Serum Protein Tumor Marker Assays: A Need for Constant Vigilance

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Learning Objectives

• Describe what comprises an ideal tumor marker and the potential clinical utilities

• Understand factors that affect tumor marker clinical performance characteristics

• Understand limitations / challenges with immunometric immunoassays (including serum tumor markers)

Estimated Cancer Deaths in the US in 2015

American Cancer Society: Cancer Facts and Figures 2015
Estimated New Cancer Cases* in the US in 2015

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>Bladder</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>Bladder</td>
<td>Bladder</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>Kidney &amp; renal pelvis</td>
</tr>
<tr>
<td>Other cancers</td>
<td>Other cancers</td>
</tr>
</tbody>
</table>

*Excludes basal cell and squamous cell cancers and in situ carcinomas except bladder.

Tumor Markers

- Definition:
  - Substance present in or produced by a tumor
  - Substance produced by the patient in response to tumor
  - Detects or differentiates tumor from normal tissue
- Presence: In tumor, serum or other body fluids

FDA-Approved Serum Protein Tumor Markers

<table>
<thead>
<tr>
<th>Serum Tumor Marker</th>
<th>Cancer Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PSA</td>
<td>Prostate</td>
</tr>
<tr>
<td>Free PSA</td>
<td>Prostate</td>
</tr>
<tr>
<td>fPSA/PSA, fPI</td>
<td>Prostate</td>
</tr>
<tr>
<td>AFP</td>
<td>Non-seminomatous Testicular</td>
</tr>
<tr>
<td>AFP-L3%</td>
<td>Hepatocellular</td>
</tr>
<tr>
<td>DCP</td>
<td>Hepatocellular</td>
</tr>
<tr>
<td>CEA</td>
<td>Colorectal, Breast, Lung</td>
</tr>
<tr>
<td>CA15-3</td>
<td>Breast</td>
</tr>
<tr>
<td>CA27.29</td>
<td>Breast</td>
</tr>
<tr>
<td>CA125</td>
<td>Ovarian</td>
</tr>
<tr>
<td>HE4</td>
<td>Ovarian</td>
</tr>
<tr>
<td>BRCA1,2 (CA12+HE4)</td>
<td>Ovarian</td>
</tr>
<tr>
<td>OVA1</td>
<td>Ovarian</td>
</tr>
<tr>
<td>TAG</td>
<td>Thyroid</td>
</tr>
<tr>
<td>CA19-9</td>
<td>Pancreas</td>
</tr>
</tbody>
</table>

**hCG is commonly used as tumor marker though this application has not been approved by the FDA.**
Potential Clinical Utility of Tumor Markers

- Detection
  - Tumor screening (e.g., CA-125 in high-risk patients)
  - Residual tumor (e.g., Tg)
  - Tumor recurrence (e.g., hCG)

- Risk stratification
  - Aid in diagnosis (e.g., AFP with ultrasound or biopsy)
  - Aid in staging – prognostication (e.g., CEA)

- Monitoring
  - Therapeutic responsiveness (Many tumor markers)

Tumor marker results should be interpreted with clinical evidence.

Ideal Tumor Marker

Clinical Performance Characteristics

- High clinical sensitivity
  - Detection of early cancer or recurrence (i.e., few FN)
  - Sensitivity = TP/(TP+FN)

- High clinical specificity
  - Absent in health and benign disease (i.e., few FP)
  - Tumor or tumor-type specific
  - Specificity = TN/(TN+FP)

- Increasing Test Values
  - Probability of correct initial diagnosis, identification of residual disease, recurrence or absence
  - Quantitatively reflect tumor burden
  - Assess cancer stage / prognostication
  - Monitor therapeutic responsiveness (short half-life)
Clinical Reality of Tumor Marker Performance

Tumor marker clinical performance characteristics are dependent on:
- Patient population
- Assay analytical performance
- Reference values

Tumor Markers: Analytical and Clinical Performance Characteristics

Clinical Sensitivity - Positivity in Disease
As you move to the RIGHT, you decrease clinical sensitivity
Clinical Specificity – Negativity in Health
As you move to the RIGHT, you increase clinical specificity
Positive and Negative Predictive Values depend on clinical sensitivity, specificity and prevalence of disease in patient population

To increase clinical sensitivity, specificity, PPV, and NPV at the same time generally necessitates a change in the analytical assay performance in order to increase the separation between Healthy and Diseased populations.
Total PSA Sensitivity and Specificity

- 1986: 4.0 ng/mL (μg/L) cutoff proposed
  - Hybritech study of 472 men w/out history of prostate cancer
- 1994: Major screening study of 6630 men 50 to 74 years old led to first FDA approval for early detection
  - Verified 4.0 ng/mL cutoff, considered aggressive position at the time
  - Sensitivity – 82%
  - Specificity – 49%
  - PPV – 32%
- No PSA cutoff level yields both high clinical sensitivity and specificity

Does PSA Screening Reduce Prostate Cancer Mortality?

- Conflicting evidence:
  - PLCO (Prostate, Lung, Colorectal and Ovarian) Screening Trial
    - 76,693 men; PSA screening vs. control arm; 7-10 yr follow-up
    - No reduction in rate of prostate cancer deaths with PSA screening
    - Contamination – PSA screening vs. U.S. PSA screening practice
  - ERSPC (European Randomized Study of Screening for Prostate Cancer)
    - 182,160 men; PSA screening vs control arm; 9 yr follow-up
    - Significant reduction in rate of prostate cancer deaths with PSA screening (relative risk ratio of 0.80)
      - To prevent 1 prostate cancer death, 1410 men would need PSA testing and an additional 48 men would need to be treated

Guidelines For Tumor Marker Clinical Utility

- Seek literature and guidelines for tumor marker clinical utility
  - Example: Guidelines for PSA Mass Screening
    - Insufficient evidence to recommend PSA screening
      - American College of Preventive Medicine (2008)
    - Recommend informed-decision making for PSA screening
      - American Cancer Society (2010)
      - American College of Physicians (2013)
      - American Urological Association (2013)
    - Recommend against PSA screening
        - Grade D recommendation (harms of screening outweigh the benefits)
      - American Academy of Family Physicians (2012)
**Immunometric Immunoassays**

- Detection Ab*  
  - [Chemiluminescent compound – ICMA](#)  
  - [Enzyme – ELISA](#)  
  - Other

- Absorbance

- [Tumor Marker](#)

- Tumor marker (serum)

- Capture Ab*

  *Antibodies (Ab) in excess

- Detection signal increases with increasing concentration of tumor marker

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**History of PSA Testing**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>Hybritech Tandem-R RIA PSA assay PDA-approved as an aid in prostate cancer management</td>
</tr>
<tr>
<td>1991</td>
<td>Automated PSA assay Calibrators traceable to Hybritech</td>
</tr>
<tr>
<td>1994</td>
<td>Origin of WHO PSA standards</td>
</tr>
<tr>
<td>1995</td>
<td>WHO IRP developed 96/670 (tPSA) 96/668 (fPSA)</td>
</tr>
<tr>
<td>1999</td>
<td>Manufacturers begin re-calibration of PSA assays</td>
</tr>
<tr>
<td>2004</td>
<td>Clinical implications of PSA result differences related to re-calibration are recognized &amp; reported</td>
</tr>
</tbody>
</table>

- Hybritech standards based on Lowry method-derived extinction coefficient
- "Stanford standards" based on mass spectrometry amino acid analysis-derived extinction coefficient
- Indicated Hybritech standards have 10-20% positive bias

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**Effect of Analytical Bias on Classification Based on Fixed Criteria**

- E.g. PSA Hybritech calibration
- E.g. PSA WHO calibration

- Fixed Decision Threshold

- 20% bias

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PSA Clinical Concordance Using WHO and Hybritech Calibrations

<table>
<thead>
<tr>
<th>WHO Calibration</th>
<th>Hybritech Calibration</th>
<th>≤3.1 ng/mL</th>
<th>&gt;4.0 ng/mL</th>
<th>Total Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤3.1 ng/mL</td>
<td>5616</td>
<td>0</td>
<td>5616</td>
<td></td>
</tr>
<tr>
<td>&gt;3.1 ng/mL</td>
<td>0</td>
<td>1014</td>
<td>1014</td>
<td></td>
</tr>
<tr>
<td>Total Samples</td>
<td>5616</td>
<td>1014</td>
<td>6630</td>
<td></td>
</tr>
</tbody>
</table>

Relative Agreement 100% 100%

- Total PSA cutoff of 3.1 ng/mL using WHO calibration is clinically equivalent to 4.0 ng/mL cutoff using Hybritech calibration

- 18% (38/208) of patients may not receive follow-up at a 4.0 ng/mL PSA cutoff with WHO-aligned calibration

- Assay-specific cutoffs may be necessary with tumor marker assays


PSA Clinical Concordance Using WHO and Hybritech Calibrations

<table>
<thead>
<tr>
<th>WHO Calibration</th>
<th>Hybritech Calibration</th>
<th>≤4.0 ng/mL</th>
<th>&gt;4.0 ng/mL</th>
<th>Total Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4.0 ng/mL</td>
<td>47</td>
<td>38</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>&gt;4.0 ng/mL</td>
<td>0</td>
<td>170</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Total Samples</td>
<td>47</td>
<td>208</td>
<td>255</td>
<td></td>
</tr>
</tbody>
</table>

Prostate Cancer confirmed by biopsy

- 18% (38/208) of patients may not receive follow-up at a 4.0 ng/mL PSA cutoff with WHO-aligned calibration

- Assay-specific cutoffs may be necessary with tumor marker assays


PSA Calibration Discordance: Clinical Implications

Patients with PSA in 4.1-5.0 ng/mL range using Hybritech calibration may have missed follow-up using PSA cutoff of 4.0 ng/mL using WHO calibration

Tumor Markers: A Need for Vigilance

• Tumor marker assays are not interchangeable
  o Check assay calibration traceability in Instructions For Use (IFU)
  o Assay-specific reference ranges may be warranted

<table>
<thead>
<tr>
<th>Tumor Marker</th>
<th>WHO Reference Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>72/225</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>89/620</td>
</tr>
<tr>
<td>CEA</td>
<td>73/611</td>
</tr>
<tr>
<td>Prolactin</td>
<td>97/714</td>
</tr>
<tr>
<td>tPSA</td>
<td>96/688</td>
</tr>
<tr>
<td>fPSA</td>
<td>96/670</td>
</tr>
</tbody>
</table>

Tumor Markers: A Need for Vigilance

• Tumor marker assays are not interchangeable
  o Patients should be monitored using the same assay
  • Important to communicate if there is a change in assay performance
  • Redetermination of patient baseline results may be necessary

Case Study 1

• Caucasian baby girl undergoing 8 wk post-natal checkup with general practitioner
  o Examination found distended abdomen, large abdominal mass and hepatomegaly
  • Referred to local hospital:
    o CT scan found left lobe intrinsic vascular mass (9X7X8 cm)
    o Differential: neuroblastoma, rhabdomyosarcoma, lymphoma, hepatic haemangioendothelioma and hepatoblastoma
    o Liver function and catecholamine tests within reference values
    o Provisional diagnosis: hepatic haemangioendothelioma
    o Patient discharged on prednisolone 2 mg/kg/day
    o AFP ordered though clinician did not follow-up with test report

Case Study 1 Continued

- Patient’s clinical condition worsened over following 2 weeks
- Evaluation by pediatric liver specialist at Academic Medical Center
  - Patient lab results (AFP age-specific reference range < 226 ng/mL):
    - AFP = 470 ng/mL (Immunoassay B, Academic Medical Center)
    - AFP = 1,917,760 ng/mL (Immunoassay A, Local hospital)
  - Repeat testing, new sample:
    - AFP = 546 ng/mL (Immunoassay B)
    - 1:10,000 dilution → AFP = 2,559,856 ng/mL (Immunoassay B)
- Diagnosis: Hepatoblastoma (AFP, liver biopsy, clinical evidence)
  - Chemotherapy: Patient exhibited decline in AFP concentration
  - Resection to be conducted following treatment

High-dose Hook Interference

Tumor Markers: A Need for Vigilance

- High-dose hook interference is a limitation of immunometric assays (including tumor marker assays)
  - Check high-dose hook interference concentration in IFU
  - Investigate patient samples with suspected high-dose hook effect
    - Repeat testing
    - Perform dilution studies (non-linear response suggests hook)
    - Test using a different manufacturer assay
Case Study 2

- Patient was diagnosed with large cell lung carcinoma with liver metastasis. Serum CEA concentrations were measured to monitor patient response to therapy.
  - CEA measured via automated immunoassay system

<table>
<thead>
<tr>
<th>Time collected/analyzed</th>
<th>IBM&lt;sup&gt;a&lt;/sup&gt; CEA result</th>
<th>X DF</th>
<th>CEA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera neat</td>
<td>Serum</td>
<td>1/10</td>
<td>Serum</td>
</tr>
<tr>
<td>At Diagnosis</td>
<td>&gt;53</td>
<td>&gt;53</td>
<td>&gt;53</td>
</tr>
<tr>
<td>+ 3 weeks</td>
<td>&gt;53</td>
<td>&gt;53</td>
<td>&gt;53</td>
</tr>
<tr>
<td>+ 6 weeks</td>
<td>&gt;53</td>
<td>&gt;53</td>
<td>&gt;53</td>
</tr>
<tr>
<td>+ 5 months&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.28</td>
<td>&gt;53</td>
<td>&gt;53</td>
</tr>
<tr>
<td></td>
<td>33.63</td>
<td>&gt;53</td>
<td>&gt;53</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sample collected at 5 months was reanalyzed

High-dose hook interference

But wait, there’s more…Analyte Carryover

- Case study 2: Lab performed re-testing of patient samples following the patient sample with grossly elevated CEA

Tumor Markers: A Need for Vigilance

- Potential analyte carryover can should be considered with automated immunoassay analyzers
  - Carryover may sometimes be missed with concomitant high-dose hook interference
  - Strategies to minimize and/or troubleshoot analyte carryover
    - Disposable pipettes safeguard against carryover
    - Conduct carryover verification studies – CLSI EP10-A2
    - Perform additional probe/cuvette washing when grossly elevated results are found with risk for carryover
    - Re-test patient samples if carryover is suspected
    - Use delta checks
Case Study 3

- 22 year old female with an unsuccessful pregnancy visited her family physician for menstrual irregularities
  - Pelvic ultrasound ruled out intrauterine or ectopic pregnancy
  - hCG – 251 IU/L (reference value < 5 IU/L; Immunoassay)
  - Repeat hCG testing: 215-278 IU/L
- Referred to Gyn/Onc specialist \(\rightarrow\) Dx: Choriocarcinoma
  - Patient treated with chemotherapy and later complete hysterectomy
  - Pathology indicated no evidence of choriocarcinoma
  - hCG remained between 232-300 IU/L
  - hCG testing using alternate method indicated hCG < 5 IU/L
- Outcome: Incorrect diagnosis of choriocarcinoma with unnecessary aggressive treatment due to false positive hCG result likely caused by interfering antibody
  - Heterophile antibody interference (e.g. HAMA)

Heterophile Antibody Interference

- Heterophile antibodies are antibodies that can bind to antigens of different species (e.g. reagent antibodies used in assays)
- Immunometric assays are subject to heterophile antibody interference
- Heterophile antibody interference is assay-specific (i.e. manufacturer) and can cause false positive or false negative results

Tumor Markers: A Need for Vigilance

- Heterophile antibody interference is a limitation of immunometric assays (including tumor marker assays)
  - Investigate patient samples with suspected heterophile antibody interference
    - Repeat testing on same sample – typically reproducible
    - Sample dilution – Presence of interfering substance may cause non-linear response
    - Heterophile blocking tubes – Ig neutralization
    - Repeat test with assay from different manufacturer


Heterophile Antibody Interference


Tumor Markers: A Need for Vigilance

Summary

- Tumor marker clinical performance characteristics are dependent on the test analytical performance, reference values and patient population tested.

- Important to understand the analytical performance characteristic requirements for cancer diagnosis and patient management decisions.

- Immunometric immunoassays are subject to limitations / challenges that necessitate vigilance in quality assurance practices.

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**Assessment Question**

Changing tumor marker reference cutoff value from C to A will cause what change(s) in clinical performance characteristics?

A. ↑ clinical specificity and ↓ clinical sensitivity
B. ↑ clinical specificity and ↑ clinical sensitivity
C. ↓ clinical specificity and ↓ clinical sensitivity
D. ↓ clinical specificity and ↑ clinical sensitivity

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**Assessment Question**

Analytical bias of -20% without change in tumor marker reference value can cause what change(s) in clinical performance?

A. ↓ FP rate and ↓ FN rate
B. ↓ FP rate and ↑ FN rate
C. ↑ FP rate and ↑ FN rate
D. ↑ FP rate and ↓ FN rate
Assessment Question

Provider calls the laboratory director due to an unexpected elevated hCG result for a 41 year old female. Imaging evidence ruled out pregnancy.

<table>
<thead>
<tr>
<th>Patient Result</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG*</td>
<td>&lt;5.0 IU/L</td>
</tr>
</tbody>
</table>

*hCG tested using automated immunoassay with disposable tips

Which is NOT a potential reason for the elevated hCG result?

A. hCG-secreting tumor with high-dose hook interference
B. “Normal” hCG concentration for peri-menopausal female
C. Analyte carryover from previous patient specimen
D. Heterophile antibody interference