Guidelines for the use of tumour markers
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Association of Clinical Biochemists in Ireland

MJ Duffy  P McGing
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Other booklets in this series:
- Guidelines on the use of biochemical cardiac markers and risk factors
- Guidelines on the use of therapeutic drug monitoring
- The biochemistry of body fluids
Preface

This is the 4th edition of the well established publication ‘Guidelines for the use of Tumour Markers’ and is part of a series commissioned and produced by the Scientific Committee of the Association of Clinical Biochemists in Ireland to promote appropriate and effective use of the laboratory service. It is intended to be a concise reference document to assist both practitioners in the Clinical Biochemistry field and those who are users of the laboratory service.

The guidelines in this new edition are based upon up to date scientific evidence and expert consensus and will provide support for consistency of test requesting. There is also a new section outlining the use of Tumour Markers in patients with a cancer of unknown primary origin and it is hoped that this section will provide useful guidance in what is considered a difficult clinical management situation.

On behalf of ACBI Council, I would like to express our gratitude to the Scientific Committee and the individual authors for their work on this project. In particular, we are honoured to have Prof Joe Duffy as the lead author. Prof Duffy has published extensively in this field and has been a lead contributor to major international guidelines for the use of Tumour Markers. Council is also grateful to Cruinn Diagnostics Ltd and Siemens Ltd for their generous financial contribution to the printing costs of this new edition.

Ms Orla Maguire,
President, ACBI

October 2010
Introduction

The purpose of this booklet is to give a brief background to enable the judicious use of seven widely performed serum cancer markers. The format used here is similar to that in previous editions. However, in this 4th edition, we provide for the first time, a section on the use of serum markers in the diagnosis of tumours of unknown primary origin.

While this booklet gives specific details relating to the most frequently used markers, we feel that it is important to make some general points about tumour marker tests.

- No serum marker in current use is specific for malignancy.
- Generally, serum marker levels are rarely elevated in patients with early malignancy. With a few exceptions, high levels are usually found only when patients have advanced disease.
- No cancer marker has absolute organ specificity. PSA however, appears to be relatively specific for prostate tissue, but not for prostate cancer.
- Apart from possibly hCG in choriocarcinoma, no marker is elevated in 100% of patients with a particular malignancy.
- Requesting of multiple markers (such as CEA and the CA series of antigens) in an attempt to identify metastases of unknown primary origin is rarely of use (see below).
- Tumour markers assays should not be carried out on biological fluids such as peritoneal fluids, pancreatic juice and ovarian cystic fluids as reliable reference ranges are currently unavailable for these types of specimen.
- Reference ranges for cancer markers are not well defined and are used only for guidance. Please note that a level below the reference range does not exclude malignancy while concentrations above the reference range does not necessarily mean the presence of cancer. Changes in levels over time are likely to be more clinically useful than absolute levels at one point in time.
- As many tumour markers lack agreed International Reference Preparations (e.g CA125, CA15-3, CA19-9), different assay kits may give different results for the same sera.
- Laboratories carrying out tumour marker tests should state the assay used on their report form.
General References on Tumour Markers

Alpha-Fetoprotein (AFP)

Structure
AFP is a 70 kDa glycoprotein homologous to albumin.

Forms in serum
AFP exhibits microheterogeneity probably due to varying levels of glycosylation. AFP produced by malignancies appears to be more highly fucosylated than that formed by normal tissues.

Physiological function
Appears to perform some of the functions of albumin in the foetal circulation.

Malignancies with elevated levels
Mainly confined to 3 malignancies, i.e.
- Non-seminomatous germ cell tumours (NSGCT) of testis, ovary and other sites.
- Hepatocellular carcinoma (HCC).
- Hepatoblastoma (in children, extremely rare in adults).
- AFP may be occasionally elevated in patients with other types of advanced adenocarcinoma.

Benign conditions which may have elevated levels
Hepatitis, cirrhosis, biliary tract obstruction, alcoholic liver disease, ataxia telangiectasia and hereditary tyrosinaemia.

Physiological conditions with elevated levels
Pregnancy and the first year of life. Infants have extremely high levels which fall to adult values between 6 months and 1 year of age. A slower than normal rate of fall may indicate the presence of a tumour.

Main clinical applications
- In combination with hCG, for monitoring patients with NSGCT (mandatory).
- Independent prognostic marker for NSGCT (e.g. of the testis).
- Diagnostic aid for hepatocellular carcinoma and hepatoblastoma. In patients with cirrhosis and a focal lesion > 2 cm with arterial hypervascularization, an AFP level >200 µg/L is suggestive of HCC, and AFP>400 µg/L is strongly suggestive of HCC.
- Screening for hepatocellular carcinoma in high risk populations (e.g. in patients with cirrhosis due to hepatitis B or C). Surveillance is recommended using 6-monthly AFP measurement and abdominal ultrasound, with AFP>20 µg/L and rising prompting further investigation.
- Note that AFP is not a useful marker for liver metastases. For liver metastases, CEA is preferable marker.

Reference range
0 - 10 kU/L or 0-12 µg/L

Half life in serum
Approx. 5 days.

Comment on assay
Existing immunoassays appear to detect total AFP and do not discriminate between different glycosylated forms.

References
Structure
CA 125 refers to the antigen originally detected by the OC-125 antibody. The protein detected by this antibody is Muc16, a mucin with a single transmembrane domain.

Forms in serum
The major forms in serum have molecular weights of 200 kDa to 400 kDa.

Physiological function
None established.

Malignancies with elevated levels
a. Epithelial ovarian cancer; 80 - 85% of all cases; but increased in only half of early (stage 1) cancer.
b. May be elevated in any adenocarcinoma with advanced disease.

Benign conditions which may have elevated levels
Endometriosis, acute pancreatitis, cirrhosis, peritonitis, inflammatory pelvic disease. The presence of ascites (of non-malignant origin) can also give rise to elevated serum levels of CA 125.

Physiological conditions with elevated levels
Menstruation and pregnancy may be associated with moderately elevated serum CA 125 (usually not more than 100 kU/L)

Main clinical applications
a. While CA125 should not be used in screening asymptomatic women for sporadic ovarian cancer, its measurement in postmenopausal patients with pelvic masses may help differentiate malignant from benign lesions.
b. The rate of decline during initial therapy is an independent prognostic indicator in ovarian carcinoma.
c. Monitoring treatment with chemotherapy.
d. Surveillance following initial treatment. The impact of this on survival is unclear.

Type of sample for assay
Serum is recommended.
CA 125 may be assayed on other fluid samples (e.g. ascitic fluid) but this cannot be recommended (outside of research projects), on analytical and interpretational grounds.

Reference range
0 - 35 kU/L (most frequently used range). Please note however, that levels may be higher in premenopausal than postmenopausal women.

Half life in serum
Approx. 5-7 days.

References
   Int J Gynecol Oncol 2005;15:679
CA 15-3

**Structure**
CA 15-3 is a transmembrane glycoprotein encoded by the MUC1 gene. It is defined by reactivity with 2 monoclonal antibodies, i.e., DF3 and 115D8 in a sandwich immunoassay.

**Physiological function**
May be involved in cell adhesion and cancer pathogenesis.

**Malignancies with elevated levels**
Breast and other adenocarcinomas, especially with distant metastasis. Rarely elevated in patients with local breast cancer.

**Benign diseases with elevated levels**
Benign liver disease, possibly benign breast disease.

**Main clinical applications:**
- a. For preclinically detecting recurrences in asymptomatic patients with diagnosed breast cancer. Use of CA 15-3 in this setting is controversial.
- b. For monitoring the treatment of patients with advanced breast cancer, especially in patients with disease that cannot be evaluated using standard criteria.

**Reference range**
0 – 25 to 0 – 40 kU/L

**Half life in serum**
Unknown

**Comment about assay**
Other assays such as BR 27.29 appear to measure the same antigen as CA 15-3

**References**
CA 19-9

**Structure**
A mucin which reacts with monoclonal antibody 111 6 NS 19-9.

**Physiological function**
May be involved in cell adhesion.

**Malignancies with elevated levels**
Most pancreatic adenocarcinomas, approx. 50% of gastric carcinomas and approx. 30% of colorectal carcinomas.

**Benign conditions which may have elevated levels**
Acute and chronic pancreatitis, hepatocellular jaundice, cirrhosis, acute cholangitis and cystic fibrosis.

**Main clinical applications**
- As a diagnostic aid for pancreatic carcinoma. Inadequate sensitivity and specificity limit the use of CA 19-9 in the early diagnosis of pancreatic cancer. However, in non-jaundiced patients, CA 19-9 may complement other diagnostic procedures.
- Monitoring treatment of patients with pancreatic adenocarcinoma.

**Other potential uses**
Diagnostic aid in gastric and cholangio carcinomas. For colorectal cancer, CEA is generally more valuable than CA 19-9.

**Reference range**
Very variable, from 0 - 37 kU/L to 0 - 100 kU/L

**Half life in serum**
Approx. 1 day but can vary from less than 1 day to 3 days.

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**References**
CEA

Structure
A 200 kDa (approx.) glycoprotein.

Physiological function
Appears to play a role in cell adhesion and inhibition of apoptosis.

Malignancies with elevated levels
Can be elevated in almost any advanced adenocarcinoma, i.e., where distant metastases are present. Almost never elevated in early malignancy.

Benign diseases which may have elevated levels
Hepatitis, cirrhosis, alcoholic liver disease, obstructive jaundice, ulcerative colitis, Crohn's disease, pancreatitis, bronchitis, emphysema and renal disease. Levels may also be mildly elevated in apparently healthy individuals who smoke.

Physiological conditions with elevated levels
None to our knowledge.

Main clinical applications
a. In surveillance following curative resection of colorectal cancer.
b. In monitoring therapy in advanced colorectal cancer. This is especially important when disease cannot be evaluated by standard criteria.

Other potential uses
May also be useful in other gastrointestinal malignancies and as a "general purpose" marker for adenocarcinomas. Please note however, that CEA is rarely elevated in patients with any type of local cancer.

Reference range
0 - 3.5 µg/L to 0 - 5.0 µg/L.

Half life in serum
Approx. 3 days but can vary from 1 to 5 days.

References
Human Chorionic Gonadotropin (hCG)

Structure
hCG is a heterodimer composed of 2 glycosolated sub-units (alpha and beta chains) non-covalently bonded. The alpha chain is almost identical to the alpha chain in TSH, FSH and LH. The beta chain is distinct from the corresponding chains in TSH and FSH but has a high degree of homology with LH over the first 75% of the amino sequence. hCG however, possesses a distinctive 24 amino acid carboxy-terminal extension.

Forms in serum
hCG can exist in multiple forms including the intact 2-chain peptide, free alpha and beta chains, as well as various degradation products (e.g., beta core fragment).

Physiological function
To maintain progesterone production by the corpus luteum during early pregnancy. hCG can be detected as early as one week after conception.

Malignancies with elevated levels
a. Virtually all patients with gestational trophoblastic disease (GTD) (i.e., complete and partial molar pregnancy, choriocarcinoma and placental site trophoblastic tumours).
   b. Non-seminomatous germ cell tumours (NSGCT) (e.g., of testis and ovary).
   c. Seminomatous germ cell tumours of testis (approx. 20%).
   d. Can be produced by a small number of other malignancies.

Benign Diseases With elevated levels
Very few, e.g., ectopic pregnancy, pituitary adenoma.

Physiological conditions with elevated levels
Pregnancy, after termination of pregnancy.

Main clinical applications
a. For monitoring patients with GTD.
   b. In conjunction with AFP, for determining prognosis and monitoring patients with NSGCT of testis, ovary and other sites.

Other potential uses
Diagnostic aid for trophoblastic disease. Serum hCG levels do not usually differentiate between trophoblastic tumours and normal pregnancy. However, very high levels outside the range for twin pregnancies may lead to suspicion of a trophoblastic tumour. For diagnosing trophoblastic tumours, hCG assays are usually used in combination with ultrasound.

Type of sample for assay
Serum or urine.

Reference range
Serum: 0 - 5 IU/L.

Half life in serum
Approx. 16 - 24 hours; decline may be biphasic with a second half life of 13 days.

Comment about assay
When used as a tumour marker, assays for hCG should detect all the main forms, especially the intact molecule and beta-subunit. Some hCG assays may give either false-positive or false-negative results. If false-positive results are suspected, then measure hCG in urine. Note: some methods for hCG may cross-react with LH.

References
Prostate Specific Antigen (PSA)

Structure
A 28.4 kDa single chain chymotrypsin-like serine protease containing 237 amino acids and a member of the glandular kallikrein family.

Forms in serum
Various molecular forms because of complex formation with protease inhibitors. Major immunoreactive form is PSA complexed with α1-antichymotrypsin (PSA-ACT). Other complexes occur such as PSA linked to α1-antitrypsin (trace quantity) and α2-macroglobulin (undetectable by current immunoassays). A non-complexed free form (fPSA) represents 5 to 40% of the “total” PSA (fPSA + α1-antichymotrypsin complex).

Physiological function
Partially responsible for the liquefaction of semen to promote the release and motility of spermatozoa.

Malignancy with elevated levels
Present data suggests that prostate cancer is the only malignancy giving rise to elevated PSA levels in serum. However, PSA has been found in cells from various cancer types and different normal tissues. PSA is thus not completely prostate specific.

Benign conditions with elevated levels
Benign prostatic hypertrophy (BPH), acute and chronic prostatitis, UTI, urinary retention.

A number of urological manipulations such as TURP, prostate biopsy, prostate massage and ejaculation may give rise to transient elevated levels. See Section Effects of Urological Manipulations on PSA Levels on the next page.

Physiological conditions with elevated levels
None described.

Main clinical applications
a. In combination with digital rectal examination PSA can aid the diagnosis of prostate cancer.
b. Determining prognosis in patients with prostate cancer.
c. Surveillance following diagnosis of prostate cancer
d. Monitoring therapy in patients with diagnosed prostate cancer.

Other potential uses
The value of PSA in screening for prostate cancer is controversial. Preliminary results from two randomized prospective trials were recently published. One trial found no significant reduction in mortality from prostate cancer, while the other found a 20% reduction in mortality, but at the expense of overdiagnosis.

Reference range:
0 - 4 µg/L (most frequently used) but some advocate age-related reference ranges as follows:

<table>
<thead>
<tr>
<th>Age Range</th>
<th>PSA µg/L</th>
</tr>
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<tbody>
<tr>
<td>40 - 49</td>
<td>0 - 2.5</td>
</tr>
<tr>
<td>50 - 59</td>
<td>0 - 3.5</td>
</tr>
<tr>
<td>60 - 69</td>
<td>0 - 4.5</td>
</tr>
<tr>
<td>70 - 79</td>
<td>0 - 6.5</td>
</tr>
</tbody>
</table>

Half life in serum
Approximately 2.5 days after radical prostatectomy. Half life after radiotherapy may be many months.

Effects of urological manipulations on PSA levels.
It is advisable to take blood for PSA measurement before rather than after any of these manipulations.

DRE May cause minor increases which are rarely of clinical significance.

Prostate massage May cause minor elevations in some patients.

Ejaculation Results conflicting but may increase PSA levels.

TURP Increases PSA levels significantly. It is recommended to wait at least 6 weeks before drawing blood for PSA assay.

Needle Biopsy As with TURP; increases PSA levels significantly. Wait at least 6 weeks before drawing blood for PSA assay.

Ultrasound Increases PSA levels in a minority of subjects.

Cystoscopy Flexible cystoscopy does not appear to increase PSA levels but rigid cystoscopy may increase levels.
Effect of drugs on PSA levels
Finasteride and Dutasteride, 5-alpha-reductase inhibitors used to treat BPH, reduce PSA levels by approx. 50%.

Comment about assay
PSA assays should detect the free and complexed forms on an equimolar basis. Furthermore, the assay should be standardised against the First International Standard for PSA.

Total PSA is stable for at least 6 hours at room temperature in uncentrifuged clotted blood. Five cycles of freezing and thawing caused no significant change.

References
Free PSA (fPSA)

Form in serum
As stated above, PSA exists in serum in both a bound and free form. The free form includes enzymically inactive pre-PSA, pro-PSA, clipped-PSA and the enzymatically active form of free PSA. The lower the %fPSA, the higher the probability of prostate cancer.

Main clinical applications
To enhance the specificity of total PSA in detecting prostate cancer, especially when total PSA values are between 4 and 10 µg/L. The use of free/total PSA in men with PSA levels can reduce the number of unnecessary biopsies.

Type of sample for assay
Serum or plasma, assay should be carried out on same sample which had total PSA determined.

Reference range
fPSA results can be used in 2 ways:

1. Use of a single cut-off point. In a large prospective multi-centre study with a cut off point of <25% fPSA (Ref 1), it was shown that unnecessary needle biopsies could be reduced by 20% while maintaining a 95% cancer detection rate with total PSA levels between 4 and 10 µg/L.

2. Probability of cancer: In the same multi-centre study, the following relationships were found between % fPSA and probability of prostate cancer.

<table>
<thead>
<tr>
<th>% fPSA</th>
<th>% Cancer Probability</th>
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<tbody>
<tr>
<td>0-10</td>
<td>56</td>
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<tr>
<td>10-15</td>
<td>28</td>
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<tr>
<td>15-20</td>
<td>20</td>
</tr>
<tr>
<td>20-25</td>
<td>16</td>
</tr>
<tr>
<td>&gt;25</td>
<td>8</td>
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Comment about assay
When using %fPSA, both the free and total PSA assay should be obtained from the same supplier. fPSA is less stable than total PSA, for medium and long-term storage, freezing at -70°C is recommended. Assay of complex PSA, i.e. PSA bound to ACT, appears to give similar information to the free/total ratio in men with PSA levels between 2 and 10 µg/L.

Effect of urological manipulations on fPSA
All the manipulations which increase total PSA levels also increase the level of free PSA as well as the percentage free.

Effect of drugs
While certain 5-alpha-reductase inhibitors, such as Finasteride and Dutasteride, reduce both total and free PSA levels, they do not significantly change the %fPSA level.

References
Cancers of unknown primary

Cancers of unknown primary origin or occult cancers are defined as histologically proven malignant metastatic tumours whose primary origin cannot be identified during pretreatment evaluation.

Overview of clinical utility

In general, serum markers are of little value in identifying the primary site. This is because, with a small number of exceptions, currently available serum markers are not organ-specific. Indeed, most of the available markers such as CEA, CA 125, CA 19-9 and CA 15-3 can be elevated in most types of advanced adenocarcinomas.

Main clinical applications

Markers that are potentially useful in diagnosing or excluding a likely site are:

- PSA in men for including or excluding prostate cancer.
- AFP and hCG for including or excluding a germ cell tumour.
- Thyroglobulin for including or excluding a differentiated thyroid tumour.

Reference

HER-2 (c-ErbB-2)

Clinical uses
a. Mandatory uses: For the identification of patients who may be treated with trastuzumab (Herceptin) in the metastatic setting, and to identify patients that may be eligible for clinical trials of trastuzumab in the adjuvant setting.
b. In combination with other factors, HER-2 may also be used to determine prognosis.
c. Insufficient data is currently available to recommend HER-2 for predicting response to endocrine therapy or any type of chemotherapy.

Recommended assay
FISH or immunohistochemistry with validated antibodies.

The extracellular domain of HER-2 can be measured in serum. Serum HER-2 levels may be used for post-operative surveillance and monitoring therapy in patients with breast cancer. Based on evidence presently available, use of serum HER-2 has no advantage over CA 15-3. It may however, be of use if CA 15-3 is not elevated. Preliminary findings suggest that serum HER-2 may be of value in monitoring Herceptin therapy.

References

Estrogen and Progesterone Receptors (ER and PR)

Clinical uses
a. For predicting response to hormone therapy in patients with either early or advanced breast cancer.
b. In combination with other factors, ER and PR may be used to determine prognosis.

Recommended assay
Immunohistochemistry with a validated antibody.

References
Urokinase Plasminogen Activator (uPA) and PAI-1

Clinical uses
a. Assay of uPA and PAI-1 can be carried out to identify lymph node-negative breast cancer patients that may not need, or who are unlikely to benefit from, adjuvant chemotherapy.

b. The prognostic value of these factors for lymph node-negative breast cancer patients has been validated using both a randomised trial and a pooled analysis.

Recommended assay
A validated ELISA

References

