Tumor Markers

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Learning Objectives

Describe when a test can be used to screen the general population for a particular disorder

Describe the pitfalls associated associated with the immunochemical methods for measuring tumor markers

IDEAL TUMOR MARKER

- Be specific to the tumor
- Level should change in response to tumor size
- An abnormal level should be obtained in the presence of micrometastases
- The level should not have large fluctuations that are independent of changes in tumor size
- Levels in healthy individuals are at much lower concentrations than those found in cancer patients
- Predict recurrences before they are clinically detectable
- Test should be cost effective

COMMON TUMOR MARKERS

Analyte	Cancer Use
CEA	Monitor colorectal, breast, lung cancer
CA-125	Ovarian cancer monitoring
CA15-3, 27. 29	Monitor recurrences of breast cancer
AFP	Germ cell tumors, liver cancer
Total PSA	Screen and monitor prostate cancer
Free PSA	Distinguish prostate cancer from BPH
HCG	Germ cell and trophoblastic tumors
Hormone receptors	Breast cancer therapy

SCREENING TESTS

- Cancer must be common
- The natural history of the cancer should be understood
- Effective treatments must be available
- The test must be acceptable to both patients and physicians
- The test must be safe and relatively inexpensive

CEA

- Described by Gold and Freedman in1965 as a marker for Colorectal Cancer
- Molecular mass of approximately 200 kDa
- Glycoprotein with a carbohydrate composition ranging from 50 - 85% of molecular mass
- CEA levels 5 - 10 times upper limit of normal suggests colon cancer
- CEA is not used to screen for colon cancer

CEA Distribution In Healthy Individuals and Patients with Non-Malignant Conditions

	% Distribution of CEA		
	ng/mL	ng/mL	ng/mL
Healthy Subjects	0-3.0	3.1-10	>10.0
Non Smokers	96	4	0
Smokers	80	19	1
Non-Malignant Diseases			
Cirrhosis	**53**	**42**	**5**
Ulcerative Colitis	**65**	**26**	**9**
Rectal polyps	**78**	**19**	**3**
Pulmonary	**52**	**39**	**9**
Gastrointestinal	76	21	3

CEA Distribution In Patients With Malignant Disease

	% Distribution of CEA		
	0-3 ng/mL	3.1-10 ng/mL	>10 ng/mL
Colorectal	28	20	52
Breast	50	27	23
Ovarian	80	16	4
Pulmonary	39	29	32

CA-125

- CA-125 glycoprotein molecular weight 200-1,000 kda
- Introduced in 1983 by Bast for ovarian cancer
- In the US, each year about 25,400 new cases will be diagnosed and 14,500 women will die as a result of this disease
- 70% of the women with ovarian cancer are over the age of 50
- One half of patients with stage 1 ovarian cancer have elevated CA-125 levels and a five year survival rate of 90%. In late stage disease, the five year survival rate is from 4-30%
- Worldwide incidence is highest in industrialized countries and lowest in Japan and India

SYMPTONS OF OVARIAN CANCER

- ASCITES
- ABDOMINAL and PELVIC PAIN
- ABNORMAL UTERINE BLEEDING
- GASTROINTESTINAL DISCOMFORT
- WEIGHT LOSS
- URINARY FREQUENCY

RISK FACTORS

INCREASED RISK	DECREASED RISK
Family History	Oral Contraceptive
Advanced Age	Breast Feeding
Infertility	Tubal Ligation
Nulliparity	

CA-125 Distribution In Healthy Subjects and Patients with Non-Malignant Conditions

	% Distribution of CA-125		
	<35 u/mL	35-65 u/mL	>65 u/mL
Healthy Individuals	98	1.7	1.3
Non-Malignant Conditions			
Pregnancy	73	22	5
Cirrhosis	30	13	57
Pulmonary Disease	94	0	6
Pelvic Inflammatory Disease	76	3	21
Endometriosis	86	11	3
Ovarian Cysts	90	7	3
Uterine Fibroids	77	13	10
Breast Fibroids	100	0	0

CA-125 Distribution In Patients With Malignant Disease

Cancers	% Distribution of CA-125 <35 u/mL	35-65 u/mL	>65 u/mL
Ovarian	14	9	77
Lung	56	19	25
Breast	82	8	10
Endometrial	70	8	22
Cervical	66	15	19
Colorectal	76	11	12

Screening Test

- Positive Predicative value PPV= ability to predict the presence of disease
- Number of true positive results= prevalence of disease x number of patients screened
- Number of false positive results= test specificity x number of non-diseased patients

$$PPV = \frac{\text{True Positive Patients}}{\text{True Positive Patients + False Positive Patients}} \times 100$$

Calculate PPV

- $PPV = \frac{\text{True Positive Patients}}{\text{True Positive Patients + False Positive Patients}} \times 100$
- Prevalence of 0.1%= .001x1000 = 1Positive Patient
- Specificity= 95% = 999x .05 =49.95 False Positive
- $PPV = \frac{1}{1 + 49.95} \times 100$
- PPV= 2 %

PITFALLS IN IMMUNOASSAYS

HETEROPHILE ANTIBODIES

HIGH DOSE HOOK EFFECT

NON-IMMUNOREACTIVE HORMONE ISOFORMS

CROSS-REACTING SUBSTANCES

HETEROPHILE ANTIBODIES

- Defined as antibodies in serum that bind antibodies of other species
- Human anti mouse antibodies (HAMA) , Human anti rabbit, anti goat and anti sheep antibodies
- Positive interference or negative interference possible

POSSIBLE CAUSES OF HETEROPHILE ANTIBODIES

- Administration of mouse monoclonal antibodies for diagnostic imaging or therapeutics
- Exposure to animals
- Vaccination
- Maternal transfer across the placenta to the unborn child

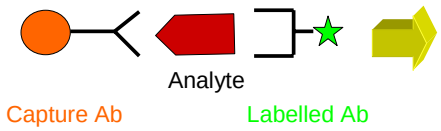
Prevalence of Heterophile Antibodies

Blood Donors	Prevalence
72/10,000	0.72%
81/2600	3.1%
91/1008	9.1%
Hospital Population	
10/295	3.4%
Patients Receiving Monoclonal Antibodies	
OC-125 11/32	34%
OKB7 15/18	28%
OKT3 14/75	19%
IMMU-4 2/63	1.6%

Analytes Affected by Heterophile Antibodies

CA-125	Troponin I
HCG	Troponin T
CEA	CKMB
PSA	TSH
Prolactin	T4
Hepatitis B Surface Antigen	T3
CRP	LH
Progesterone	FSH

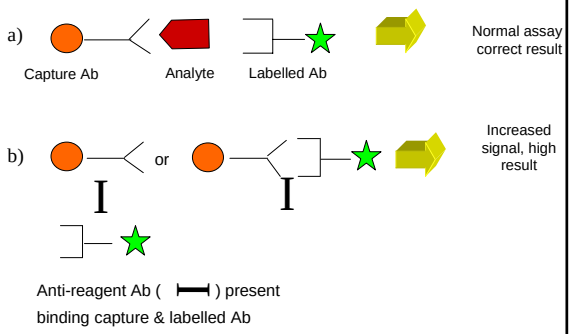
Two-site Immunometric Assay



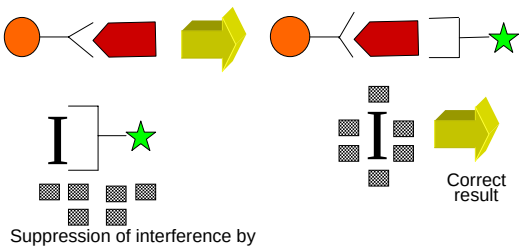
Analyte

Capture Ab Labelled Ab

Interference From Heterophile Antibodies



Effect of Adding Non-Immune Mouse IgG



Suppression of interference by addition of non-immune IgG ( )

Consequences of a Falsely Elevated HCG Result

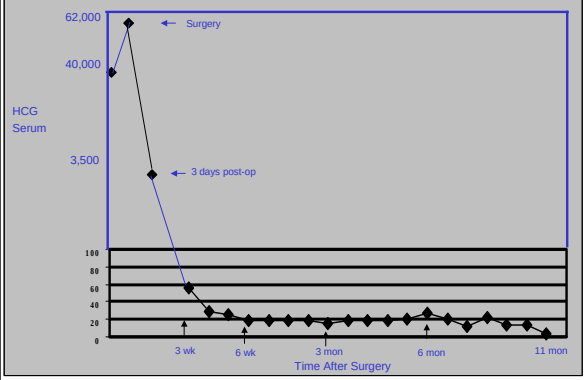
A 37 year-old woman was examined at 12 weeks gestation. She had abdominal pain during the pregnancy, some diarrhea but no bleeding. The uterus was distended with a heterogeneous mass with multiple cystic spaces. Fetal heart rate was absent. There is one corpus luteum cyst on the right side and no evidence of theca lutein cysts. A transabdominal and transvaginal ultrasound at 16 weeks of gestation showed a heterogeneous mass with cystic degeneration.

Pesce, Clin Chem 2003,49,92-93.

The serum HCG level was 60,128 U/L. The elevated HCG level and untrasound results are consistent with a molar pregnancy. Suction curretage (D&C) was performed. The tissue was sent to pathology. The specimen consists of enlarged villi with grapelike appearance. The histological features are consistent with a complete Hydatidiform mole.

The D&C procedure was successful and serum HCG levels were moniitored to determine if the evacuation of the Hydatidiform was complete. The serum HCG levels decreased to 18 IU/L at 1.2 months after surgery, but remained at levels between 12 and 27 IU/L for the next 9½ months.The possibility of HAMA interference was investigated as the cause of the low HCG levels.

HCG Levels Measured with the AXSYM Assay Before and After Surgery in the Serum of a Patient with a Molar Pregnancy



Serum HCG Levels Measured with the AXSYM, Immulite 2000, Elecsys 2010 and Centaur Procedures Following Evacuation of the Hydatidiform Mole

	HCG Levels, IU/L			
Time After Surgery in Months	AXSYM	IMMULITE 2000	Elecsys 2010	Centaur
2.5	16	<1.0	<0.5	<2.0
3.0	18	<1.0	<0.5	<2.0
4.0	27	<1.0	<0.5	<2.0
4.8	21	<1.0	<0.5	<2.0
5.9	12	<1.0		
6.8	22	<1.0		
8.9	14	<1.0		
9.8	13	<1.0		
11.0	4	<1.0		

These results show that HCG is not detected in the serum of this patient with the Immulite 2000, Elecsys 2010, and Centaur procedures. The low level of HCG results obtained with the AXSYM assay suggest that HAMA are present in the samples.

Antibody Characteristics of the AXSYM, Immulite 2000, Elecsys 2010 and Centaur HCG Assays

	Solid Phase Antibody	Capture Antibody
AXSYM	monoclonal mouse	polyclonal goat
Immulite 2000	monoclonal mouse	polyclonal ovine
Elecsys 2010	monoclonal mouse	monoclonal mouse
Centaur	monoclonal mouse	polyclonal goat

The HCG reagents for the Immulite 2000, Elecsys 2010 and Centaur are formulated to minimize the risk from HAMA antibodies. The AXSYM antibody reagents do not contain any immunoglobulins that would bind the HAMA antibodies.

Urine HCG Levels Measured with the AXSYM, Immulite 2000, Elecsys 2010 and Signify POC Procedures Following Evacuation of the Hydatidiform Mole

	HCG Levels, IU/L			
Time After Surgery in Months	AXSYM	Immulite 2000	Elecsys 2010	Signify
2.5	<2.0	<1.0	<0.5	undetectable
3.0	<2.0	<1.0	<0.5	"
4.8	<2.0	<1.0	<0.5	"
5.9	<2.0	<1.0		"
8.9	<2.0	<1.0		"
9.8	<2.0	<1.0		"
11.0	<2.0	<1.0		"

The undetectable urine HCG results obtained with the AXSYM, Immulite 2000, Elecsys 2010 and Signify assay suggest that the serum HCG levels obtained with the AXSYM are due to the presence of HAMA.

THE HCG PROBLEM

- Since 1980 there have been about 100 reported cases of false-positive hCG results that have led to misdiagnosis of
  - Gestational trophoblastic disease
  - Choriocarcinoma
  - Ectopic pregnancy
- And unnecessary procedures & treatment, like
  - Exploratory surgery
  - Chemotherapy
  - Hysterectomy

THE hCG PROBLEM

- In most cases, the clinical data were non-specific and imaging studies were inconclusive
- The HCG results were relatively low (25-500 mIU/mL) and relatively stable
- Pathological hCG levels are usually much higher and tend to change with time or with therapy
- However, low and stable hCG levels have been reported in pathological samples.

Consequences of a False Positive HCG Result

A 23 year-old woman who had one unsuccessful pregnancy had serum HCG levels measured because of menstrual irregularities. HCG was measured with the AXSYM analyzer from Abbott laboratories. Her HCG concentration was 251 IU/L. Pelvic ultrasound and diagnostic laparoscopy ruled out intrauterine or ectopic pregnancy.

HCG measured for 11 months was between 215-278 IU/L. She was treated with methotrexate,followed by actinomycin D. HCG levels remained between 232-300 IU/mL. She was given combination chemotherapy, but the HCG was still high.She underwent total abdominal hysterectomy and removal of both ovaries. Pathological examination showed no evidence of choriocarcinoma.

After the hysterectomy a PET scan showed suspicious spots on the right upper lobe of her lung. A thoracotomy was performed.Biopsies showed normal lung tissue. HCG was measured 44 times with the AXSYM system. HCG was always elevated. After a year of aggressive cancer therapy,a HCG test from a different vendor gave a normal HCG level.

The patient sued Abbott laboratories and the University of Washington. A jury awarded the patient and her husband 16.2 million dollars. Abbott laboratories and the University are appealing the decision.

CASE HISTORY

- 36 year-old woman, 1 prior unsuccessful pregnancy
- Serum hCG = 385 mIU/mL
  - ◇ preoperative testing for unrelated surgery
- Regular menses; no medications
- Pelvic ultrasound - no evidence of normal or ectopic pregnancy
- D&C - no products of conception
- Diagnostic laparoscopy - no ectopic pregnancy
- CT & MRI of chest, abdomen, pelvis - no metastases

CASE HISTORY

- hCG rose to 463 mIU/Ml
- Diagnosis - choriocarcinoma
- Treatment:
  - ◇ 4 courses of methotrexate
    - hCG remained at 287-374 mIU/mL
- Repeat pelvic MRI - uterine endometrial lesion suggestive of invasive trophoblastic tumor
  - ◇ Treatment - vaginal hysterectomy

CASE HISTORY

- **Increased hCG persisted**
- **Patient started on combined chemotherapy with etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine**
- **Patient admitted to hospital in coma due to methotrexate induced pancreatitis**
- **hCG dropped to below 100 mIU/mL**
- **Chemotherapy resumed, but w/o methotrexate**
- **Further testing at this point provided strong evidence for false-positive HCG**
  - ◊ Urine hCG negative; serum hCG by other methods negative

CASE HISTORY

Incorrect diagnosis of choriocarcinoma resulting in inappropriate chemotherapy (with complications) and hysterectomy due to false positive HCG result probably caused by an interfering antibody.

Rotmensch and Cole. Lancet 2000;355:712-5.

INVESTIGATING POSITIVE HCG

- Test urine
  - Serum HCG is more sensitive than urine HCG
  - Interfering Ab are rarely present inurine
- Use heterophile/HAMA-blocking reagents
- Perform systematic dilution method
- Assay by a different method
- Perform recovery testing
- Precipitate antibodies with PEG, ethanol

Summary of Clinical Findings

Patient	Reason for HCG Test	Surgical Treatment	Chemotherapy
1	Incidental	D&C, laparoscopy, TAH	MTX, EMAC, Vincristine
2	Menstrual Irregularity	D&C, laparoscopy, TAH Thoracotomy	MTX, EMAC, Vincristine
3	Vaginal bleeding	D&C, laparoscopy, TAH	MTX
4	Abdominal pain	D&C, laparoscopy, TAH	
5	Menstrual Irregularity	D&C, laparoscopy	MTX
6	Incidental	D&C, laparoscopy	MTX
7	Abdominal pain	D&C, laparoscopy	MTX
8	Incidental	D&C, laparoscopy	
9	Incidental	D&C	

Measurement of Serum HCG Levels with 4 Different Immunochemical Systems from 9 Patients That Gave False Positive HCG Results

Patient	AXSYM		ACCESS	Chiron	IMMULITE
			IU/L		
		Range			
1	68	(68-463)	4.6	<2	<2
2	215	(215-300)	<2	<2	<2
3	17	(17-89)	ND	ND	ND
4	150		ND	ND	ND
5	110	(45-135)	6.6	4.5	4.2
6	145	(145-351)	ND	ND	ND
7	33		ND	ND	<2
8	32	(5-205)	<2	<2	<2
9	93		ND	ND	ND

High Dose Hook Effect

Definition: A sample with an extremely high analyte concentration that produces a result below that of the highest calibrator.

- Occurs with hCG, Prolactin, LH, FSH, PSA, AFP and CA-125 assays.
- Measured analyte levels are significantly lower than expected.

PATIENT HISTORY

A 65 year-old woman was diagnosed with Stage III,IV ovarian carcinoma. She was treated with Cis-Platinum and Cytoxan for 12 months and was switched to a Taxol protocol for 8 months, after which an abdominal and pelvic CAT scan was performed to determine the status of her disease. This evaluation showed a significant progression of her disease with increased ascites, presence of new liver metastatic lesions in both lung fields. Because her disease was progressing, Taxol therapy was discontinued and she was treated with 5-FU and leucovorin.

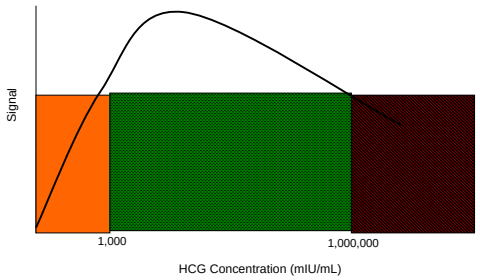
Pesce Clin Chem1993, 39,1347.

CA-125 Levels From This Patient That Were Monitored for 5 Months

Time, (weeks)	CA-125 Levels Neat U/mL	Expected
-	734	9830
3	608	9400
6	644	8303
10	484*	9650
13	422*	14100
17	470*	11622
18	575	12480
19	447*	12650
20	472*	22160
22	462*	22080

*A Hook Effect was observed in 6 out of 10 specimens from this patient at CA-125 levels of 10,000 U/mL

One Step Immunometric Assay=

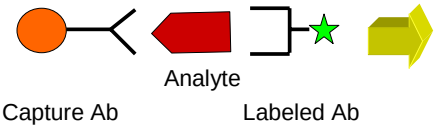


No problem is assumed to exist when concentration <1,000 mIU/mL

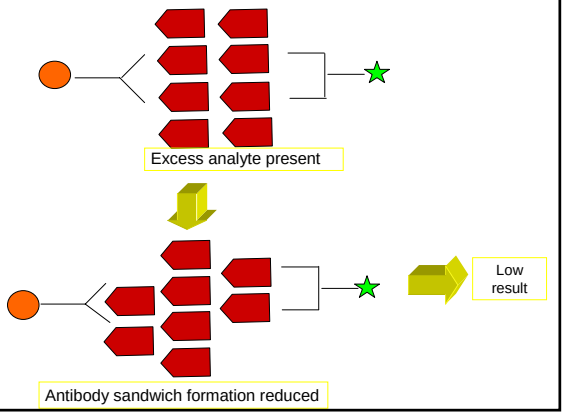
Concentration is between 1,000 & 1,000,000 mIU/mL an over-range message is generated.

Concentration is >1,000, assay results are falsely low, but no error flag occurs. High dose hook is a problem here.

Two-site Immunometric Assay



Mechanism of High-Dose Hook Effect



Hook Effect

- If undetected, a significantly lower analyte value will be reported which can result in mismanagement of patients.
- The Hook Effect can be eliminated by development of a two step immunochemical assay.
- The laboratory should have a dilution protocol in place to test for the Hook Effect

CA-125 Proficiency Survey

Assay System	CA-125 Concentration u/mL
Abbott AXSYM	77
TOSOH	68
Centaur	55
Immulite	52
Access	52
Vitros	49
Elecsys	47
Centocor	36

GUIDELINES FOR ORDERING/ INTERPRETING TUMOR MARKER TESTS

- Never rely on the result of a single test
- Order every test from the same laboratory
- Consider presence of HAMA antibodies when the test result is inconsistent with clinical findings or there is an unexplained change from a previous result.
- Consider the Hook Effect