

Finding the enemy withintumor markers in breast cancer

Continuing Education Seminar

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Learning objectives

Briefly describe the types and characteristics of tumor markers used in clinical setting

Provide an overview of breast cancer diagnostic work-up

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Elucidate the utility of breast cancer tumor markers in the clinical chemistry laboratory

Outline



Part-1 Basics of Breast Cancer

What is cancer?

• Cancer

- Uncontrolled cell division
- Invasion of surrounding and distant tissues
- Oldest description: 3000 BC Egypt
- In 2021 ~1.9 million individuals will be diagnosed with cancer
 - Men \rightarrow 970,250 cases
 - Women \rightarrow 927,910 cases
- About 608,570 cancer-related deaths will occur in 2021



https://www.sciencemag.org; Novikov et al, British Journal of Cancer (2021)



https://webpath.med.utah.edu/histhtml/normal/norm005.html https://www.cdc.gov/cancer/breast/images/breast1_566_838.jpg

Part-2 Breast Cancer Diagnosis



• Higher grade = more aggressive = worse outcomes

Rakha et al, BCR (2010)

Breast cancer staging

Tumor size, lymph node involvement, metastasis



https://pathology.jhu.edu/breast/staging-grade/

Breast cancer screening

- To identify asymptomatic disease
- Tumor markers are **NOT** sensitive or specific enough
- Mammography-based screening
- Benefit vs risk debatable
 - Unnecessary treatment
 - Psychological stress
- Self-exam by women



Part-3 Tumor Markers in Breast Cancer

What are tumor markers?

- Tumor markers (TM)
 - Biomarkers found in body tissues, blood, or urine that can be elevated by the presence of one or more types of cancer

- Proteins, enzymes, hormones, mRNA, CTC, ctDNA
- Produced by the tumor or by the body in response to the tumor



https://www.cusabio.com/c-20631.html

Types of tumor markers

| Screening | Identify subclinical disease |
|-----------------------|--------------------------------------|
| Diagnostic | Cancer diagnosis |
| Prognostic | Estimate survival outcomes |
| Predictive | Therapeutic response prediction |
| Monitoring therapy | Tumor shrinkage or cancer recurrence |
| Preventative | Risk prediction |

Tumor markers performance characteristics

• Perfect test



• Questionable test



• In reality...



Courtesy: Dr. Lisa Johnson

Sensitivity

• The ability of a test to correctly identify the population with disease (cancer)



For tumor markers

- Good Sensitivity- Someone with cancer produces a high tumor marker result
- Poor Sensitivity- Someone with cancer produces a low/no tumor marker result
 - False negative results

SN = TP/(TP + FN)

Courtesy: Dr. Lisa Johnson

Specificity

• The ability of a test to correctly exclude the population without disease (no cancer)



For tumor markers

- Good Specificity-Someone without cancer produces a low/no tumor marker result
- Poor Specificity- Someone without cancer produces a high tumor marker result
 - Increased tumor marker for benign conditions
 - False positive results

SP = TN/(TN + FP)

How is the cutoff determined?



 $https://marlin-prod.literatumonline.com/cms/attachment/34661288-1f8f-459e-b8b4-936efc49e9bc/fx1_lrg.jpg$

Kaplan-Meier survival curve

• Evaluate biomarker relationship with patient outcomes



https://www.graphpad.com/support/faq/prism-3-kaplan-meier-survival-analysis/

Breast cancer development



Normal sexual function

E = estrogen; P = progesterone; EGF = epidermal growth factor

Images prepared with biorender.com

Predictive biomarkers



Breast cancer therapies



Images prepared with biorender.com

Outcomes based on prediction



Wang et al, Oncology Letters (2019)

Breast cancer related deaths are declining



- Biomarkers \rightarrow HER2
- Improved the rapies \rightarrow Herceptin
- Increased understanding of the disease

Cancer facts and figures, ACS (2021)

mRNA-based markers

• *BIK* is an estrogen sensitive gene involved in apoptosis

Pandya et al, Oncotarget (2016)

Oncotype Dx and MammaPrint

MammaPrint – Microarray

Kwa et al, Nature Reviews Clinical Oncology (2017)

DNA-based tumor markers

- **BR**east **CA**ncer genes 1 and 2
- Tumor suppressor genes
 - Do not cause cancer
 - Prevent cancer
- About 1:400 individuals (0.25%) carry BRCA1/2 mutations
 - Family history
- Common population: ~12% women will develop breast cancer
- Women with BRCA1 mutations: 55-65%
- Women with BRCA2 mutations: ${\sim}45\%$
- The Angelina Jolie effect
 - Preventative double mastectomy

https://www.nationalbreastcancer.org/what-is-brca; https://www.verywellhealth.com/non-brca-gene-mutations-4173768

Serum-based tumor markers

- CA 15-3 and CA 27.29
 - Upregulated in breast cancer
 - Shed by epithelial cells
 - Stage II and III \rightarrow early detection of recurrence
 - Stage IV \rightarrow monitoring therapy response
 - Can be elevated in other malignancies
- Carcinoembryonic antigen (CEA)
 - Produced by GI tissue during fetal development
 - Very low levels in healthy adults
 - Maybe elevated in breast, colon and lung cancers
- HER2/neu by ELISA
 - Human Epidermal Growth Factor Receptor
- All serum-based markers are used to monitor therapy response
 - **NOT** for diagnosis \rightarrow **poor specificity**

Therapy response

Tietz textbook of clinical chemistry and molecular diagnostics, 6th edition (2018)

Pre-analytical considerations

- Serum or plasma are usually of choice
 - Recommended to store specimens at 4°C or –20 °C
- Timing of specimen collection \rightarrow not too critical
 - Avoid collection immediately after surgery \rightarrow false elevations
- Avoid testing for unfocused requests such as "tumor marker screen" or suspicion of "malignancy" from ED
- Lack of sensitivity and specificity for specific cancers should be reiterated for consults on abnormal results

Analytical interferences

- High-dose hook effect
 - Antigen in vast excess
- Specimen carryover
- Heterophile antibodies
 - Affect non-competitive sandwich immunoassays

Preissner et al, JCEM (2003); Tietz textbook of clinical chemistry and molecular diagnostics, 6th edition (2018)

Summary of breast cancer management

Part-4 Case Study

- 38-year-old female
- She felt a peanut-sized lump in her right breast and ignored it thinking a swollen node due to a recent cold
- 3-months later the lump grew to the size of a walnut
- She had persistent headache and mood swings
- Visited her PCP who referred her to an oncologist

- 1. What is the oncologist likely to do?
 - A. Perform surgery to remove the tumor
 - B. Order a biopsy to screen for breast cancer
 - C. Prescribe chemotherapy
 - ✓ D. Perform imaging studies to visualize tumors

- Imaging studies followed by biopsy analysis found a primary tumor of 3.5 cm in diameter and a 1.2 cm lesion in the axillary lymph node
- Diagnosed with grade 2 stage II breast cancer
- Breast cancer subtype: Triple negative (TNBC)

- 2. What is the clinical team likely going to do next?
 - A. Surgically remove the primary tumor and initiate chemotherapy
 - B. Ask the patient to go home as this is not serious
 - C. Collect a pre-surgical serum specimen to determine the baseline levels of CA 15-3
 - ✓ D. Both A and C
 - Pre-surgical levels of CA 15-3 were 489 U/mL (RI: 0-31)
 - * 1 month later \rightarrow 35 U/L
 - 2 months later \rightarrow 34 U/L
 - 6 months later \rightarrow 15 U/L with no radiological abnormalities

- 3. What do these results likely mean?
 - A. She never had cancer
 - \checkmark B. Her cancer is responding to therapy and is in remission
 - C. The tumor is growing back
 - D. She will never have cancer recurrence
- The patient will be monitored over the next few months and put on surveillance to detect recurrent disease activity