# Clinical Practice Guidelines


# On


# Serum Tumour Markers


# 2003

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**Reasons for producing clinical practice guidelines of serum tumour markers**

Many clinicians have the misconception that serum tumour markers can be used reliably to screen for and diagnose malignancies. Many laboratories in Malaysia include serum tumour markers as part of wellness packages, executive screening profiles, admission profiles, etc. This practice is not supported by the evidence available in the scientific literature. Most of the commonly requested serum tumour markers are produced by many different tissues in the body and, hence, suffer from non-specificity. Such tumour markers are, therefore, not of much use in screening and diagnosis of specific malignancies. The indiscriminate use of serum tumour markers in screening packages results, in many instances, in unnecessary investigations leading to increased healthcare costs to the government and/or patient. The finding of a slightly raised serum carcinoembryonic antigen (CEA), for instance, for which there can be several causes, could lead to investigation of a patient for colorectal carcinoma, involving a colonoscopy, CT abdomen or an MRI abdomen to rule out a malignancy. Besides the expense incurred undue anxiety is experienced by the patient.

The Expert Committee hopes that these clinical practice guidelines on the more commonly requested serum tumour markers will help doctors to practise evidence-based medicine as far as tumour markers are concerned.

**LEVELS OF EVIDENCE**

We have used the evidence-grading system for clinical practice recommendations of the American Diabetes Association to grade the levels of evidence of these Clinical Practice Guidelines (Diabetes Care 2003; 26: S33-S50):

| Level of evidence | Description |
|---|---|
| A | Clear evidence from well-conducted, generalisable, randomised controlled trials that are adequately powered including: <br>• Evidence from a well-conducted multicentre trial <br>• Evidence from a meta-analysis that incorporated quality ratings in the analysis <br>• Compelling non-experimental evidence, i.e., "all or none" rule developed by the Centre for Evidence Based Medicine at Oxford" <br><br>Supportive evidence from well-conducted randomised controlled trials that are adequately powered including: <br>• Evidence from a well-conducted trial at one or more institutions <br>• Evidence from a meta-analysis that incorporated quality ratings in the analysis |
| B | Supportive evidence from well-conducted cohort studies <br>• Evidence from a well-conducted prospective cohort study or registry <br>• Evidence from a well-conducted prospective cohort study <br>• Evidence from a well-conducted meta-analysis of cohort studies <br><br>Supportive evidence from a well-conducted case-control study |
| C | Supportive evidence from poorly controlled or uncontrolled studies <br>• Evidence from randomized clinical trials with one or more major or three or more minor methodological flaws that could invalidate the results <br>• Evidence from observational studies with high potential for bias (such as case series with comparison to historical controls) <br>• Evidence from case series or case reports |
| E | Expert consensus or clinical experience |

## INTRODUCTION

**What are tumour markers?**

Tumour markers are substances related to the presence or progress of a tumour.

**Classification of tumour markers**

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 makers which are structural molecules are carcinoembryonic antigen (CEA), mucins like CA 19-9, CA 15-3 and CA125, â2-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), paraproteins 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.

**What is an ideal tumour marker?**

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.
- easy and cheap to measure, from a laboratory point of view.

At present, no tumour marker fulfills all of the above criteria.

## Clinical uses of tumour markers

Tumour markers are used for one of more of the following uses:
- Screening )
- Diagnosis )                        *of limited value*
- Prognosis )
- Monitoring response to therapy }       *valuable*
- Detection of early recurrence    }       *functions*

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 serum in a patient who has had a tumour diagnosed histologically then the tumour marker is useful in monitoring response to therapy and detection of early 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. If the laboratory were to measure a certain tumour marker for reasons other than those which have been approved by the Food and Drug Administration (FDA) the laboratory concerned will not receive any payment for the analysis.


## PSA

### Introduction

Prostate cancer 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 lack sensitivity, often only becoming abnormal in the presence of metastasis and have been largely replaced by serum prostate specific antigen (PSA).[1]

PSA was first identified in the prostate[2] and later found to be immunologically similar to a seminal plasma protein which had been identified earlier.[3] The development of a serum assay for PSA[4] quickly led to its evaluation and use as a marker for prostate cancer.[5]

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 á1-antichymotrypsin, a protein that inactivates its proteolytic activity. The third form of serum PSA is bound to á$_2$-macroglobulin. This third form is not detected by commercially available PSA immunoassays as all its epitopes are concealed by the binding protein.[6] The serum half-life of PSA has been estimated to be 2 to 3 days.[5,7]

**Standardisation of PSA assay**

Numerous commercial immunoassays for PSA are available and variations among them are inevitable. International standards for free and total PSA for the calibration of different assays have been produced.[8-10] 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 and this has been attributed to a higher prevalence of benign prostatic hyperplasia (BPH) in the elderly male population.[11,12] However, this observation is not seen across all Asian populations. The Koreans, for example, have reported a poorer correlation of PSA levels with age[13] contrary to the findings in Taiwanese.[14,15] Attempts to correlate PSA levels with volume of hyperplastic tissue have not been conclusive[16-18] probably due to a large variation of epithelial contribution to the total mass of hyperplastic tissue.[19] In general, serum PSA levels appear to fall substantially following surgical resection for BPH.[5] Less invasive ablation techniques produce a moderate fall in serum PSA levels.[20] Finasteride causes a 50% decrease in PSA levels[21] but herbal products such as saw palmetto (*Serenoa repens*) in its pure form[22] and Permixon[23] do not decrease serum PSA levels significantly. All lower tract endoscopic manipulation can result in a rise in serum PSA concentration.[17,18] Transrectal and transperineal prostate biopsy also increase PSA levels.[24] PSA levels should not be measured after acute urinary retention as marked elevations have been reported.[25] Most workers agree that the rise of PSA after digital rectal examination (DRE) is insignificant.[24,26] Prostatic massage also causes a transient PSA elevation.[5] Similarly, prolonged cycling can cause a rise in PSA,[27] probably from the pressure on the perineum by the bicycle seat.[28] Serum PSA levels rise transiently after sexual activity, peaking approximately 1 hour after ejaculation and returning to baseline within approximately 24 hours.[29] PSA has been reported to be markedly raised in acute prostatitis with levels up to 100 ng/ml.[30]

**Clinical usefulness of PSA in prostate cancer**

*Screening*

Most clinicians have adopted the reference range of 0 to 4 ng/ml using the Tandem R[®] PSA assay.[31] In screening programmes, approximately 30% of Western men who are asymptomatic with PSA levels above 4 ng/ml will have prostate cancer.[11,32] With the additional finding of an abnormal DRE, an incidental PSA of > 4 ng/ml is associated with a cancer predictive value close to 50%.[33] Similar studies have not been conducted in the Malaysian male population but local reports suggest a lower prevalence of prostate cancer in the local population.[34-36]

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 serum 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. Full population screening for prostate cancer in Malaysia will be very difficult to justify as the disease is not as prevalent and the cost implications on the healthcare system will be enormous.

## *Staging*

In general, PSA levels correlate with both clinical and pathological stages.[31] In localised cancers, incidence of capsular penetration on histological examination is higher in patients presenting with PSA levels of greater than 10 ng/ml.[37,38] The finding of high grade PIN in a man with an elevated serum PSA level implies the probable existence of an occult invasive cancer.[39]

## *Monitoring of treatment*

Most PSA assays received approval in the USA only for monitoring of prostate cancer after treatment. Serum PSA levels generally fall 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.[40,41] The absolute serum levels, nadir and rate of fall of PSA after androgen withdrawal have been correlated with disease prognosis.[42-44] Following radical prostatectomy for organ-confined prostate cancer, serum PSA levels should become undetectable within 2 to 4 weeks. Similarly, serum PSA levels fall after definitive radiotherapy but the actual nadir that predicts absence of clinical progression remains a matter for debate.

## *Detection of early recurrence*

Rising serum PSA levels invariably denote disease recurrence. The time interval between a rise in PSA levels and eventual clinical disease can be considerable. For example, the median time to metastases was 8 years from the time of serum PSA level elevation in the Johns Hopkins series.[45]

## **Improving performance of PSA as a tumour marker**

In spite of its increasing popularity, the limitations of PSA are all too obvious. Approximately a quarter of all newly diagnosed cancers in the West present with normal serum PSA levels. Conversely, some two thirds of men with abnormal serum 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.[34-36]

Various surrogates of PSA assays have been suggested to increase PSA performance. Use of age-specific serum PSA cut-off levels have been suggested.[12,46] Generally, PSA increases with age. This is likely to be due to the fact that the incidence of prostate pathology (including cancer) increases with age. A lower serum 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, in the setting of a screening programme for prostate cancer, the overall number of years of life saved, using age-specific cut-off levels, would be reduced.[47]

Men with large prostate glands tend to have higher serum PSA levels and PSA density was initially enthusiastically proposed and shown to be more useful than serum PSA levels.[48-50] However, subsequent studies failed to reproduce this advantage.[51,52] 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.

The rate of PSA increase (PSA velocity) has been shown to be higher in cancer patients than in controls. [53] 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 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 in order to improve the performance of PSA in cancer detection.[6,54] In a multicentre trial, Catalona et al. (1997)[55] reported the use of free-to-total PSA ratio in 622 men with total PSA levels of 4.0 to10.0 ng/ml with no abnormal DRE findings. They 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[56] 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.[57] Results from batch processing at centralised laboratories should, therefore, be interpreted with caution.


## Recommendations

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 measurement and DRE. This is 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 PSA should be explained to patients.

- All males above 40 years of age with the risk factor of having a first degree relative with prostate cancer diagnosed at a young age (<60 years) may be screened. **(E)**
- PSA should be used in combination with DRE to enhance early detection. **(B)**
- The improved sensitivity and specificity of age-specific PSA ranges is at best modest and, hence, not recommended. **(C)**
- PSA velocity (0.75 ng/ml/yr) has not improved the sensitivity and specificity of the test and is probably not useful as first line assessment for prostate cancer. **(C)**
- PSA density is not recommended as there is no improvement in the sensitivity and specificity over the PSA level. **(B)**
- Free to total PSA ratio is helpful as the sensitivity and specificity for detecting prostate cancer is higher in patients with a serum PSA level of between 4 and 10 ng/ml. **(B)** The optimal cut-off level is still being investigated.
- Population screening for prostate cancer among Malaysian men is not recommended. **(E)**
- The appropriate threshold serum PSA level for case detection is 4.0 ng/ml. **(B)**
- Volunteers and/or referred patients with abnormal PSA results and/or suspicious DRE are recommended to undergo prostate biopsy. **(A)**
- PSA is useful in monitoring response to treatment. **(B)**
- PSA is useful in detection of early recurrence. **(B)**

## 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

Serum CA 19-9 levels may be raised in patients with 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, cholestasis, cirrhosis, acute cholangitis and cystic fibrosis [Bates, 1991, Duffy, 1998].[58,59] 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.

CA 19-9 is most useful in pancreatic cancer.

**Clinical usefulness of CA 19-9 in pancreatic cancer**

*Screening*

Screening is not useful. **(C)** The sensitivity of CA 19-9 in early (small) pancreatic cancers is low.[60]

*Diagnosis*

Elevated CA 19-9 is of limited use in the clinical diagnosis of pancreatic cancer. **(B)** 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. **(B)** It may be useful in guiding resectability of the tumour. Very high levels usually predict presence of unresectable tumours.[59] **(B)**

*Monitoring response to treatment*

CA 19-9 is useful for monitoring progress and response to therapy of patients being treated for pancreatic carcinoma.[61,62,63] **(B)** It may be useful in monitoring patients with gastric carcinoma as well. **(C)**

*Detection of early recurrence*

CA 19-9 is useful in detection of early recurrence following pancreatectomy, when levels begin to rise.[64] **(B)**

## Carcinoembryonic Antigen (CEA)

**Introduction**

CEA is a 200 kDa glycoprotein and was first described in 1965 by Gold and Freeman[65] when they identified an antigen that was present in both foetal colon and colon adenocarcinoma but that was absent 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.[66] 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 [Wilson, et al 1999].[67]

Its greatest usefulness is in colorectal cancer.

**Clinical usefulness of CEA in colorectal cancer**

*Screening*

A sensitivity of 36% and specificity of 87% have been calculated for early colonic cancer (Dukes A and B).[68] The low sensitivity limits its value in colorectal cancer screening. **(B)**

*Diagnosis*

In symptomatic patients, the 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. **(C)**

*Prognosis*

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

*Monitoring response to treatment*

CEA measurements are recommended for monitoring response to treatment. **(B)** Longitudinal CEA measurements detect recurrent cancer with a sensitivity of 80% and specificity of 70%.[68] An elevated CEA can help diagnose hepatic metastases with high accuracy.[71]

*Detection of early recurrence*

It is less helpful in predicting loco-regional recurrences. In asymptomatic patients, CEA is the most frequent indicator of recurrences.[71] **(B)**

## CA 125

**Introduction**

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 blood stream. 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. It 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.[72,73]

**Conditions where CA 125 levels may be elevated**

This marker is raised in both benign and malignant conditions other than ovarian cancer as shown in Table 1.[74]

Table 1. Conditions in which a raised serum CA 125 level may be found.

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

**Clinical usefulness of CA 125 in ovarian cancer**

*Screening*

CA 125 should not be used to screen for ovarian cancer since its level can be raised in many benign and malignant conditions.[75] **(B)** A normal value does not exclude ovarian cancer.

*Diagnosis*

Due to its poor specificity CA 125 should not be used to diagnose ovarian cancer.[75] **(B)**

*Prognosis*

In patients with invasive ovarian cancer where the CA 125 level is elevated preoperatively it is valuable is assessing progressive disease and tumour response to chemotherapy.[75] **(B)** It appears to be an independent predictor of survival. If the CA 125 reactive determinant is not expressed preoperatively but present during or after therapy this is a poor prognostic sign.

*Monitoring response to treatment*

A raised CA 125 level after therapy is indicative of residual disease.[75] **(B)** In such instances second-look procedures could be avoided and definitive treatment like aggressive chemotherapy, secondary cyto-reductive surgery or palliative therapy can be considered. However, normal CA 125 level does not exclude small-volume residual disease.

*Detection of early recurrence*

Elevation of CA 125 level can precede clinical or radiographic evidence of recurrence by 3 months, particularly in paracaval or retrocaval lymph nodes.[75] **(B)** After chemotherapy is completed, the assay can be done at regular intervals in order to detect recurrence early.

## 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 occasionally 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.[76,77] 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 of CA 15-3 in breast cancer**

*Screening*

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.[78] **(B)**

*Diagnosis*

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.[75] **(B)** 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.[75]

*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.[75] However, it is still not clear whether CA 15-3 is an independent prognostic indicator. 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.[75] **(B)**

*Monitoring response to treatment*

Tumour marker sensitivity in patients with advanced breast cancer is significantly higher than in loco-regional disease.[79,80] 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. **(B)** 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.

*Detection of early 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. **(B)** 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.[81] 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.[81] 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.

**Recommendations**

Based on current evidence, it is recommended that CA 15-3 be used only in the monitoring of response of breast cancer patients to therapy, especially systemic therapy. **(B)** Moreover, it should be used in conjunction with other markers of response, e.g. clinical evaluation and imaging studies.

We cannot at present recommend the routine use of CA 15-3 in the screening, prognostication and the detection of early recurrence of breast cancer. **(B)**

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

</div>

**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.[82]

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.[75] 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.[83] 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 etiology of liver cancer is well established.[84]

**Clinical usefulness of AFP in hepatocellular carcinoma**

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).[85,86]

*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.[75,87-90] Several trials which have used cirrhotics of various aetiologies[91] and patients who are HBsAg positive[92] have established the benefits of screening of high risk patients with AFP and US. McMahon et al.[92] who studied HBsAg-positive Alaska 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.**(A)** Screening with AFP is not recommended in populations who are not at high risk of developing HCC.**(E)**

*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.[85,86] 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.[85] In malignancy, however, concentrations remain high or even increase. The assay of AFP every 2-3 weeks may therefore eliminate falsely-raised values.[75]

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.[75] **(B)**

*Monitoring response to treatment*

After successful surgical resection of HCC, serum AFP levels 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.[93] 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.[94]

Serial measurement of AFP may be used to monitor response to chemotherapy and may be better than imaging techniques such as CT.[94,95] **(B)**


*Detection of early recurrence*

AFP may be used to detect early recurrence. **(B)** If there is tumour recurrence AFP levels start to rise, often before clinical evidence of disease.[95]


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

### Introduction

hCG is a sialoglycoprotein with a molecular weight of about 36,500 Da.[96] It is initially secreted by the trophoblastic cells of the placenta, shortly after implantation of the fertilised ovum into the uterine wall.[97-100] The physiological source of hCG is the placenta. It contains á and â subunits, á being identical to the á subunit of LH, FSH and TSH. The amino acid residues specific for the â subunit of hCG confer its immunospecificity.[101,102]


### 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.[103-105] The source of hCG in tumours is trophoblast-like cells.[98] Elevated levels are found in germ cell tumours such as choriocarcinoma (always), teratoma (frequently - 40-60%), seminoma (sometimes - 5-10%) and dysgerminoma (sometimes - 3%).[96]


### Clinical usefulness of hCG in choriocarcinoma

*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.[96] 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.[96] **(B)** 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.[99] **(B)** As the tumour arises from placental trophoblasts, it usually produces hCG in quantities that reflect the bulk of the tumour.

*Prognosis*

Serum âhCG is one of the several factors taken into account for prognostication of choriocarcinoma which, in turn, determines the chemotherapy regimen for individual patients. **(B)** The prognosis is poor if serum âhCG level is >40,000U/L at the time treatment is started.[96]

*Monitoring of treatment*

Serum hCG is also useful in monitoring response to treatment and detection of recurrence.[96] **(B)** Contraception should be advocated during the entire follow-up interval to avoid misinterpretation of elevated hCG by pregnancy. Monthly determinations of âhCG is recommended until the level is normal for 12 months.

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

AFP, hCG and its β subunit (βhCG), and, lactate dehydrogenase (LDH) are well-established tumour markers integral to the successful management of patients with testicular and other germ cell tumours.[75,106] 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.[75] 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 tesicular and other GCTs.[106]

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

*Screening*

AFP and hCG should not be used to screen for GCTs.[75, 106] **(B)**

*Diagnosis*

Some 20-60% of patients with germ cell tumours will have raised levels, depending on tumour stage.[107] 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.[96] 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.[75]

Serum hCG and alpha-fetoprotein (AFP) measurement may be helpful in the diagnosis of patients suspected of having GCTs.[75,106] **(B)** Tumour marker measurements, together with testicular ultrasound, may be used to help in the differential diagnosis of a painless swelling of one testis.[75]**(B)** Cerebrospinal fluid (CSF) hCG measurement may improve diagnostic efficiency if metastasis to the brain is suspected.[75] **(E)**


*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.[75]

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.[75]

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. **(A)** 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.[75] In addition, cerebrospinal fluid determinations of AFP and hCG may be helpful in the diagnosis and monitoring of intracranial GCT.[75,108] **(C)** 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.[106] 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.[75,108] **(B)**

**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 (multiple of upper limit of reference range) |
| Good (S1) | <1000 | <5000 | <1.5 x (RR) |
| Intermediate (S2) | ≥1000 & ≤10,000 | ≥5000 & ≤50,000 | ≥1.5 x (RR) & ≤10 x (RR) |
| Poor (S3) | >10,000 | >50,000 | >10 x (RR) |

The system also takes into account tumour site (testis, retroperitoneal, mediastinal) and the presence or absence of non-pulmonary visceral metastases.[75,108]

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.[75] Half-life can be estimated using only 2 measurements or using linear regression[75,109] 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.[75]

While guidelines[75,106,108] have recommended the use of half-life of markers, it should be noted that at least one multi-centre, randomised control 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.[110] **(B)**

*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.[100] **(B)**

For patients undergoing chemotherapy, marker determinations are mandatory prior to each cycle.[75] **(B)** 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).[106]

*Stage 1A and 1B 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.[75] **(B)**

*Stage II disease:* Surveillance should include tumour markers with physical examination and chest X-ray every month during the first year, every second month during the second year, and every third month for the third year.[75] **(B)**

*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.[96] **(B)**

AFP and hCG are useful in monitoring the response to treatment in patients with GCTs.**(B)** Post-treatment monitoring is essential for optimal care and tumour marker normalisation is required to assess the response to chemotherapy. 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.[96] Chemotherapy may damage the liver producing a rising AFP level in a patient with a purely hCG-producing tumour.[75,96] 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.

*Detection of early recurrence*

Preoperative measurement of hCG in all patients with possible germ cell tumours will help in the detection of residual disease post-operatively.[75] **(B)** 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.[111-114] 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.[96] 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).[115] 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 10U/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.[116] 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.[100] 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.[100]

Monthly measurements be made for 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.[96] **(B).** After this, all patients should be monitored for life, with a frequency such that recurrent disease is identified before it is difficult to eradicate. **(B)** 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.

## References

1. Lowe FC, Trauzzi SJ. Prostatic acid phosphatase in 1993: Its limited clinical utility. Urol Clinics North America 1993; 20:589-95.
2. Wang MC, Valenzuela IA, Murphy GP, et al. Purification of a human prostate specific antigen. Invest Urol 1979; 17:159-63.
3. Hara M, Inorre T, Fukuyama T. Some physico-chemical characteristics of gamma-seminoprotein, an antigenic component specific for human seminal plasma. Jpn J Legal Med 1971; 5:322.
4. Kuriyama M, Wang MC, Papsidero LD, et al. Quantification of PSA in serum by a sensitive enzyme immunoassay. Cancer Res 1980; 40:46-58.
5. Stamey TA, Yang N, Hay AR, et al. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987; 317:909-16.
6. Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex alpha-antichymotrypsin. Clin Chem 1991; 37:1618-25.
7. Oesterling JE, Chan DW, Epstein JI, et al. Prostate specific antigen in the preoperative and postoperative evaluation of localized prostatic cancer treated with radical prostatectomy. J Urol 1988; 139:766-72.

8.  Stamey TA. Second Stanford conference on international standardization of PSA immunoassays. Urology 1995; 45:173-84.
9.  Rafferty B, Rigsby P, Rose M, et al. Reference reagents for prostate-specific antigen (PSA): establishment of the first international standards for free PSA and PSA (90:10). Clin Chem 2000; 46:1310-7.
10. Chan DW, Sokoll LJ. WHO first international standards for prostate-specific antigen: the beginning of the end for assay discrepancies? Clin Chem 2000; 46:1291-2.
11. Brawer MK, Chetner MP, Beattie J, et al. Screening for prostate carcinoma with prostate specific antigen. J Urol 1992; 147:841-5.
12. Oesterling JE, Jacobsen SJ, Chute CG, et al. Serum PSA in a community-based population of healthy men. JAMA 1993; 270:860-4.
13. Ku JH, Ahn JO, Lee CH, et al. Distribution of serum prostate-specific antigen in healthy Korean men: influence of ethnicity. Urology 2002; 60:475-9.
14. Lin WY, Gu CJ, Kao CH, Changlai SP, Wang SJ. Serum prostate-specific antigen in healthy Chinese men: establishment of age-specific reference ranges. Neoplasma 1996; 43(2):103-5.
15. Kao CH. Age-related free PSA, total PSA and free PSA/total PSA ratios: establishment of reference ranges in Chinese males. Anticancer Res 1997; 17:1361-5.
16. Partin AW, Carter HB, Chan DW, et al. Prostate specific antigen in the staging of localised prostate cancer: Influence of tumour differentiation, tumour volume and benign hyperplasia. J Urol 1990; 143(4):747 –52
17. Stamey TA. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. Monogr Urol 1989; 10:49.
18. Stamey TA, Kabalin JN, McNeal JE, et al. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. II: Radical prostatectomy treated patients. J Urol 1989; 141(5):1076 – 82.
19. Weber JP, Oesterling JE, Peters CA et al. The influence of reversible androgen deprivation on serum prostate-specific antigen levels in men with bening prostatic hyperplasia. J Urol. 1989;141:987-92.
20. Shingleton WB, Terrell F, Kolski J, May W, Renfroe DL, Fowler JE. Prostate specific antigen measurements after minimally invasive surgery of the prostate in men with benign prostatic hypertrophy. Prostate Cancer Prostatic Dis 2000; 3:200-2.
21. Pannek J, Marks LS, Pearson JD, et al. Influence of finasteride on free and total serum prostate specific antigen levels in men with benign prostatic hyperplasia. J Urol 1998; 159:449-53.
22. Gerber GS, Zagaja GP, Bales GT, et al. Saw palmetto (Serenoa repens) in men with lower urinary tract symptoms: effects on urodynamic parameters and voiding symptoms. Urology 1998; 51:1003-7.
23. Carraro JC, Raynaud JP, Koch G, et al. Comparison of phytotherapy (Permixon) with finasteride in the treatment of benign prostate hyperplasia: a randomized international study of 1,098 patients. Prostate 1996; 29:231-40.
24. Yuan JJ, Coplen DE, Petros JA, et al. Effects of rectal examination, prostatic massage, ultrasonography and needle biopsy on serum prostate specific antigen levels. J Urol 1992; 147:810-4.

25. McNeill SA, Hargreave TB. Efficacy of PSA in the detection of carcinoma of the prostate in patients presenting with acute urinary retention. J R Coll Surg Edin 2000; 45:227-30.

26. Crawford ED, Schutz MJ, Clejan S, et al. The effect of digital rectal examination on prostate-specific antigen levels. JAMA 1992; 267:2227-8.

27. Rana A, Chisholm GD. He sold his bike for a low prostate specific antigen. J Urol 1994; 151:700.

28. Crawford ED 3rd, Mackenzie SH, Safford HR, Capriola M. The effect of bicycle riding on serum prostate specific antigen levels. J Urol 1996; 156:103-5.

29. Tchetgen MB, Song JT, Strawderman M, et al. Ejaculation increases the serum prostate specific antigen concentration. Urology 1996; 47:511-6.

30. Neal DE Jr, Clejan S, Sarma D, et al. Prostate-specific antigen and prostatitis I: Effect of prostatitis on serum PSA in the human and non-human primate. Prostate 1992; 20:105-11.

31. Myrtle JF, Klimley PG, Ivor LP, et al. Clinical utility of prostatic specific antigen (PSA) in the management of prostate cancer. Advances in cancer diagnostics 1986; 14:1.

32. Catalona WJ, Smith DS, Ratliff TL, et al. Detection of organ-confined prostate cancer is increased through PSA-based screening. JAMA 1993; 270:948-54.

33. Catalona WJ, Richie JP, Ahmann FR et al. Comparison of DRE and serum PSA in the early detection of prostate cancers: results of a multicenter clinical trial of 6,630 men. J Urol. 1994;151:1283-90.

34. Ng LG, Yip S, Tan PH, Yuen J, Lau W, Cheng C. Improved detection rate of prostate cancer using the 10-core biopsy strategy in Singapore. Asian J Surg 2002; 25:238-43.

35. Jeng HS, Huang SP, Chou YH, Huang CH. Detection of prostate cancer - experience of seven years in KMUH and review of literature. Kaohsiung J Med Sci 2002; 18:281-8.

36. Yu HJ, Lai MK. The usefulness of prostate-specific antigen (PSA) density in patients with intermediate serum PSA level in a country with low incidence of prostate cancer. Urology 1998; 51:125-30.

37. Lange PH, Ercole CJ, Lightner DJ, et al. The value of serum prostate-specific antigen determinations before and after radical prostatectomy. J Urol 1989; 141:873-9.

38. Ellis WJ, Brawer MK. Repeat prostate biopsy: Who needs it? J Urol 1995; 153:1496-8.

39. Brawer MK, Lange PH. PSA in the screening, staging and monitoring of prostatic adenocarcinoma. World J Urol 1989: 7:711.

40. Young CYF, Montgomery BT, Andrews PE, et al. Hormonal regulation of prostate specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCaP. Cancer Res 1991; 31:3748-52.

41. Henttu P, Liao S, Vihko P. Androgens up-regulate the human prostate-specific antigen messenger ribonucleic acid (mRNA) but down-regulate the prostatic acid phosphatase mRNA in LNCaP cell line. Endocrinology 1992; 130:766-72.

42. Cooper EH, Armitage TG, Robinson MRG, et al Prostate specific antigen and the prediction of prognosis in metastatic prostate cancer. Cancer 1990; 66:1025-8.

43. Siddall JKL, Hetherington JW, Cooper EH, et al. Biochemical monitoring of carcinoma of the prostate treated with an LHRH analogue (Zoladex). Br J Urol 1986; 58: 676-82.

44. Mecz Y, Barak M, Lurie A, et al. Prognostic importance of the rate of decrease in prostate specific antigen (PSA) levels after treatment of patients with carcinoma of the prostate. J Tumour Marker Oncol 1989; 4:323-8.

45. Pound CR, Partin AW, Eisenberger MA, et al. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 1999;281:1591-7.

46. Dalkin BL, Ahmann FR, Kopp JB. PSA levels in men older than 50 years without clinical evidence of prostate carcinoma. J Urol 1993; 150:1837-9.

47. Brawer MK. How to use PSA in the early detection or screening for prostate carcinoma. CA Cancer J Clin 1995; 45:148-64.

48. Benson MC, Whang IS, Olsson CA, et al. The use of prostate specific antigen density to enhance the predictive value of intermediate levels serum prostate specific antigen. J Urol 1992; 147:817-21.

49. Barzinet M, Meshref AW, Trudel C, et al. Prospective evaluation of prostate specific antigen density and systematic biopsies for early detection of prostate carcinoma. Urology 1994;43:44-51.

50. Rommel FM, Augusta VE, Breslin JA, et al. The use of PSA and PSAD in the diagnosis of prostate cancer in a community based urological practice. J Urol 1994; 151:88-93.

51. Brawer MK, Aramburu EAG, Chen GL, et al. The inability of PSA index to enhance the predictive value of PSA in the diagnosis of prostatic carcinoma. J Urol 1993; 150:369-73.

52. Mettlin C, Littrup PJ, Kane RA, et al. Relative sensitivity and specificity of serum PSA level compared with age-referenced PSA, PSA density and PSA change. Cancer 1994; 74:1615-20.

53. Carter H, Morrell CH, Pearson JD, et al. Estimation of prostatic growth using serial prostate specific antigen measurements in men with and without prostate disease. Cancer Res 1992; 52:3323-8.

54. Stenman UH, Leinonen J, Alfthan H, et al. A complex between prostate-specific antigen and á1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991; 51:222 – 6.

55. Catalona WJ, Partin AW, Slawin KM, et al. A multicenter clinical evaluation of free PSA in the differentiation of prostate cancer from benign disease. J Urol. 1997;157:111A.

56. Roth HJ, Stewart SC, Brawer MK. A comparison of three free and total PSA assays. Prostate Cancer Prostatic Dis 1998; 1:326-31.

57. Sokoll LJ, Bruzek DJ, Dua R, et al. Short-term stability of the molecular forms of prostate-specific antigen and effect on percent complexed prostate-specific antigen and percent free prostate-specific antigen. Urology 2002; 60:24-30.

58. Bates SE. Clinical application of serum tumor markers. Ann Intern Med 1991; 115:623-38.

59. Duffy MJ. CA 19-9 as a marker for gastrointestinal cancers. Ann Clin Biochem 1998; 35: 364-70.

60. Steinberg W. the clinical utility of the CA 19-9 tumor-associated antigen. Am J gastroenterology 1990; 85: 350-5.

61. Willett CG, Daly WJ, Warshaw AL. CA19-9 is an index of response to neoadjunctive chemoradiation therapy in pancreatic cancer. Am J Surgery 1996; 172: 350-2.

62. Heinemann V, Schermuly MM, Stieber P, et al. CA 19-9: A predictor of response in pancreatic cancer treated with gemcitabine and cisplatin. Anticancer Res 1999; 19:1-3.

63. Saad ED, Machado MC, Wajsbrit D, et al. Pretreatment CA19-9 level as a prognostic factor in patients with advanced pancreatic cancer treated with gemcitabine. Int J Gastrointest Cancer 2002; 32: 35-41.

64. Steinberg GJ, Kurtzman SH, Steinberg SM, Sindelar WF. Evaluation of the utility of a radioimmunoassay for serum CA 19-9 levels in patients before and after treatment of carcinoma of the pancreas. J Clin Oncol 1988; 6: 462-8.

65. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 1965; 121: 439-62.

66. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clinical Chemistry 2001; 47: 624-30.

67. Wilson APM, van Dalen A, Sibley PEC, Kasper LA, Durham AP, El Shami AS. Multicenter tumor marker reference range study. Anticancer Res 1999; 19: 2749-52.

68. Fletcher RH. Carcinoembryonic antigen. Ann Intern Med 1986; 104: 66-73.

69. McLeod HL, Murray GI. Tumor markers of prognosis in colorectal cancer. Br J Cancer 1999; 79:191-203.

70. Fillela X, Molina R, Pique JM, et al. CEA as a prognostic factor in colorectal cancer. Anticancer Res 1994; 14:705-8.

71. Pietra N, Sarli L, Costi R, Ouchemi C, Grattarola M, Peracchia A. Role of follow-up in management of local recurrence of colorectal cancer, a prospective randomized study. Dis Colon Rectum 1998; 228:1127-33.

72. Bast RC, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Eng J Med 1983; 309: 883.

73. Kenemaus P, Van Kamp GJ, Oehr P, et al: Heterologous double-determinant immunoradiometric assay CA125II: Reliable second-generation immunoassay for determining CA 125 in serum. Clin Chem 1993; 39:2509.

74. Topalak O, Saygili U, Soyturk M, et al. Serum, pleural effusion and ascites CA 125 levels in ovarian cancer and non ovarian benign and malignant diseases: a comparative study. Gynecol Oncology 2002; 85:108.

75. European Group on Tumour Markers: Consensus Recommendations. Anticancer Res 1999; 19: 2785-820.

76. Hayes DF, Zurawski VR, Kufe DW. Comparison of circulating CA15-3 and carcinoembryonic antigen levels in patients with breast cancer. J Clin Oncol 1986; 4: 1542-50.

77. Colomer R, Ruibal A, Genolla J, et al. Circulating CA 15-3 levels in postsurgical follow-up of breast cancer patients and in non-malignant diseases. Breast Cancer Res Treat 1989; 13: 123-33.

78. Tumour Marker Expert Panel. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996; 14: 2843-2877.

79. van Dalen A, Heering KJ, Barak V, et al. Treatment response in metastatic breast cancer. A multicentre study comparing UICC criteria and tumour marker changes. Breast 1996; 5: 82-8.

80. Soletormos G, Nielsen D, Schioler V, et al. Tumor markers cancer antigen 15.3, carcinoembryonic antigen, and tissue polypeptide antigen for monitoring metastatic

breast cancer during first-line chemotherapy and follow-up. Clin Chem 1996; 42: 564-75.

81. Molina R, Zanon G, Filella X, et al. Use of serial carcinoembryonic antigen and CA 15.3 antigen and CA 15.3 assays in detecting relapse in breast cancer patients. Breast Cancer Res Treat 1995; 36: 41-8.

82. Beastall GH, Cook B, Rustin GJS and Jennings J. A review of the role of established tumour markers 1991; 28: 5-18.

83. Okuda K. Early recognition of hepatocellular carcinoma. Hepatology 1986; 6: 279-738.

84. Bosch FX, Ribes J, Boras J. Epidemiology of primary liver cancer. Seminars in Liver Dis 1999; 19: 271-85.

85. Dusheiko GM. Diagnosis of hepatocellular carcinoma, pp 395-397. Ann Intern Med 1988; 108: 390-401.

86. Dusheiko GM. Hepatocellular carcinoma associated with chronic viral hepatitis – aetiology, diagnosis and treatment. Br Med Bull 1990; 46: 492-511.

87. McMahon BJ, London T. Workshop on screening for hepatocellular carcinoma. J Natl Cancer Inst 1991; 83: 916-9.

88. Colombo M. Early diagnosis of hepatocellular carcinoma in Italy. J Hepatol 1992; 14: 401-3.

89. Colombo M. Screening for hepatocellular carcinoma. Digestion 1998; 59 (suppl): 70-1.

90. Haydon GH, Hayes PC. Screening for hepatocellular carcinoma. Eur J Gasterol & Hepatology 1996; 8: 856-60.

91. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of AFP in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology 1994; 19: 61-6.

92. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, Dunaway E, Williams J. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology 2000; 32: 842-6.

93. Trojan J, Raedle J, Zeuzem S. Serum tests for diagnosis and follow-up of hepatocellular carcinoma after treatment. Digestion 1998; 59 (suppl 2): 72-4.

94. Yang WT, Johnson PJ. Monitoring response to treatment in liver tumours. Balliére's Clinical Gastroenterology 1999. 13: 637-54.

95. Johnson PJ. Chemotherapy for unresectable liver cancer. Asian J Surgery 2000; 23: 9-15.

96. Beastall GH, McAllister EJ. Tumour markers. In: Marshall WJ, Bangert SK (eds.) Clinical biochemistry: metabolic and clinical aspects. Churchill Livingstone, Edinburgh 1995, pp705-16.

97. Braunstein GD, Rasor J, Adler D, Danzer H, Wade ME. Serum human chorionic gonadotropin levels throughout normal pregnancy. Am J Obstet Gynecol 1976;126: 678-81.

98. Brody S, Carlstrom G. Immunoassay of human chorionic gonadotropin in normal and pathologic pregnancy. J Clin Endocrinol Metab 1962; 22: 564-74

99. Mann K. Tumour markers in testicular cancers. Urology 1990; A 29: 77-86.

100. Doherty AP. Bower M. Christmas TJ. The role of tumour markers in the diagnosis and treatment of testicular germ cell cancer. Br J Urol 1997; 79: 247-52.

101. Swaminathan N, Bahl OP. Dissociation and recombination of the subunits of human chorionic gonadotropin. Biochem Biophys Res Commun 1970; 40: 422-7.

102. Human Reproduction Unit, World Health Organization, Geneva, Switzerland. Assay of protein hormones related to human reproduction: Problems of specificity of assay methods and reference standards. Acta Endocrinol 1972; 71: 625-37.

103. Saxena BB, Landesman R. Diagnosis and management of pregnancy by the radioreceptor assay of human chorionic gonadotropin. Am J Obstet Gynecol 1978; 131: 97-107.

104. Kadar N. DeVore G, Romero R. Discriminatory hCG zone: its use in the sonographic evaluation for ectopic pregnancy. Obstet Gynecol 1981; 58; 156-61.

105. Kadar N. Caldwell BV, Romero R. A method of screening for ectopic pregnancy and its indications. Obstet Gynecol 1981; 58: 162-6.

106. Sturgeon C. Practice guidelines for tumour marker use in the clinic. Clin Chem 2002; 48: 1151-9.

107. Klein EA. Tumour markers in testis cancer. Urol Clin North Am 1993; 20: 67-73.

108. International Germ Cell Cancer Collaborative Group (IGCCCG): International Germ Cell consensus classification: a prognostic factor-based staging system for metastatic germ cell cancer. J Clin Oncol 1997; 15: 594-603.

109. Horwich A, Peckham MJ. Serum tumour-marker regression rate following chemotherapy for malignant teratoma. Eur J Cancer Clin Oncol 1984; 20: 1463-70.

110. de Wit, R, Sylvester R, Tsitsa C, de Mulder PH, Sleyfer DT, ten Bokkel Huinink WW, Kaye SB, van Oosterom AT, Boven E, Vermeylen K, Stoter G. Tumour marker concentration at the start of chemotherapy is a stronger predictor of treatment failure than marker half-life: a study in patients with disseminated non-seminomatous testicular cancer. Br J Cancer 1997; 75: 432-5.

111. Mazumdar M, Bajorin DF, Bacik J, Higgins G, Motzer RJ, Bosl GJ. Predicting outcome to chemotherapy in patients with germ cell tumors: The value of the rate of decline of human chorionic gonadotrophin and alpha-fetoprotein during therapy. J Clin Oncol 2001; 19: 2534-41.

112. Horwich A, Peckham MJ. Transient tumor marker elevation following chemotherapy for germ cell tumors of the testis. Cancer Treat Rep 1986; 70: 1329-31.

113. Vogelzang NJ, Lange PH, Goldman A, et al. Acute changes of á-fetoprotein and human chorionic gonadotrophin during induction chemotherapy of germ cell tumors. Cancer Res 1982; 42: 4855-61.

114. Horwich A, Peckham MJ. Serum tumour marker regression rate following chemotherapy for malignant teratoma. Eur J Cancer Clin Oncol 1984; 20: 1463-70.

115. Lamerz R. Alpha-fetoprotein. In: Clinical Laboratory Diagnostics, 1ˢᵗ Ed., L. Thomas (ed.) Frankfurt am Main. TH-Books Verlagsgesellschaft, 1998, pp 941-5

116. De Bruijn HWA, Sleijfer DTH, Koops HS, Suurmeijer AJH, Marrink J, Oekhuizen T. Significance of human chorionic gonadotrophin, á-fetoprotein, and pregnancy-specific â1-glycoprotein in the detection of tumor relapse and partial remission in 126 patients with non-seminomatous testicular germ cell tumors. Cancers 1985; 55: 829-35.