>82,112</sup> In clinical Stage I disease a second marker determination should be performed 5-6 days post-operatively for the determination of marker half-life. Stage I classification can be confirmed retrospectively if marker concentrations decline according to half-life.

**Prognosis:** Pre-treatment concentrations/activities of AFP, hCG and lactate dehydrogenase (LDH) contribute to the classification of metastatic GCTs as having good, intermediate or poor prognosis.<sup>110</sup> The International Germ Cell Cancer Collaborative Group (IGCCCG) has proposed a prognostic factor-based staging system for metastatic GCT (both seminomatous and non-seminomatous) that allows the classification of these tumours into good, intermediate and poor as outlined in Table 2.<sup>82,112</sup>

The system also takes into account tumour site (testis, retroperitoneal, mediastinal) and the presence or absence of non-pulmonary visceral metastases.<sup>82,112</sup>

Determination of the half-lives of AFP and hCG is recommended for monitoring treatment, normalisation of both markers (AFP within 5 days, hCG within 1-2 days) indicating favourable prognosis.<sup>82</sup> Half-life can be estimated using only 2 measurements or using linear regression.<sup>82,113</sup> After 2 cycles of chemotherapy, patients for whom half-lives are >7days for AFP and >3 days for hCG have significantly lower survival rates than those with normal tumour marker half-lives.<sup>82</sup>

While guidelines<sup>82,110,112</sup> have recommended the use of half-life of markers, it should be noted

that at least one multi-centre, randomised controlled clinical trial of patients with disseminated non-seminomatous testicular cancer found that half-lives of AFP and hCG during induction chemotherapy were inaccurate parameters for the prediction of treatment failure. In contrast, initial serum concentrations of AFP and hCG were highly significant in the prediction of unfavourable treatment outcome.<sup>114</sup>

**Monitoring response to treatment:** The choice of chemotherapy regimen depends on the prognosis of the patient which is in part determined by the pre-chemotherapy AFP and hCG levels.<sup>104</sup>

For patients undergoing chemotherapy, marker determinations are mandatory prior to each cycle.<sup>82</sup> The decreases in tumour marker concentrations after orchidectomy contribute to the management of patients with GCTs and can be determined from serial marker measurements. The rate of decrease should be calculated and compared with the normal rates of disappearance of AFP (half-life < 7days) and hCG (half-life <3 days).<sup>110</sup>

**Stage IA and IB disease:** Surveillance alone is suggested rather than retroperitoneal lymph node dissection (RPLND) following inguinal orchidectomy. Together with chest X-ray and clinical examination, routine measurements of tumour markers should be made monthly during the first year post-orchidectomy, and then every second month during the second and third years. Abdominal CT scans are desirable every 2 to 3 months throughout the first 3 years. If AFP or hCG remain elevated and half-lives prolonged with no evidence of residual disease on CT, this is highly suggestive of occult metastases distant from the retroperitoneum, and systemic chemotherapy (rather than RPLND) should be considered.<sup>82</sup>

**Stage II disease:** Surveillance should include tumour markers with physical examination and

**TABLE 2. Prognostic classification of patients with metastatic GCT based on pre-treatment tumour marker concentrations**

Prognostic group	Tumour Marker concentration		
	AFP (KU/l)	hCG (U/l)	LDH (multiples of the upper limit of the reference range)
Good (S1)	<1000	<5000	<1.5 x
Intermediate (S2)	≥1000 & ≤10,000	≥5000 & ≤50,000	≥1.5 x & ≤10 x
Poor (S3)	>10,000	>50,000	>10 x

chest X-ray every month during the first year, every second month during the second year, and every third month for the third year.<sup>82</sup>

**Advanced Stage II and Stage III disease:** The rate of decline in tumour marker levels following chemotherapy predicts response to treatment. Persistently elevated marker levels or prolonged tumour marker half-lives in the first 6 weeks post-chemotherapy specifically indicate resistance to chemotherapy and poor prognosis. Patients with residual masses following chemotherapy may be considered for post-chemotherapy surgery, but where serum tumour marker levels are still elevated salvage chemotherapy should be recommended instead since disease is likely to be surgically unresectable.<sup>100</sup>

AFP and hCG are useful in monitoring the response to treatment in patients with GCTs. The rapidity of decreases in tumour marker concentrations in the first 6 weeks of chemotherapy can predict the potential for relapse months later, and weekly measurements during chemotherapy are recommended.

The timing of surgery is important as it is likely to have the best outcome at the lowest tumour marker level that can be achieved with chemotherapy. Chemotherapy should be continued even after hCG levels have 'normalised' in order to eradicate all tumour cells. It has been estimated that  $10^5$  tumour cells may persist at an hCG level of only 1 U/L.<sup>100</sup> Chemotherapy may damage the liver producing a rising AFP level in a patient with a purely hCG-producing tumour.<sup>82,100</sup> Therefore, availability of these tumour markers in the majority of teratoma patients makes it easier to modify treatment of patients with good prognosis in order to minimise toxicity.

**Early detection of recurrence:** Preoperative measurement of hCG in all patients with possible germ cell tumours will help in the detection of residual disease post-operatively.<sup>82</sup> Following surgery, if the disease is confined to the testis or ovary, serum hCG levels should reduce to normal with an apparent half-life of 1-2 days.<sup>115-118</sup> If hCG remains elevated or a metastasis is identified radiologically, further treatment is required. The ratio of serum:CSF hCG is a sensitive method for detecting brain metastases. A ratio of <10:1 is diagnostic of brain metastases; ratios between 10:1 to 60:1 are suggestive but metastases are unlikely if the ratio is >60:1.<sup>100</sup> There is general agreement that rising concentrations of tumour markers are incompatible with tumour regression and often indicate progressive disease months

before clinical evidence of recurrence (lead-time 1-6 months).<sup>119</sup> In the follow-up of metastatic tumour, rising AFP and/or HCG levels provide the first indicator of relapse in about 50% of patients. Combined determination of AFP (cut-off 10 U/L) and hCG (cut-off 5 U/L) yielded a diagnostic sensitivity of 86% for tumour recurrence and partial response in conjunction with 100% diagnostic specificity and positive/negative predictive values of 100% and 87%, respectively.<sup>120</sup> Discordant behaviour (decline in serum marker concentrations while tumour burden increases) has been reported and attributed to selective chemotherapeutic destruction of marker-producing cancer cells.<sup>104</sup> In contrast, false positive tumour marker results may also occur transiently due to tumour lysis on initiation of chemotherapy or (for AFP) due to hepatic damage.<sup>104</sup>

Monthly measurements should be made in the first year after treatment for advanced disease, with measurements every 2 months in the second year, every 2 or 3 months in the third year, and then every 6 months up to 5 years.<sup>100</sup> After this, all patients should be monitored for life, with a frequency such that recurrent disease is identified before it is difficult to eradicate. Frequency of follow-up depends on the time since diagnosis, the type of treatment and whether the patient is thought to be cured or to retain a focus of disease.

## CATECHOLAMINES AND THEIR METABOLITES

### Introduction

In man, adrenaline is derived principally from the adrenal medulla, where it forms about 80% of the catecholamines, while noradrenaline is the primary catecholamine produced by the sympathetic nervous system.<sup>121</sup> Both adrenaline and noradrenaline are found in many other tissues, largely reflecting the sympathetic innervation of the organs concerned.<sup>121</sup>

### Conditions in which elevated levels are found

Phaeochromocytoma is a rare, functioning catecholamine-producing tumour arising from the sympathetic nervous system, most commonly in the adrenal medulla.<sup>121,122</sup> Neuroblastoma/ganglioneuroma is an uncommon tumour of prenatal life, infancy and childhood, commonest in boys under 3 years of age, arising in the adrenal medulla or from the sympathetic chain.<sup>121</sup> Physiological conditions which may raise plasma catecholamines include noise, stress, discomfort, position of the body, coffee and nicotine.<sup>121-124</sup>

**Clinical usefulness****Screening and diagnosis of phaeochromocytoma:**

24-hour urinary catecholamines and metanephrines are useful for the screening and diagnosis of patients suspected of having phaeochromocytoma, including young hypertensives, those with a positive history of multiple endocrine neoplasia (MEN) 2, those with refractory or extremely labile hypertension (especially if accompanied with phaeochromocytoma-associated symptomatology), and those with hypertension and evidence of glucose intolerance.<sup>121</sup>

24-hour urinary metanephrines have around 90% sensitivity and specificity.<sup>121-124</sup> Urinary-free catecholamines are also of value although small intermittently-secreting tumours may be missed. Urinary noradrenaline levels are more often raised (80-90%) than adrenaline (50-70%) and the overall sensitivity of urinary-free catecholamines is over 90%.<sup>121</sup> Urinary HMMA (4-hydroxy-3-methoxymandelic acid) is less accurate with only 60% sensitivity, and, depending on the method of measurement, patients may need to be on vanilla- and phenolic acid-free diet for 72 hours prior to urine collection, to reduce the likelihood of false positive results.<sup>121</sup>

Plasma catecholamines (under resting conditions) which exceed 10 nmol/L for noradrenaline or 1.5 nmol/L for adrenaline are also around 90% sensitive and specific.<sup>121</sup> Small tumours remain difficult to diagnose.<sup>121</sup> Nevertheless, since plasma catecholamine levels fluctuate considerably depending on whether the patient is stressed, etc., plasma catecholamines are less useful than 24-hour urinary catecholamines and metanephrines.

Elevated plasma adrenaline levels or increased urinary metanephrine secretion generally suggest a tumour of adrenal origin, whereas exclusively noradrenaline-secreting tumours suggest either a very large adrenal tumour or a paraganglioma.<sup>121,122</sup>

**Diagnosis of neuroblastoma/ganglioneuroma:**

75% of cases produce excess catecholamines and are detected as for phaeochromocytoma, though dopamine secretion is often prominent.<sup>121</sup>

**CALCITONIN****Introduction**

Calcitonin (CT) is a hormone produced by the parafollicular or C-cells of the thyroid gland and to a smaller extent by the gastrointestinal tract.<sup>121</sup>

**Conditions in which elevated levels are found**

Medullary thyroid carcinoma (MTC)

**Clinical usefulness**

**Screening:** MTC affects 100% of patients with Multiple Endocrine Neoplasia type 2 (MEN 2). Most patients with sporadic MTC have high basal plasma levels but some with familial MTC have normal levels.<sup>121-125</sup> Provocative tests involving stimulation of CT secretion with calcium infusion or pentagastrin injection, have been developed for family screening.<sup>121-125</sup> Thus, as one form of MTC is hereditary, all close family members of a patient should be screened with basal and/or stimulated CT measurement.

**Diagnosis:** Calcitonin is essential to the diagnosis of MTC and is close to being a 'perfect' tumour marker for this rare condition.<sup>126</sup> Using modern immunoassays with low detection limits, CT has 100% sensitivity for MTC and at a cut-off of 20 ng/L, the diagnostic specificity is almost 100%.<sup>121,126</sup> In addition, CT is elevated at an early stage in tumour development, often before there is a focal lesion and well before any clinical symptoms.

**Detection of residual tumour:** The reduction in CT following surgery is an excellent predictor of residual tumour.<sup>126,127</sup>

**Monitoring of response to treatment:** CT monitoring should be continued for life in a treated patient, a rising level is an indication of recurrence and the need to consider re-operation.<sup>121,126,127</sup>

**Determining prognosis:** Although the CT level correlates with tumour bulk, it cannot give an indication of tumour dissemination. Therefore, the CT level is of limited value in determining prognosis.<sup>121</sup>

**THYROGLOBULIN****Introduction**

Thyroglobulin is a large glycoprotein synthesized by the thyroid follicular cells and stored in the colloid space.<sup>121</sup> It is present in all thyroid tissue and does not discriminate between benign and malignant disease.<sup>121</sup>

**Conditions in which elevated levels are found**

Benign and malignant diseases of the thyroid gland.<sup>121</sup>

**Clinical usefulness**

**Screening and Diagnosis:** It has no role in screening for or diagnosis of thyroid cancer and has no value as a prognostic indicator.

**Monitoring response to treatment:** As a marker of thyroid tissue it has an important role in monitoring patients with thyroid carcinoma who are treated by total thyroid ablation (surgery and/or radioiodine). In such patients, serum thyroglobulin level should be <10 µg/L some weeks after ablation and remain low.<sup>121</sup> An elevated or rising thyroglobulin is evidence of residual thyroid tissue or recurrence. Measurements are more reliable when the patient is off thyroid hormone replacement, although elevated levels on replacement indicate persistent or metastatic disease. Thyroglobulin measurement is an important alternative to <sup>131</sup>I-scanning, since it can detect non-functioning metastases.<sup>121</sup>

## PARATHYROID HORMONE

### Introduction

Parathyroid hormone (PTH) is a polypeptide comprising 84 amino acids.<sup>121</sup> The biological activity of PTH resides in the N-terminal 1-34 amino-acid sequence of the hormone.<sup>121</sup> PTH is secreted by the parathyroid glands in response to a fall in plasma (ionised) calcium concentration.

### Conditions in which elevated levels are found

- Primary hyperparathyroidism – primary diseases of the parathyroid usually due to parathyroid adenoma, less often to diffuse hyperplasia of the glands and rarely to carcinoma.<sup>121</sup>
- Secondary hyperparathyroidism – increased PTH secretion as an appropriate physiological response, e.g. chronic renal failure and vitamin D deficiency.<sup>121</sup>
- Tertiary hyperparathyroidism – autonomous PTH secretion as a result of prolonged hypocalcaemic stimulus, e.g. hypercalcaemia develops in end-stage renal failure.<sup>121</sup>
- Parathyroid adenoma is associated with MEN 1 (parathyroid disease, pituitary and pancreatic tumour) and MEN 2 (parathyroid disease, pheochromocytoma, medullary thyroid carcinoma ± mucosal neurofibromatosis).<sup>121</sup>

### Clinical usefulness

#### **Diagnosis of primary parathyroid diseases:**

Serum PTH levels are useful in the diagnosis of primary parathyroid diseases, including parathyroid tumours such as parathyroid adenoma and a functioning parathyroid carcinoma. The serum PTH level should always

be interpreted in relation to the serum calcium level. For instance, if the serum calcium level is high but the serum PTH level is in the upper end of the reference range, i.e. within the reference range, it is, nevertheless, inappropriately high for the serum calcium level. A normal response to a high serum calcium would be to suppress PTH secretion.

**Screening for MTC:** Parathyroid adenoma may be familial and occur as part of one of the MEN syndromes. Parathyroid disease is the most frequent manifestation of MEN 1 and the most common reason for seeking medical attention. There is evidence of parathyroid involvement in 20-60% of patients at the time of diagnosis of MEN 2A; 84% of these have hyperplasia while 16% have adenomata.<sup>121</sup> Parathyroid involvement in MEN 2B is rare.<sup>121</sup> The diagnosis is made by the demonstration of simultaneously elevated plasma calcium and PTH levels or inappropriately elevated PTH level in the face of hypercalcaemia.

## PROLACTIN

### Introduction

Prolactin is a 198 amino-acid polypeptide hormone produced by the anterior pituitary gland.<sup>121</sup> Its principal physiological action is to initiate and sustain lactation. Prolactin secretion is controlled by the hypothalamus through the release of dopamine (PIH – prolactin inhibiting hormone) which inhibits lactation. Both thyrotrophin-releasing hormone (TRH) and vasoactive intestinal peptide stimulate prolactin secretion.

### Conditions in which elevated levels are found

Prolactinoma (prolactin secreting pituitary tumour) and other pituitary tumours may obstruct blood flow from the hypothalamus and, thus, reduce dopamine-dependent inhibition of prolactin secretion.<sup>128,129</sup>

Serum prolactin levels are elevated during major stress such as hypoglycaemia but the commonest cause of hyperprolactinaemia is pregnancy and lactation.<sup>128,129</sup> Any drug with dopamine antagonist effects will cause hyperprolactinaemia such as anti-emetics (e.g. metoclopramide, prochlorperazine) and most major tranquillizers (e.g. chlorpromazine and other phenothiazines, haloperidol, etc.).<sup>128</sup> Primary hypothyroidism, renal failure and polycystic ovary syndrome are also associated with hyperprolactinaemia.

**Clinical usefulness**

**Diagnosis of prolactinoma and functionless pituitary tumours:** A plasma prolactin level of >5000 mU/L is almost always due to a prolactinoma and levels may reach several hundred thousand.<sup>121</sup> Plasma levels of below 5000 mU/L may be due to a prolactinoma, a functionless pituitary tumour (adenoma, craniopharyngioma, etc) or any of the other causes.<sup>121</sup> It is almost unlikely for a large macroprolactinoma to be associated with prolactin levels in this range.

**Monitoring response of prolactinoma to dopamine agonist therapy:** The aim of dopamine agonist therapy is to suppress plasma prolactin levels into the normal range.<sup>121</sup> The dose of dopamine agonist is usually slowly increased with sequential prolactin measurements until the normal range is achieved, followed by prolactin level monitoring in subsequent clinic visits. Failure of plasma prolactin to suppress fully with high or maximally tolerated dose of dopamine agonists may be due to non-compliance or dopamine resistant tumours.

**ADRENOCORTICOTROPIC HORMONE (ACTH)****Introduction**

ACTH is a polypeptide with a molecular weight of 4.5 kDa and comprises a single chain of 39 amino acids.<sup>130</sup> Its biological function is to stimulate adrenal glucocorticoid secretion. ACTH is secreted by the anterior pituitary gland, and its release is controlled by a hypothalamic peptide, corticotrophin-releasing hormone (CRH).

**Conditions in which elevated levels are found**

ACTH-dependent Cushing's syndrome due to either (a) pituitary Cushing's disease, usually associated with a corticotroph pituitary microadenoma or to (b) ectopic ACTH secretion (and very rarely ectopic CRH secretion) by a variety of tumours elsewhere.<sup>121,131</sup> Possible causes of ectopic ACTH production are small cell carcinoma of the bronchus (commonest source of ectopic ACTH), neuroendocrine tumours such as carcinoid tumours of the lungs and mediastinum, pancreatic endocrine tumours, pheochromocytoma and medullary thyroid carcinoma. ACTH secretion is also greatly increased by stress, depression and obesity.

**Clinical usefulness**

**Diagnosis of ACTH-dependent Cushing's syndrome:** Plasma ACTH levels are elevated in ACTH-dependent Cushing's syndrome but suppressed in an adrenal cause of Cushing's syndrome such as adrenal tumour. In pituitary-dependent Cushing's disease, plasma ACTH levels are often <100 ng/L but higher values (up to 400 ng/L, in some reports) are not infrequently seen.<sup>121</sup> Although the plasma ACTH and cortisol levels in ectopic ACTH secretion are generally higher than in pituitary disease, there is an overlap of the results between the two conditions.<sup>121</sup>

The CRH stimulation test is able to help differentiate between pituitary-dependent Cushing's disease and ectopic ACTH secretion. Following administration of CRH (hCRH-41, 100 ug iv.) patients with pituitary disease almost invariably show a rise in plasma ACTH and, hence, plasma cortisol. In about 80% of cases of pituitary disease, the cortisol response is exaggerated.<sup>121</sup> In contrast, exaggerated response to CRH in ectopic ACTH is extremely rare.

The petrosal sinus sampling catheter for ACTH is able to ascertain directly the source of ACTH secretion via sampling from the central veins. After a technically successful procedure, the vast majority (all in most series) of patients with Cushing's disease are found to have central:peripheral gradients of ACTH levels of 3:1 or more, which is diagnostic of pituitary disease.<sup>121</sup>

**OESTROGEN AND PROGESTERONE RECEPTORS****Introduction**

Oestrogen and progesterone receptors (ER and PgR) are members of a family of nuclear receptors that bind DNA and a class of small hydrophobic ligands including steroid hormones, thyroid hormone, vitamin D and retinoic acid.<sup>132</sup>

ER is an intracellular receptor that mediates oestrogen activity. Oestrogens pass through the cell membrane and bind with ER, transforming the receptor into an active transcription factor, which binds DNA as a dimer at specific oestrogen response elements, and regulates the expression of a variety of genes.

**Method of determination**

The immunohistochemical technique for detection of oestrogen and progesterone receptors utilises monoclonal antibodies directed against both the oestrogen and progesterone

receptors.<sup>133,134</sup> This method has been widely adopted.

Newer methods of measuring the variants of the ER and PgR such as polymerase chain reaction, mRNA and enzyme immunoassay, may prove to be better in predicting the success of hormonal therapy or even the success of cytotoxic chemotherapy or the prognosis for a particular patient.<sup>135,136</sup>

### Status in primary tumour

Hormone receptor status (ER and PgR) is used as a prognostic marker. Those with ER and PgR positive tumours tend to have a better prognosis than those with ER or PgR negative tumours. The hormone receptor status test is also used to help determine treatment options, including endocrine therapy, when a primary tumour has been removed, or to help guide treatment decision when a tumour recurs. ER status is a very important factor in the management of breast cancer because:

- 1) ER is useful in predicting survival.<sup>137,138</sup> ER-negative tumours are associated with early recurrence and poor patient survival compared to ER-positive tumours.
- 2) ER is useful in predicting response to endocrine therapy.<sup>139-141</sup> Patients with ER-negative tumors rarely respond to endocrine therapy. There appears to be a correlation between the level of ER expression and endocrine response. In patients with breast cancer containing high levels of ER and PgR, adjuvant endocrine therapy is more effective than in patients without these receptors. The response in patients with metastatic disease is shown in Table 3.<sup>142,143</sup>

**TABLE 3. Response to hormonal therapy in patients with metastatic breast cancer according to ER and PgR status.**

Receptor status	Response to Hormonal Therapy
ER + PgR +	75%
ER + PgR -	35%
ER - PgR +	25%
ER - PgR -	10%

Patients with primary tumours that are rich in ER and PgR experience longer disease-free periods after mastectomy, have fewer episodes of recurrent cancer and longer overall survival than those with receptor-poor cancers.

The duration of response to hormonal therapy in patients with ER levels > 50 fmol/mg protein is significantly longer than that for patients with lower ER levels.<sup>144</sup> The disease-free survival at 5 years is 74% for ER + and 66% for ER – patients. PgR alone does not appear to offer independent prognostic information.

### Recommendation

ER and PgR are recommended to be measured on every primary breast cancer and may be measured on metastatic lesions if the results would influence treatment planning.<sup>145</sup>

In both pre- and post-menopausal patients, ER and PgR receptor status may be used to identify patients most likely to benefit from endocrine forms of adjuvant therapy and therapy for recurrent or metastatic disease.

### p53

#### Introduction

The protein p53 is critical in maintaining ordered proliferation, growth and differentiation of normal cells. It is a 393 amino-acid nuclear phosphoprotein coded by the TP53 gene, which is located on human chromosome 17p13. In the normal cell cycle, TP53 is actually inactive. It is in damaged cells that TP53 acts to regulate growth. Damage to cellular DNA initiates increased expression of TP53, which leads to arrest of the cell cycle. This interruption permits DNA repair to occur before the cell resumes the cell cycle and normal cell proliferation. If DNA repair is not successful, the cell then undergoes apoptosis.

When TP53 mutates, DNA-damaged cells are not arrested in G<sub>1</sub> and DNA repair does not take place. This failure to arrest DNA-damaged cells will be repeated in subsequent cell cycles, permitting other mutations to accumulate, culminating in neoplastic transformation and cancer. The mutation of TP53 is probably the most common mutational event and most significant genetic change characterising the transformation of cells from normalcy to malignancy. It is also thought to be the most common somatically mutated gene in sporadic cancers. Loss of function of both alleles is usually required for complete transformation; one through deletion and the other through a point mutation.

#### Method of determination

The majority of studies have utilised immunohistochemistry (IHC) as an indirect

measure of p53 mutation. This technique can be used because the majority of p53 mutant alleles are mis-sense mutations and encode a mutant protein with prolonged half-life that accumulates intracellularly and can be detected by IHC.<sup>146,147</sup>

A combination of direct sequencing with polymerase chain reaction (PCR) coupled to DNA mobility shift assays such as single strand conformation polymorphism (SSCP), with constant denaturing gel electrophoresis (CDGE), have been used.<sup>148</sup>

IHC has some benefit over DNA sequencing when employed in large studies because it is considerably less labour-intensive and can be used on archival paraffin-embedded material.

### Status in primary tumours

#### Breast Cancer

In human breast cancer, alteration in p53 at the protein or DNA levels has been detected in 25-50% or 15-35% of cases respectively. Such aberration causes either loss of function or dominant negative action which disrupts the native growth-regulatory role of p53 thereby contributing to tumourigenesis. Aberrant p53 function can lead to derangement in differentiation and cell cycle control, including disruption of the G1-S checkpoint, resulting in loss of the apoptotic response to DNA damage and untoward DNA replication with an unrepaired genome.

Low grade cancers of the breast (tubular, mucinous, papillary and invasive cribriform types) do not express p53 proteins.<sup>149</sup> However, p53 expression is very common (60%) in medullary carcinoma, grade 3 carcinoma and carcinoma that lack steroid receptors.<sup>150</sup> The 8-year survival is 82% in p53 negative and 66% in p53 positive lymph node-negative breast cancer patients; and 56% in p53 negative and 20% in p53 positive lymph node-positive patients.<sup>151</sup>

#### Colon cancer

p53 is mutated in approximately 75% of colon carcinomas and 7-50% of colon adenomas.<sup>152,153</sup> p53 expression is related to size (7% for adenoma < 1 cm vs 35% for adenoma > 2cm) and dysplasia (11% for low grade vs 52% for diffuse high grade).<sup>153</sup>

Colon carcinoma patients with p53 positive tumours have significantly poorer prognosis than those with p53 negative tumours. (5 year-survival 58% vs 76% respectively).<sup>154</sup> In Dukes C carcinoma, p53 positive patients have a survival rate of 59% vs 89% for p53 negative patients.<sup>154</sup>

#### Prostate Cancer

p53 expression is associated with higher Gleason grade (0% of Gleason grade 2 vs 18% of Gleason grade 3 or greater)<sup>155</sup> and tumour progression.<sup>156</sup>

### Recommendation

According to the American Society of Clinical Oncology, present data are insufficient to recommend the use of p53 expression or mutation for management of patients with breast cancer, or for screening, diagnosis, staging, surveillance or monitoring treatment of patients with colorectal cancer.<sup>145</sup>

### HER-2 / c-erbB2

#### Introduction

HER-2 proto-oncogene (*c-erb2*, also known as *neu* in rat) encodes the production of a 185 kDa transmembrane glycoprotein known as HER-2 protein. This HER2 protein has intrinsic tyrosine kinase activity that resembles the receptor for epidermal growth factor (EGF).<sup>157,158</sup>

HER-2 is normally expressed at low levels in a variety of human secretory epithelial tissues.<sup>159</sup> The HER-2 receptor has no known ligand; but HER-2 has been shown to form heterodimers with HER-1 (the epidermal growth factor receptor, EGFR), HER-3 and HER-4 in a complex with the ligands for these receptors. Heterodimer formation results in the activated HER-2 receptor transmitting growth signals from outside the cell to the nucleus, thus controlling aspects of normal cell growth and division.<sup>160</sup>

#### Method of determination

The FDA has approved two main ways to test the HER-2 status: immunohistochemistry (IHC) and fluorescent *in situ* hybridisation (FISH).

IHC is used to detect the presence of HER-2 protein in tissue samples (fresh, frozen or formalin-fixed, paraffin-embedded) obtained from fine needle aspiration, needle biopsy or surgical biopsy. It uses antibodies to HER-2 and a chemical detection method to stain HER-2 protein in the tissue samples.

FISH is a direct method to detect the actual HER-2 gene amplification. It uses fluorescent DNA probes to identify increased copies of the HER-2 gene.

IHC is currently the most widely used initial testing method. If the result is indeterminate or negative, then the FISH method is often done as a follow-up test.

### Status in primary tumours

In tumour cells, amplification of the HER-2 gene leads to an over-expression of HER-2 protein, resulting in increased cell division and a higher rate of cell growth. Over-expression of HER-2 protein is frequently found in tumours arising from many sites, especially the breast and ovary, where it correlates with poor patient prognosis.

Amplification of HER-2 has been implicated as an important event in the genesis of human breast cancer. Approximately 25-30% of human breast cancers have HER-2 protein over-expression<sup>161</sup> often in conjunction with mutation in p53.

The presence of HER-2 mRNA and elevated HER-2 protein levels in primary human breast tumour specimens was demonstrated to have a shortened disease-free survival and shortened overall survival for breast cancers with node-positive patients, and for ovarian cancers when HER-2 is expressed at high levels (>5-fold increase).<sup>161</sup>

### Recommendation

The 2000 Update of Recommendations on Clinical Practice Guidelines of the American Society of Clinical Oncology has recommended that every primary breast cancer should be evaluated for HER-2 over-expression either at the time of diagnosis or at the time of recurrence.<sup>145</sup> Measures of HER-2 gene amplification may also be of value.

### BRCA1 and BRCA2

#### Introduction

In 1990, the first breast cancer susceptibility gene, Breast Cancer Gene 1 (BRCA1), was localised to chromosome 17q by linkage analysis of multiple families affected by early onset breast and ovarian cancers.<sup>162</sup> Four years after its localisation, BRCA1 was identified by positional cloning. At about the same time that BRCA1 was cloned, a second breast cancer susceptibility gene, Breast Cancer Gene 2 (BRCA2), was localised to chromosome 13q and cloned shortly thereafter.<sup>163,164</sup>

BRCA1 is a tumour suppressor gene that appears to be involved in the double-stranded DNA error correction function. BRCA2 is also a tumour suppressor gene, but little is known about its actual function. Normally, they are thought to be involved in cell growth regulation, mutations in these genes can change their normal function leading to an increased chance of

developing cancer.

BRCA1 and BRCA2 are two genes that are linked with hereditary breast and ovarian cancers.

#### Method of determination

DNA sequencing is the most sensitive test for BRCA1 and BRCA2 mutation detection, as it determines the nucleotide sequence of the coding regions of genes.<sup>165</sup> It is estimated that sequencing will uncover nearly 98% of all the mutations in the coding regions of BRCA1 and BRCA2, but will consistently miss mutations in non-coding region of genes and large genomic deletions.

#### Status in primary tumours

Mutations in the genes BRCA1 and BRCA2 account for almost 85% of all inherited breast and ovarian cancers.<sup>166</sup> An estimated 10-15% of all breast cancers are inherited. If a mutation is detected in one of these genes, there is a high probability that breast cancer will develop. An individual with a BRCA1 or BRCA2 mutation has a 50% chance of passing down that alteration to his or her children independent of the sex of the child.

Mutations in BRCA1 are responsible for approximately 50% of all inherited predisposition to breast cancer.<sup>166</sup> The estimated lifetime risk for developing breast cancer from BRCA1 mutation is 55-85% and 20-40% lifetime risk for ovarian cancer. The cumulative risk of developing breast or ovarian cancer by age 70, given a mutation in the BRCA1 gene, was estimated to be 80% and 44% respectively.<sup>167,168</sup> Phelan, et al<sup>169</sup> and Rebbeck, et al<sup>170</sup> found that about 61% of the hereditary breast cancers were due to mutations in BRCA1. Men who inherited a BRCA1 mutation have a mildly elevated lifetime risk for prostate cancer. BRCA1 mutation carriers may also have an elevated lifetime risk for colon cancer.

Mutations in BRCA2 account for approximately 35% of the remaining hereditary breast cancers. BRCA2 mutations appear to confer a similar female breast cancer risk to that seen with BRCA1 mutations.<sup>166</sup> However, the risk of ovarian cancer for BRCA2 mutation carriers appears to be lower, with up to a 27% lifetime risk for developing ovarian cancer. Male breast cancer is much more common in BRCA2 families. Male BRCA2 mutation carriers have up to a 6% lifetime risk for developing breast cancer.<sup>166,171</sup> In addition, detailed pedigree analyses of BRCA2 families have identified other malignancies that include laryngeal, prostate, pancreatic and gastrointestinal cancers,

which may occur with increased frequency compared with that of the general population.<sup>172</sup>

The involvement of BRCA1 and BRCA2 in sporadic breast cancers has not been demonstrated. The risk of a woman with a mutation in BRCA1 developing breast cancer is difficult to quantify because the frequency of mutations in the general population is unknown and penetrance of different mutations may vary.

### Recommendation

In 1996, the American Society of Clinical Oncology recommended that only women with a strong family history of breast cancer or those who have developed breast cancer at an early age may be eligible for BRCA genetic testing.<sup>145</sup> Candidates for BRCA testing include women with:

- Breast cancer in two or more close relatives, such as a mother and two sisters.
- Early onset of breast cancer in family members, often before 50 years of age.
- History of breast cancer in more than one generation.
- Cancer in both breasts in one or more family members.
- Frequent occurrence of ovarian cancer
- One or more BRCA positive relatives.
- Male breast cancer. Note: testing limited to BRCA2.

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