Wednesday Evening Case Studies

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Case #1
Cure Worse than The Disease

- A healthy 22 year old female college student goes to the university health services with symptoms of a urinary tract infection (frequent, painful urination)
- A urine dipstick confirms the presence of white blood cells (leukocyte esterase) and bacteria (nitrites)
- She is discharged with a prescription for an antibiotic (Bactrim)

A Few Days Later

- The patient returns to the clinic
- Urinary symptoms are much improved
- But she feels very fatigued and has even begun to sense shortness of breath
- What would you do?

Physician's Evaluation

- physical examination is unremarkable
- lab tests:
  - to check on UTI: dipstick
  - to check for anemia: CBC
  - ***assuming anemia is present, she also orders***
    - reticulocyte count: is she making new RBCs?
    - if reticulocyte count is inappropriately low, is anemia 2° to deficiency?
      - Fe and transferrin
      - B_{12} and folate
    - if reticulocyte count is appropriately high, is anemia 2° to hemolysis?
      - LDH
      - haptoglobin
      - bilirubin (total/direct/indirect)
      - Direct Antiglobulin Test (DAT)

All Tests Come Back Normal
(those in blue were not needed, and should not have been ordered up front)

- physical examination is unremarkable
- lab tests:
  - to check on UTI: dipstick ➔ NEGATIVE
  - to check for anemia: CBC ➔ Normal Hemoglobin, RBC, Hct
  - assuming anemia is present, she also orders
    - reticulocyte count: is she making new RBCs?
    - if reticulocyte count is inappropriately low, is anemia 2° to deficiency?
      - Fe and transferrin
      - B_{12} and folate
    - if reticulocyte count is appropriately high, is anemia 2° to hemolysis?
      - LDH
      - haptoglobin
      - Bilirubin (total/direct/indirect)
      - Direct Antiglobulin Test (DAT)

Next Steps?

- physician is puzzled:
  - UTI is resolving (normal dipstick)
  - patient seriously fatigued and "short of breath"
  - but absolutely no evidence of anemia
  - arranges for patient to see hematologist
  - earliest appointment is 2 weeks later

Patient's Course

- four days later, finishes course of antibiotics
- two days after that, begins to feel better:
  - with more energy
  - less shortness of breath
- does internet search:
  - "discovers" that sulfa drugs, including Bactrim, have been reported to cause "methemoglobinemia
- calls her physician with this information
- physician calls lab to order methemoglobin
  - lab does co-oximetry on recent blood sample
  - methemoglobin = 22%

Problem (Partially) Solved

- visit to hematologist not canceled
  - focus will be on patient's possible predisposition to methemoglobinemia
  - e.g., G6PD deficiency
- causes of methemoglobinemia:
  - congenital
    - Some abnormal hemoglobins
    - Some enzyme defects
  - acquired: oxidant drugs that overwhelm even normal reducing systems
    - nitrites
    - local anesthetics (e.g. "hurricane spray" used for intubation)

Case #2: Too Much of a Good Thing

- 68 year old woman, relatively healthy, brought to Emergency Room by her family because of somnolence

- her physical examination is unremarkable
- routine lab tests are unremarkable:
  - CBC: no evidence of anemia or infection
  - normal glucose, BUN, creatinine, electrolytes
  - normal calcium, magnesium, phosphate

- Admitted to hospital for further work-up

A Closer Look

- physicians on the floor review her chart carefully
- their assessment is that the clinical picture is most suggestive of hypercalcemia
- they note that calcium from Emergency Room
  - was indeed “normal”: 10.2 mg/dL
  - but was right at the upper limit of “normal” (8.4 – 10.3 mg/dL)
- they decide to check “corrected” and “ionized” calcium, ordering:
  - repeat total calcium
  - total protein and albumin
  - “ionized” calcium

Corrected Calcium

- repeat total calcium: 10.1 mg/dL
- albumin: 3.0 g/dL
- “ionized” calcium:

- corrected calcium:
  - For each 1.0 g/dL change in albumin from 4.5 g/dL there’s a 0.8 mg/dL change in calcium in the opposite direction (i.e., if albumin is low, effective (unbound) calcium is higher)
  - So, (4.5 - albumin) + calcium = corrected calcium
  - In this case: (4.5 – 3.0) + 10.1 = 11.6 mg/dL
  - That is, if patient had a normal albumin, her calcium would be 11.6!

“Ionized” Calcium

- a misnomer:
  - all the calcium we measure is ionized
  - we’re really talking about free (or unbound) calcium
  - this is the fraction that’s physiologically active
  - usually, about ~50% of total
- measured values:
  - total calcium: 10.2 mg/dL
  - “ionized” calcium: 1.50 mmol/L (reference interval 1.12-1.32)
  - ➔ about 20% rather than 50%
- but it should be high in this case
  - low albumin, high total calcium

Proportion “Ionized” Calcium

- check those units carefully
- measured values:
  - total calcium: 10.2 mg/dL
  - “ionized” calcium: 1.50 mmol/L (reference interval 1.12-1.32)

- one is conventional, one is SI
  - convert total calcium to SI:

    [(10.2 mg Ca/dL) / (40 mg Ca/mmol)] X 10 dL/L = 2.55 mmol/L

  - Ionized/total is now correctly calculated as :

  - 1.50 mmol/L / 2.55 mmol/L = 59%

Genuine Hypercalcemia

- after initiating treatment (hydration), the search for causes begins

- initial test is PTH (parathyroid hormone)
  - if PTH is high (inappropriate),
    ➔ hyperparathyroidism
  - if PTH is low (appropriate),
    ➔ most likely, malignancy
    (excess Vitamin D possible, too)

- in this case, PTH is undetectable

Patient's Course

- Patient undergoes:
  - numerous (and expensive) imaging studies
  - many blood tests for tumor markers
- ➔ no evidence of malignancy found

- 25-OH vitamin D:
  - patient's level: 150 ng/mL (375 nmol/L)
  - reference interval: 20-50 ng/mL (50-125 nmol/L)
- ➔ vitamin D toxicity

Vitamin D: Clinical Background

- The advertising:

  The cure for everything, from osteoporosis to cancer

- The facts:

  In temperate climates like Boston (not San Diego) most people are probably Vitamin D deficient

Vitamin D: Clinical Background

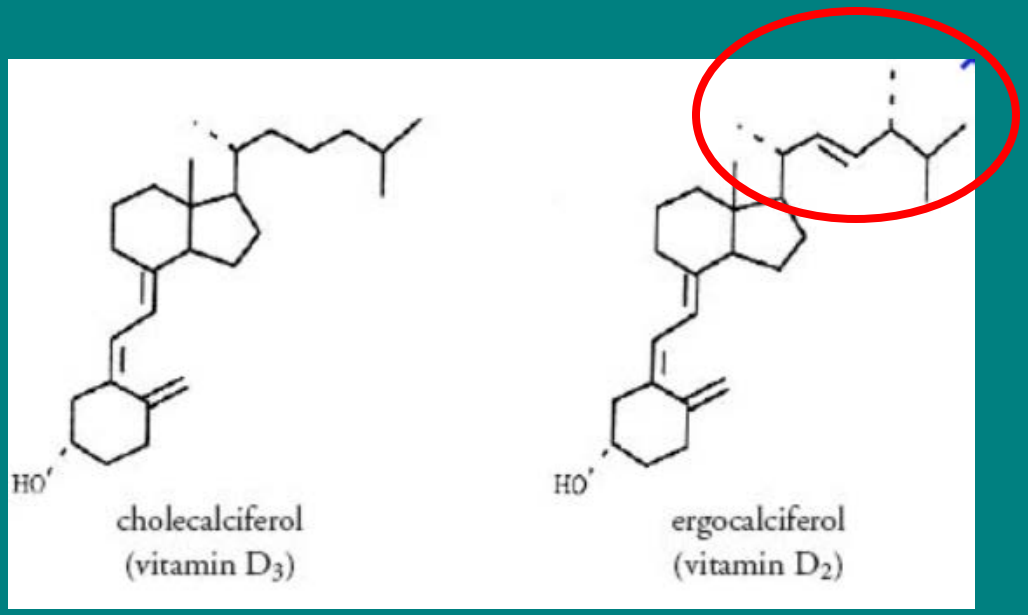
- Osteoporosis is a big problem
  - Many people (in the US) have been taking supplements of calcium and vitamin D
  - in retrospect, calcium supplementation not needed
  - rather, the problem has always been too little vitamin D
  - 400 IU/day (current official RDA) is inadequate; probably need ~1000 IU/day
- Vitamin D testing is rarely needed (my lab does 150 vitamin D assays each day!)
  - Most people are deficient
  - Just take vitamin D supplements, about 1000 IU/day

Vitamin D Metabolism

- Vitamin D is made in skin (or ingested in food)
- Gets hydroxylated in liver to 25-OH Vitamin D
- Gets an additional hydroxylation in the kidney to form 1,25 Dihydroxy Vitamin D
- Vit D ➔ 25-OH Vit D ➔ 1,25-OH Vit D
- To monitor dietary adequacy, measure 25-OH Vitamin D

Vitamin D Testing

- two forms:
  - Cholecalciferol **(D_3)** found in mammals
  - Ergocalciferol **(D_2)** found in plants



- mass spectrometry can distinguish them
- immunoassays cannot
  - as long as antibodies have 100% cross-reactivity to both D_2 & D_3, this may not matter too much
  - this is not the case with all assays
  - depending on specific assay, one may (seriously) underestimate the amount of 25-OH D2

Resolution of Patient's Problem

- patient made an uneventful recovery
- on further questioning, her physicians discovered:
  - patient had had a recent annual physical exam
  - her physician had ordered a 25-OH vitamin D level, which came back markedly low
  - he gave her a prescription for 50,000 IU of Vitamin D, to be taken **weekly** for 8 weeks
  - patient apparently had gotten confused, and had taken the high dose Vitamin D **daily**

Case #3: Is It There, or Isn't It?

- 45 year old man, with a diagnosis of intermediate grade non-Hodgkin's lymphoma, is admitted to the hospital for chemotherapy

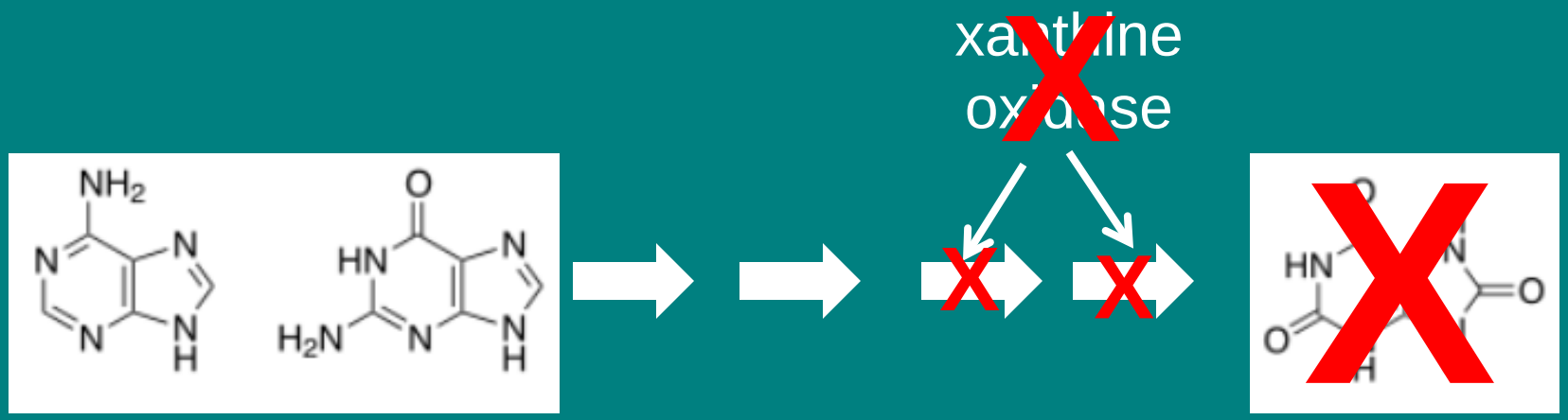
- his baseline serum creatinine was 1.2 mg/dL (106 umol/L), with an estimated GFR of >60 mL/min/1.73m^2

- two days following initiation of therapy,
  his urine output decreased markedly, and
  his serum creatinine increased to 3.6 mg/dL (318 umol/L), with an estimated GFR of 18 mL/min/1.73m^2 (AKI)

The Culprit

- his physicians order a uric acid level:
  - patient's value: 16.4 mg/dL (0.97 mmol/L)
  - reference interval: 3.4-7.0 mg/dL (0.20-0.41 mmol/L)
- confirming their diagnosis: Tumor Lysis Syndrome
- they initiate rasburicase therapy

Purine Metabolism



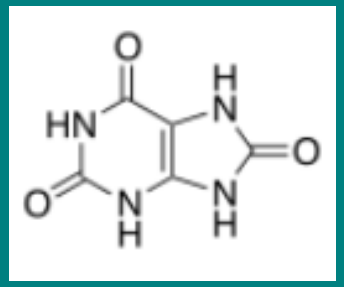
Adenine Guanine

Uric Acid

(insoluble at acid pH)

- in some cases (Burkitt's lymphoma), rapid cell death can be expected with chemotherapy
- one can then pre-medicate with **allopurinol**, a xanthine oxidase inhibitor, so uric acid does not form
- in other cases, such rapid cell turnover is not expected

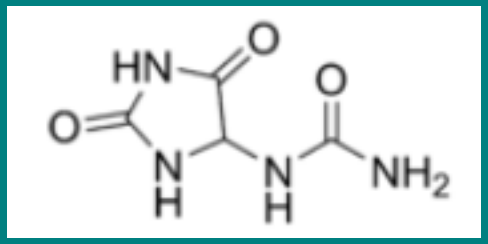
Dealing with Uric Acid Precipitates After They Have Formed



Uric Acid

(insoluble at acid pH)

urate oxidase



Allantoin

(15-20 times more soluble)

- urate oxidase is present in most species, except primates (which includes human beings)
- recombinant form is marketed as the drug rasburicase, which can dissolve uric acid and its crystals

Our Patient's Serial Blood Levels

Time from Admission (hours)	Creatinine (mg/dL)	Uric Acid (mg/dL)
0	1.2	3.2
24	1.3	4.2
48	3.2	16.4
72	5.4	0.2

- uric acid = 0.2: rasburicase is working, right?
- but why is creatinine still increasing?
- this could be a pre-analytic problem . . .

Sample Handling is Critical

- rasburicase works in vitro as well as in vivo
- samples must be kept at 4ºC, from the time they are drawn until they are analyzed
- otherwise, a low uric acid level may represent in vitro conversion

Our Patient

- a properly handled sample showed that his in vivo uric acid level remained elevated at 8.4 mg/dL (0.50 mmol/L)
- this prompted additional doses of rasburicase
- ongoing blood samples (handled properly) showed uric acid levels of 0.4 mg/dL (0.02 mmol/L)
- serum creatinine levels gradually improved

Case #4: Where's Waldo?

- The week before his annual routine physical examination, a healthy, 62 year old man, has several lab tests done
- He receives a call from his physician the next day, asking him to come in immediately because of "some abnormal test results"
- Specifically, his serum creatinine, which for many years has been ~1.3 mg/dL (115 umol/L), was measured as 2.9 mg/dL (256 umol/L)

Office Visit

- He tells his physician that, aside from feeling a little more tired than usual, he really has no complaints or symptoms

- His physical examination is unremarkable

- The physician orders several lab tests:
  - dipstick urinalysis (done in office)
  - CBC
  - Basic Metabolic Profile (glucose, BUN, creatinine, electrolytes)
  - urine Na and creatinine (to calculate FE(Na))

Test Results

- urinalysis: totally normal
  ➔ no blood, white cells, bacteria, glucose, or protein
- CBC:
  ➔ anemia (Hct = 30); otherwise normal
- BMP:
  ➔ creatinine = 3.0 mg/dL (265 umol/L)
  BUN = 54 mg/dL (19.3 mmol/L)
  otherwise normal
- FE(Na) = 3.4%
  ➔ intrinsic renal disease

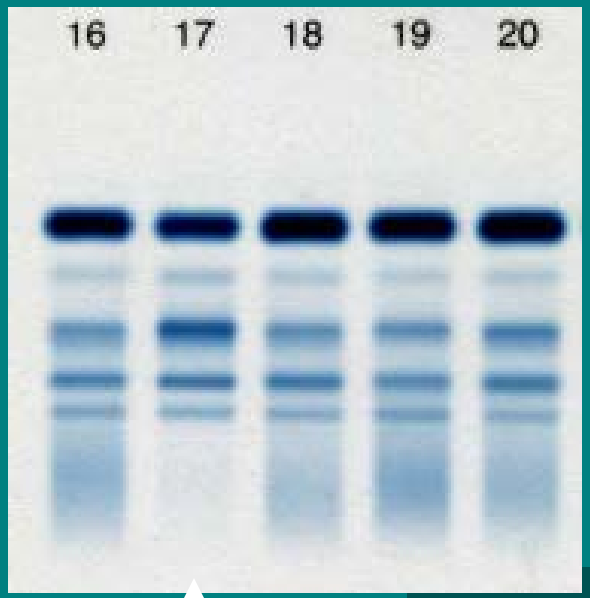
- concerned about AKI, his physician refers the patient to a nephrologist

Nephrologist Consultation

- The nephrologist confirms the patient's history
- She finds nothing new on the physical examination
- She repeats the urinalysis herself,
  but, again, she finds no abnormalities

- Worried about AKI, she orders many additional
  blood tests, too numerous to list here
- Among all of these tests, only one comes back as
  even mildly abnormal

Serum Protein Electrophoresis

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----------------------PROTEIN AND IMMUNOELECTROPHORESIS-----
            PEP          IgG          IgA          IgM
                       700-1600     70-400       40-230
                        mg/dL        mg/dL        mg/dL
01/29     HYPOGA        400*         63*          38*
10:00P          1
     1 HYPOGAMMAGLOBULINEMIA
       IF CLINICALLY INDICATED, SUBMIT URINE FOR PEP
       INTERPRETED BY GARY L. HOROWITZ, MD
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New Urine Sample Obtained

- The nephrologist is puzzled by the report:
  - she had checked the urine herself: there was no protein present
- But she arranges to get a new sample
  - repeats her dipstick analysis, which is again totally normal
  - sends the new sample to the lab for UPEP
- Results:
  - total protein = 425 mg/dL (4250 mg/L) (!)
  - urine PEP/IFE reveal Bence-Jones

Urine PEP and IFE

Light Chain Myeloma

- 15-20% of all case of multiple myeloma
- Free Light Chains (Bence-Jones):
  - not detected by urine dipstick protein test
  - is detected by standard clinical chemistry test for urine protein
- PEP reveals abnormal band
- on IFE, stains only with light chain anti-sera
- Deposition of these proteins in the kidney leads to “myeloma kidney”, which includes renal failure (reduced GFR)

Predictive Value of Laboratory Tests

- One of the most important, but least understood, concepts in laboratory medicine

- ***Diagnostic Sensitivity*** = "Positivity in Disease"

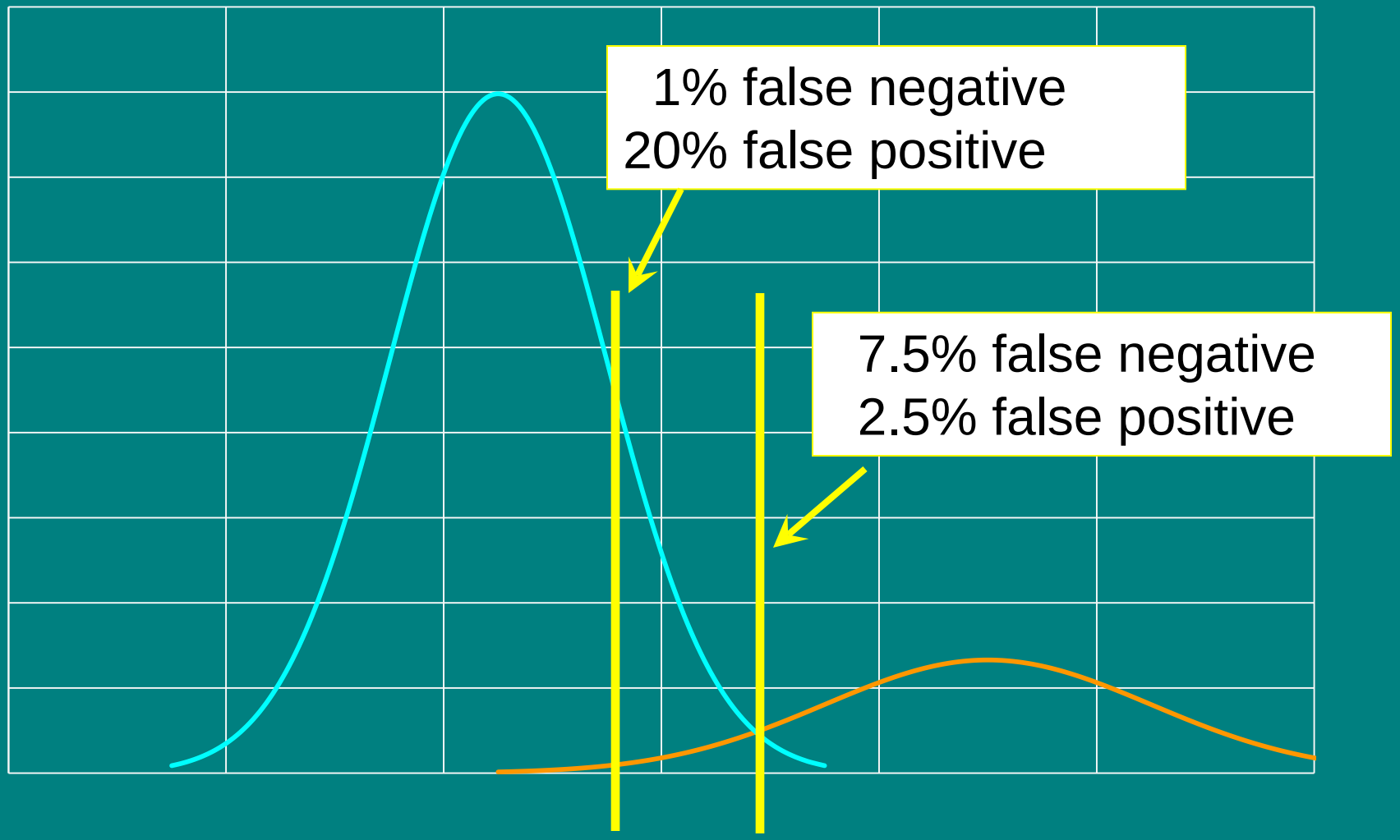
  $$\frac{\text{number of people with positive test}}{\text{total number of people with disease}}$$

- ***Diagnostic Specificity*** = "Negativity in Health"

  $$\frac{\text{number of people with negative test}}{\text{total number of people without disease}}$$

- ***Predictive Value*** depends on ***disease prevalence*** as well as sensitivity and specificity

Sensitivity and Specificity Depend on Cut-Off



Receiver Operating Characteristic (ROC) Curves

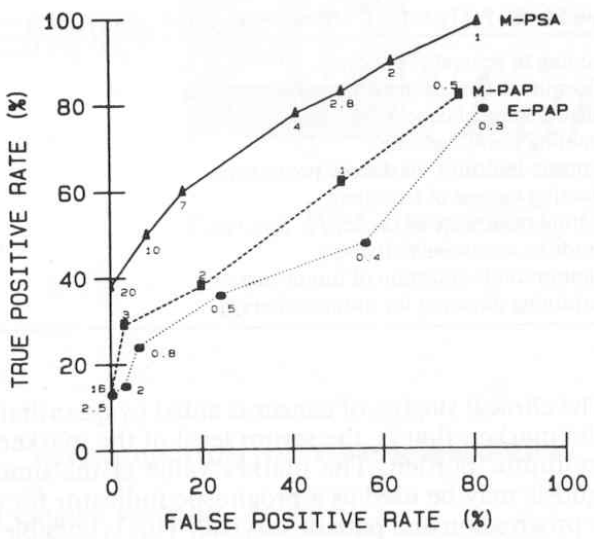


Figure 20–1. Receiver operating characteristic curves for prostate-specific antigen (M-PSA) and prostatic acid phosphatase (M-PAP) by monoclonal immunoassay, and enzymatic prostatic acid phosphatase (E-PAP). The data for all 128 patients with prostatic disease are plotted, with several quantitative decision levels (as indicated in the figure) for each assay. Units are μg/L for M-PAP and M-PSA, and U/L for E-PAP. (From Rock, R.C., Chan, D.W., Bruzek, D.J., et al.: Evaluation of a monoclonal immunoradiometric assay for prostate-specific antigen. Clin. Chem., *33*:2257–2261, 1987.)

Theoretical Example: New Diagnostic Test
typical publication in medical journal

- Sensitivity = 95%
- Specificity = 90%

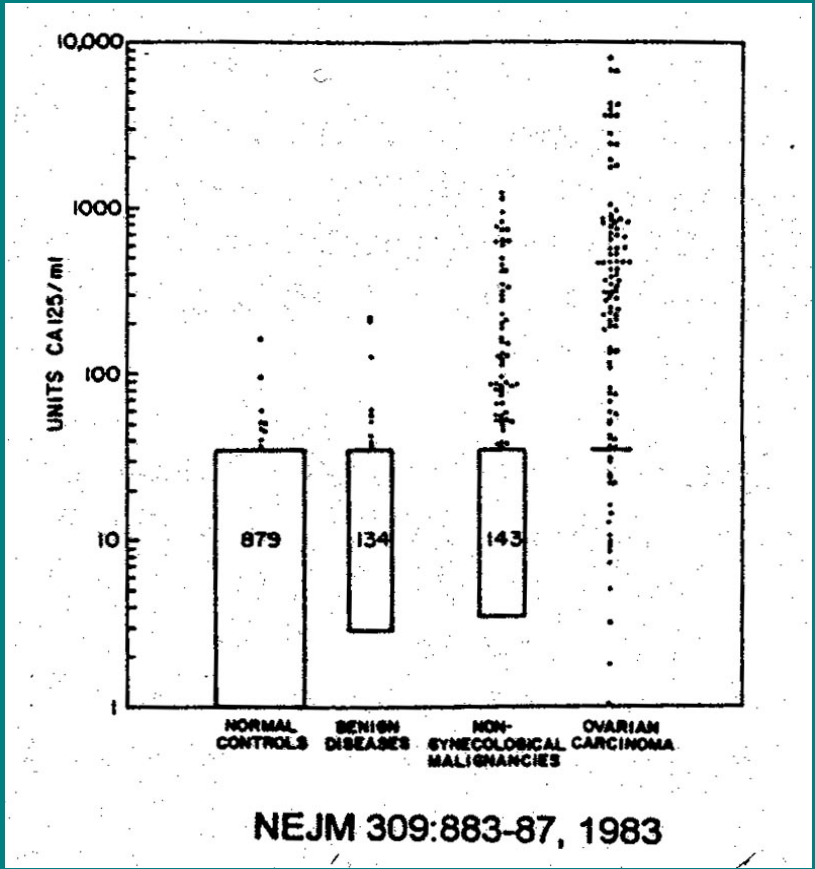
	# of patients	Test +	Test -	
with disease	100	95	5	Sensitivity= 95/100= 95%
without disease	100	10	90	Specificity= 90/100= 90%
	Prevalence= 100/(100+100) =50%	PV_+= 95/(95+10)= 90.5%	PV_-= 90/(90+5)= 94.7%	

Theoretical Example: New Diagnostic Test
note performance change when used indiscriminately

- Sensitivity = 95%
- Specificity = 90%

	# of patients	Test +	Test -	
with disease	100	95	5	Sensitivity= 95/100= 95%
without disease	1900	190	1710	Specificity= 90/100= 90%
	Prevalence= 100/(100+1900)= 5%	PV_+= 95/(95+190)= 33.3%	PV_-= 1710/(5+1710)= 99.7%	

Statistically Different Means vs Overlap/Prevalence



NEJM 309:883-87, 1983

Real Example #1: PSA & Prostate Cancer

- For a 40-year old man,
  with a life expectancy of 25 years,
  lifetime risk of prostate cancer is
  - microscopic disease: 42%
  - clinically evident disease: 10%
  - fatal prostate cancer: 3%
- Let's assume 10% prevalence

Sensitivity & Specificity of PSA

- Typical figures (Tietz), for men > 50 yrs old:

Cut-Off	Sensitivity	Specificity
4.0	78% 22% of cases missed!	33%
2.8	92%	23%

Predictive Value: PSA Screening

	# of patients	Test +	Test -	
with disease	1000	780	220	Sensitivity= 780/1000= 78%
without disease	9000	6030	2970	Specificity= 2970/9000= 33%
	Prevalence= 1000/(1000+9000)= 10%	PV_+= 780/(780 +6030)= 11%	PV_-= 2970/(220+2970)= 93%	

With realistic numbers for sensitivity, specificity, & prevalence, 89% of positive results are false positives!

What additional tests/procedures are you willing to subject these men to?

“The PSA Conundrum”

The poor specificity of PSA testing results in a high probability of false positives requiring prostate biopsies and lingering uncertainty about prostate cancer risk, even with initially negative biopsy findings.[3]

Although we now know that aggressive surgical treatment of prostate cancers largely detected the “old fashioned way” without screening has a modest benefit, with about 18 cancers needing to be removed to prevent 1 death over 10 years,[4] that benefit comes at a considerable price in terms of sexual dysfunction and incontinence.

The key question is whether early detection and subsequent aggressive treatment of prostate cancers found through PSA screening prevents enough morbidity and mortality to overcome these disadvantages—it will have to work to some degree just to “break even.”

Although recent reductions in population-based prostate cancer mortality in the United States have suggested a benefit, at the regional level those reductions have not correlated with the intensity of screening,[5] and reductions have also been seen in other countries, such as the United Kingdom, without widespread screening.[6]

Barry MJ. Arch Intern Med 2006;166:7-8

Real Example #2: CA125 & Ovarian Cancer

- Predictive Value variables:
  - CA125 **sensitivity**: 80% (best case)
  - CA125 **specificity**: 98% (18/855 healthy subjects)
  - **prevalence** (ovarian carcinoma) = 14/100,000

Predictive Value: CA125 Screening

	# of patients	Test +	Test -	
with disease	14	11	3	Sensitivity= 11/14= 80%
without disease	99986	2000	97986	Specificity= 97986/99986= 98%
	Prevalence= 14/(100,000)= 0.014%	PV_+= 11/(11 +2000)= 0.5%	PV_-= 97986/(97989)= 100%	

With realistic numbers for sensitivity, specificity, & prevalence, 99.5% of positive results are false positives!

What additional tests/procedures are you willing to subject these women to?