Discovery and Validation of Biomarkers for Cancer: 15 Years of Experience with the Early Detection Research Network

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What are Biomarkers?

- measured in body tissue or fluids
- diagnosis/screening e.g. PSA
- prognosis e.g. Genomic Health Recurrence Score
- risk prediction e.g. BRCA1 gene mutation

What is the Early Detection Research Network (EDRN)?

Created

- 2000 by NCI
- collaborative network to facilitate bench to bedside

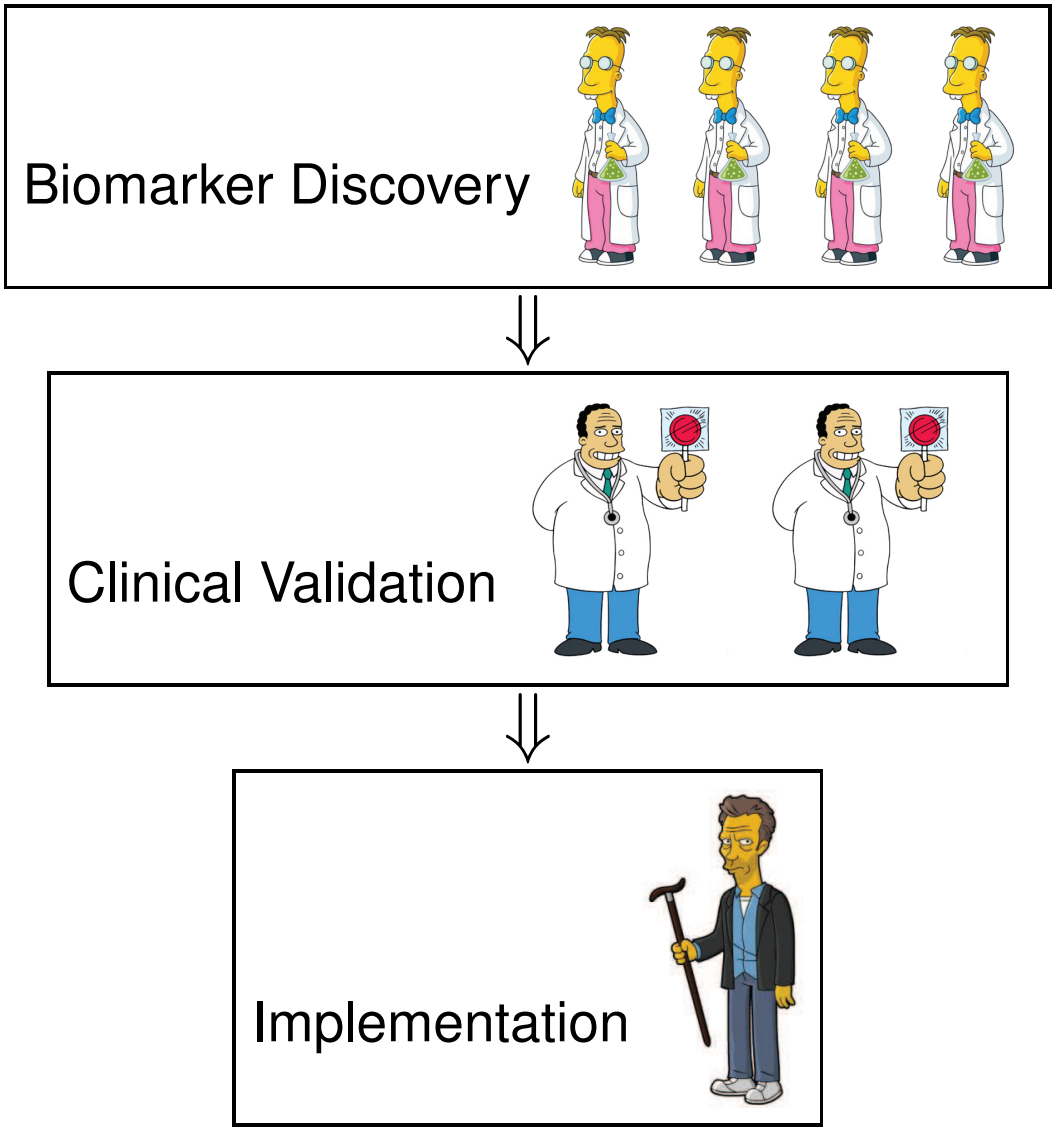
Components

- 22 development+8 reference laboratories
- 8 clinical validation centers
- data management and coordinating center
- organized around organ-specific collaborative groups

Early Detection of Ovarian Cancer

- symptomatic only in late stage
- hard to treat in late stage
- easy to treat with surgery in early stage
- incidence = 25/100,000
- seek blood based biomarker for ovarian cancer screening

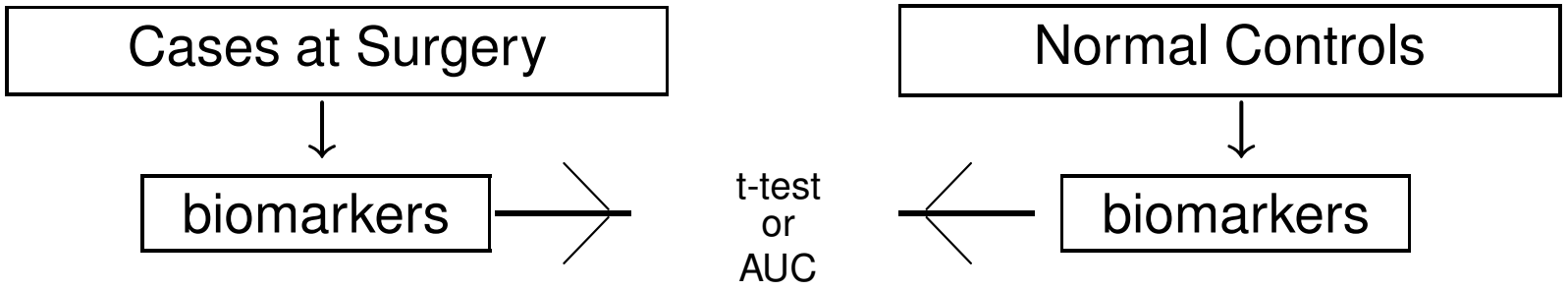
Phases of Biomarker Development



Pepe et al. JNCI 2001 93:1054–1061

- Focus initially on design of clinical validation studies.

Typical Study Design



The research question: How well does biomarker detect presymptomatic ovarian cancer?

Issues with design

- biased samples: cases and controls from different settings
- biased samples: preclinical disease not addressed
- AUC = $P(Y_{\text{case}} > Y_{\text{control}})$ is not clinically relevant
- $\bar{Y}_{\text{case}} - \bar{Y}_{\text{control}}$ is not clinically relevant

Rigorous Design for Clinical Validation

- PRoBE
- **P**rospective enrollment, sample collection and outcome ascertained for a clinically relevant **p**opulation
- **R**etrospective **r**andom selection of cases and controls from the cohort
- **B**linded specimen handling and assays
- **E**valuation with relevant statistical methods

Pepe et al. JNCI 2008 100:1432–1438.

Components of the PRoBE Design

(i) Clinical Context

(ii) Clinical Performance Criteria

(iii) Biomarker Test

(iv) Data analysis and sample sizes

Detailed checklists for each aspect (Pepe et al. JNCI 2008 100:1432-1438).

PRoBE for Ovarian Cancer Screening Biomarkers

Clinical Context (Intended use drives design)

- cohort = healthy asymptomatic women
- definitions
  - case = ovarian cancer 6–18 months from sample
  - control = healthy cancer free 5 years from sample
  - other groups to account for whole population
- consequences of a positive test
  - ultrasound followed by surgery if indicated

$\Rightarrow$ stored blood samples from large healthy cohort, followed prospectively

Clinical Performance Criteria

- ρ = case prevalence = 25/100,000 for age 55–59
  TPR = $P(Y \text{ positive} \mid \text{case})$
  FPR = $P(Y \text{ positive} \mid \text{control})$
- $B =$ benefit of work-up to a case
  $C =$ cost of work-up to a control
- Expected benefit
  $= B\,\text{TPR}\rho - C\,\text{FPR}(1-\rho) > 0$
- $$\frac{\text{TPR}}{\text{FPR}} > \frac{1-\rho}{\rho}\frac{C}{B}$$

How to Solicit C/B

Approach #1: How many false positives are worth a true positive?

- e.g. 300 mammograms for 1 breast cancer detected

Approach #2: Risk Threshold (r)

- expected benefit: $BP(D = 1|Y) - CP(D = 0|Y)$
- risk $> r \Rightarrow$ work-up warranted
  risk $< r \Rightarrow$ work-up not warranted
  therefore $Br - C(1 - r) = 0$
  $\Rightarrow C/B = r/(1 - r)$

  e.g. $r = 20\% \Rightarrow C/B = .20/.80 = 1/4$

Application to Ovarian Cancer

- In ovarian cancer: "10 surgeries should yield at least 1 cancer."
- $r = 0.10$ for the Biomarker + Ultrasound test
- $\text{TPR}_{B+\text{US}} = P(Y\text{positive and USpositive}|\text{ case})$
  $= P(Y\text{positive}|\text{case}) \times P(\text{US positive}|\text{case})$
  $= \text{TPR} \times 0.755$
- $\text{FPR}_{B+\text{US}} = \text{FPR} \times 0.018$

$$\frac{\text{TPR}_{B+\text{US}}}{\text{FPR}_{B+\text{US}}} > \frac{1-\rho}{\rho} \times \frac{r}{1-r} = \frac{1-.00025}{.00025} \times \frac{1}{9} = 444$$

$$\Rightarrow \frac{\text{TPR}}{\text{FPR}} > 444 \times \frac{0.018}{0.755} = 10.6$$

Sample Size Calculations

- notation: ROC(f) = TPR corresponding to biomarker positivity threshold that yields FPR = f
- conclude biomarker useful if ROC$(0.05) \geq 0.53$
- H_0 : ROC$(0.05) = 53\%$ versus H_1 : ROC$(0.05) = 0.73$
  - 0.73 is based on preliminary data
  - details in Pepe (2003) textbook
- $n_{\text{cases}} = 40$ and $n_{\text{controls}} = 160$ yields 71% power
  - Stata software
  - DABS FHCRC website

Results of EDRN-PLCO Collaborative Study

	ROC(0.05)			
Marker	Phase 2 preliminary data (160 cases)	≤ 6 months (45 cases)	$6-12$ months (22 cases)	$12-18$ months (17 cases)
CA-125	0.73	0.80	0.32	0.12
HE4	0.57	0.60	0.23	0.06
MMP7	0.47(?)	0.20	0.14	0.18
Spondin 2	0.28	0.11	0.14	0.06
CA72-4	0.40	0.44	0.14	0.20
MIF	0.15	0.18	0.09	0.00

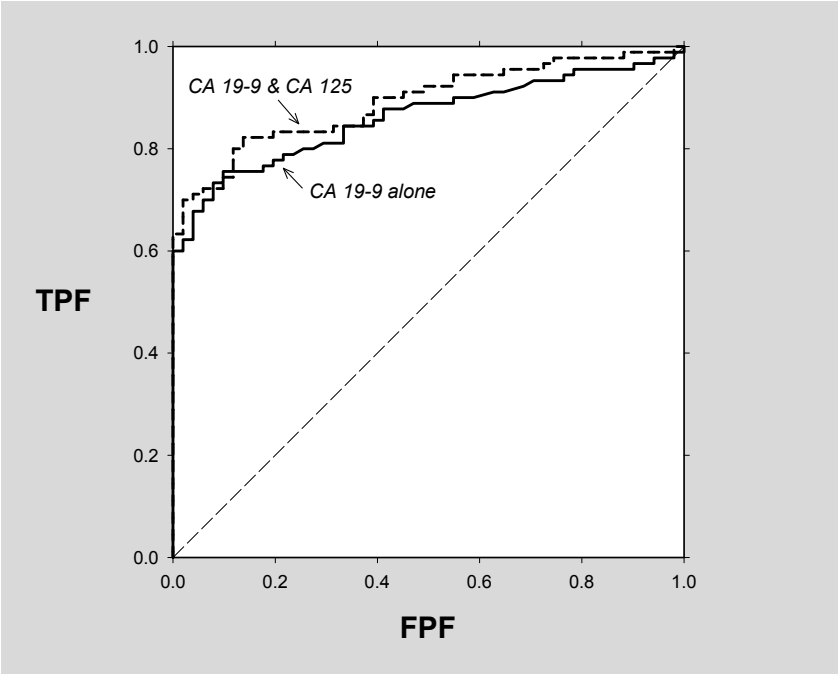
Cramer et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prevention Research 2011; 4:365–74

When a Biomarker Test X Already Exists

Examples: PSA, CA-125, mammography

Incremental value

- performance of (X, Y) combined versus X alone
- ROC(0.05) improved from 0.68 to 0.71



- not possible if X already in use (verification bias)
- cautions: independent data to evaluate improvement versus to combine markers
- use a "proper" statistics e.g., ΔROC(0.05),not NRI

The NRI Statistic can be Misleading

$$NRI = \{P(\text{risk}(X, Y) > \text{risk}(X)|\text{case}) - P(\text{risk}(X, Y) < \text{risk}(X)|\text{case})\}$$
$$+ \{P(\text{risk}(X, Y) < \text{risk}(X)|\text{control}) - P(\text{risk}(X, Y) > \text{risk}(X)|\text{control})\}$$

Pencina et al Stat in Med 2008, 2010

Table: Rates at which the null hypothesis of no performance improvement is rejected in favor of the one-sided alternative hypothesis that prediction is improved by adding the four biomarker panel to the baseline clinical score*

Dataset	NRI‡	LR‡	ΔAUC‡
Training set (n = 420)			
Using training set risks, TR-TR	63.0%	5.3%	9.8%
Test set (n = 420)			
Using training set risks, TR-TS	23.2%	—	1.1%
Using re-estimated risks, TS-TS	19.4%	4.7%	1.5%
Test set (n = 840)			
Using training set risks, TR-TS	34.4%	—	0.6%
Using re-estimated risks, TS-TS	18.8%	5.1%	1.8%

* Because the biomarkers have no association with the outcome in the population, all rejections are false-positive results.

† AUC = change in the area under the receiver operating characteristic curve; LR = likelihood ratio; NRI = Net Reclassification Index; TR = training dataset; TS = test dataset.

‡ Five thousand simulated studies in which the biomarkers have no association with outcome. Nominal rejection rates are 5.0%.

Pepe et al JNCI 2014

Use a Clinically Relevant and Valid Statistic

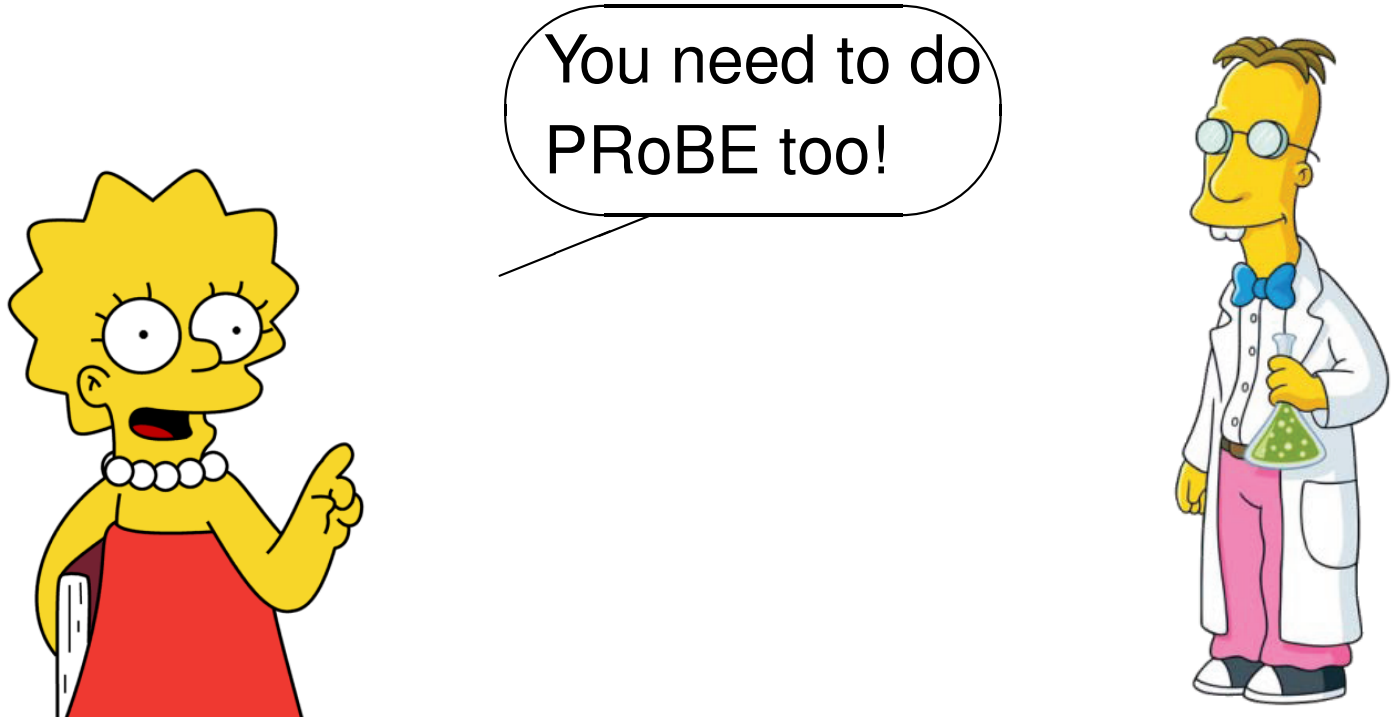
- AUC — not relevant
- NRI — not relevant (usually)
- TPR at pre-specified low FPR — relevant in screening
- FPR at pre-specified high TPR — relevant in diagnosis
- Net Benefit = $B \times \text{TPR} \times \rho - C \times \text{FPR} \times (1 - \rho)$
  Standardized NB = $\text{TPR} - (\frac{C}{B})\text{FPR}\frac{(1-\rho)}{\rho}$
  - Vickers and Elkin Med Decision Making (2004)
  - meaningful as discounted TPR

Discovery Research

- Not producing biomarkers that validate
- Biased designs are common in discovery research
- Yield biomarkers of non-disease related differences between cases and controls
  - anesthesia, medication use, stress, ...
  - aging, other medical conditions, ...
- Yield biomarkers that look great
  - in severe cases, at diagnosis . . . .

Discovery Research

- Should use PRoBE designs too.



Pepe MS, Li CI, Feng Z. Improving the quality of biomarker discovery research: the right samples and enough of them. Cancer Epidemiol Biomarkers Prev. 2015

Sample Size Calculation for Colocare Study

Colocare

- stage 1 colon cancer
- markers for 'high' risk of recurrence within 2 years $\rho =$ overall recurrence rate = 10%
- 'high risk' = 30%$= r$, warrants chemotherapy
- useful marker: TPR/FPR $\geq (\frac{1-\rho}{\rho}(\frac{r}{1-r}) \approx 3.9$
- fix FPR=10%
- # candidate biomarkers = 9,000

Operating Characteristics

- False leads expected:% FLE=proportion of null markers filtering in = 2% say
- Discovery power: proportion of useful markers filtering in = 95% say
- Filter in criterion: p-value for biomarker $< C$

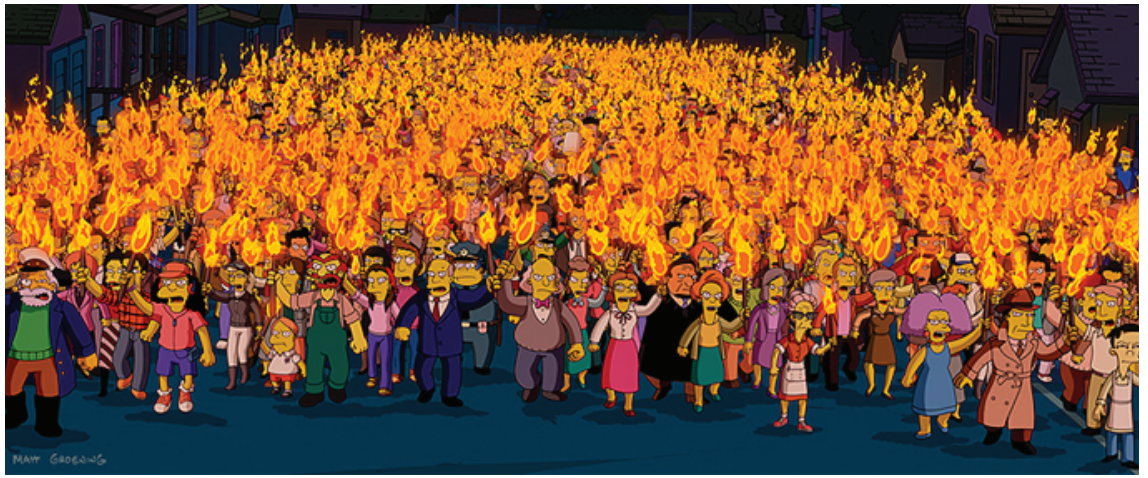
Calculations

- Fix % FLR = 2% by choosing $C = 2\%$
- works in theory, not always in practice with small samples
- simulations to refine C
  simulations to calculate discovery power
- not computationally intensive: vary # cases and # controls
- 40 cases, 160 controls, $C = 1\%$ yields FLE% = 2.3% and Discovery Power = 95%

Summary

- phases of research
- PRoBE ideal design for validation
- PRoBE ideal design for discovery
- many basic statistical issues
  - measures of performance
  - how to accommodate covariates ?
  - is matching a good idea?
  - failure time event data?
  - etc.
- DMCC provides leadership and excellent implementation

Colleagues at EDRN



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