

Novel Prostate Biopsy Test in the Diagnosis Between Benign and Cancerous Prostate by Fractionated Sub-cellular Protein Apoptosis Biomarker

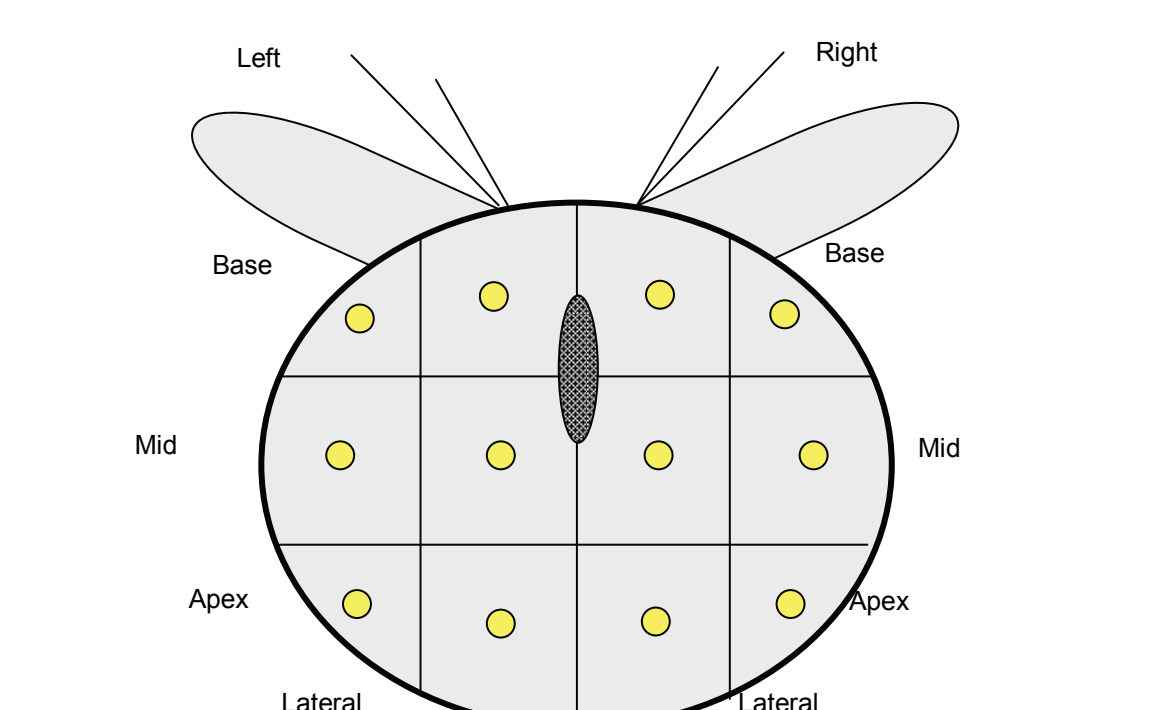
Joseph V. DiTrollo, MD; Michael D. LaSalle, MD; Rahuldev Bhalla, MD

St. Barnabas Medical Center, Livingston, NJ; New Jersey Medical School, Newark, NJ; Stony Brook University Medical Center, East Setouket, NY

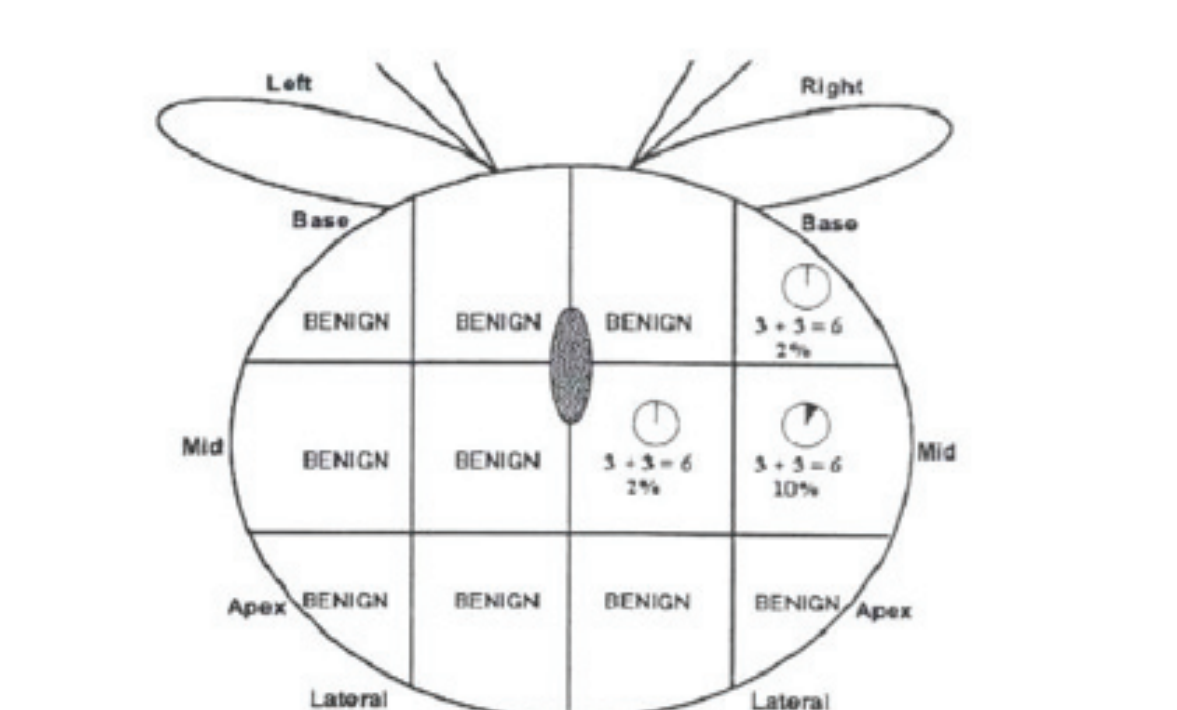
Introduction: Prostate cancer (PCa) detection using PSA, digital rectal exam (DRE), PCA3+, and standard twelve core ultrasound guided biopsies (TRUS) are not always accurate in predicting diagnosis. EDGE*TEST (ET) is a new subcellular fractionation/statistical analysis technology. ET was developed based on Edge™ technology (Enhanced density gradient extraction) and Prospect Biosystems' proprietary relative distribution ratio profiling to statistically differentiate between diseased and healthy tissue or cell samples. The objective is to evaluate if ET can predict PCa biological activity using an apoptosis biomarker Grp78 throughout the entire prostate rather than individually targeted biopsies.

Method:

- Twenty-four (24) patients (age 48-80) with elevated PSA, abnormal DRE and/or elevated PCA3+ genetic test were chosen by their urologist for this study.
- Twelve core TRUS, six from the right gland and six from the left gland were collected for pathological evaluation by a single urologist. PSA and PCA3+ analyses were also done on each of the patients.
- At time of biopsy, under informed consent from the patient, an extended procedure was performed by the urologist on the patient. One additional biopsy from the right gland and another from the left gland were taken from random locations for EDGE*TEST.
- EDGE*TEST samples from each patient were collected into ice cold buffered saline, combined, rinsed and stored at -80°C.
- Samples were thawed and homogenized using a glass dounce. Nuclei were removed and the post-nuclear supernatants were fractionated into four subcellular density fractions (8.5%, 15%, 35% and 50%) using Edge 200 Separation System.
- All fractions from each sample were subjected to western blot analysis using the GRP78 marker.
- GRP78 distribution profiles within each sample were statistically analyzed to determine the optimal distribution ratios.
- EDGE*TEST results of patients' distribution ratios were blinded against all clinical and pathology data.
- Negative PCa biopsy patients were followed up for 18 months to compare predictive value of EDGE*TEST to traditional screening and biopsy.

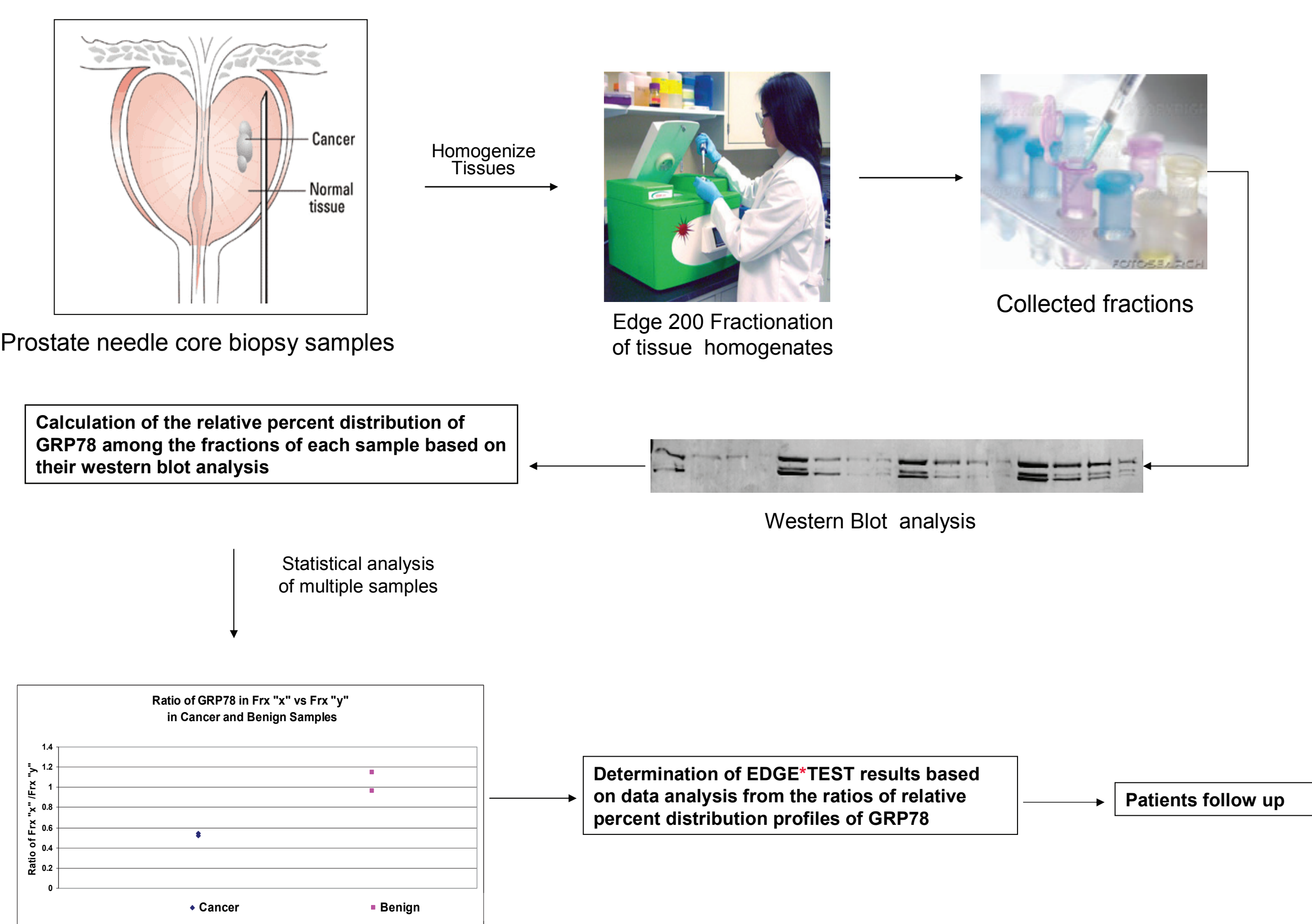


Twelve directed biopsies in specific areas of the prostate were collected for pathological evaluation. Needle cores are taken from the right and left sides of the prostate gland near the apex, near the base, and from the central portion of the prostate gland. An additional core (not shown) was randomly taken from each side of the prostate for this study.



Prostate Biopsy Map: Pathological evaluation result from one of the PCa patients.

EDGE*TEST Process



Results:

Statistical Analysis of GRP78 Distribution

Example of Western Blots of GRP78: all fractions were subjected to western blot analysis based on standard procedures.

Patient	Arbitrary Units of GRP78 in Each Density Fraction				Relative % Distribution of GRP78 in Each Density Fraction				Ratio of Relative % of GRP78 in Frx 8.5% vs Frx 15%
	8.5%	15%	35%	50%	8.5%	15%	35%	50%	
A	2498	465	583	261	65.60	12.24	15.31	6.85	5.36
B	1175	122	177	305	66.05	8.86	9.95	17.14	9.63
C	2006	340	138	0	80.76	13.89	5.96	0.00	5.90
D	3297	911	0	0	78.20	21.80	0.00	0.00	3.59
E	2468	1514	368	158	54.75	33.58	8.16	3.50	1.63
F	8142	3420	821	855	61.50	25.83	6.20	6.46	2.38
G	1556	159	135	0	84.11	8.59	7.30	0.00	9.79
H	2797	1096	471	109	62.28	24.67	10.80	2.45	2.52
I	3384	745	1331	936	52.91	11.65	20.81	14.63	4.54
J	912	155	0	0	85.39	14.61	0.00	0.00	5.85
K	8910	5994	4049	4834	36.52	23.20	17.31	20.67	1.46
L	3810	591	0	0	86.57	13.43	0.00	0.00	6.45
M	1460	535	514	1443	36.94	13.54	13.01	36.51	2.73
N	2142	227	189	0	83.74	8.87	7.39	0.00	9.44
O	3910	1078	312	297	69.88	19.20	5.58	5.31	3.63
P	2002	1114	1046	736	40.87	22.74	21.36	15.03	1.80
Q	4476	1129	387	0	74.70	18.94	6.46	0.00	3.96
R	913	160	0	0	85.09	14.91	0.00	0.00	5.71
S	6450	2993	686	285	63.41	26.80	8.85	2.85	2.36
T	8034	1978	810	757	69.38	17.08	7.00	6.54	4.06
U	8787	3841	1731	1611	55.02	24.05	10.84	10.09	2.29
V	4697	930	296	159	78.48	13.80	4.98	2.86	5.65
W	1483	397	64	0	76.29	20.42	3.29	0.00	3.74
X	4745	1285	568	189	69.91	18.93	8.37	2.78	3.69

* Western blot analysis was done by ImageJ software. The relative percent distribution of GRP78 in each fraction has been calculated by dividing the arbitrary units of a fraction by the total arbitrary units of all fractions within the sample.
** A specific ratio (Frx 8.5%/ Frx 15%) of the relative percent of GRP78 in each sample was selected based on a statistical analysis.

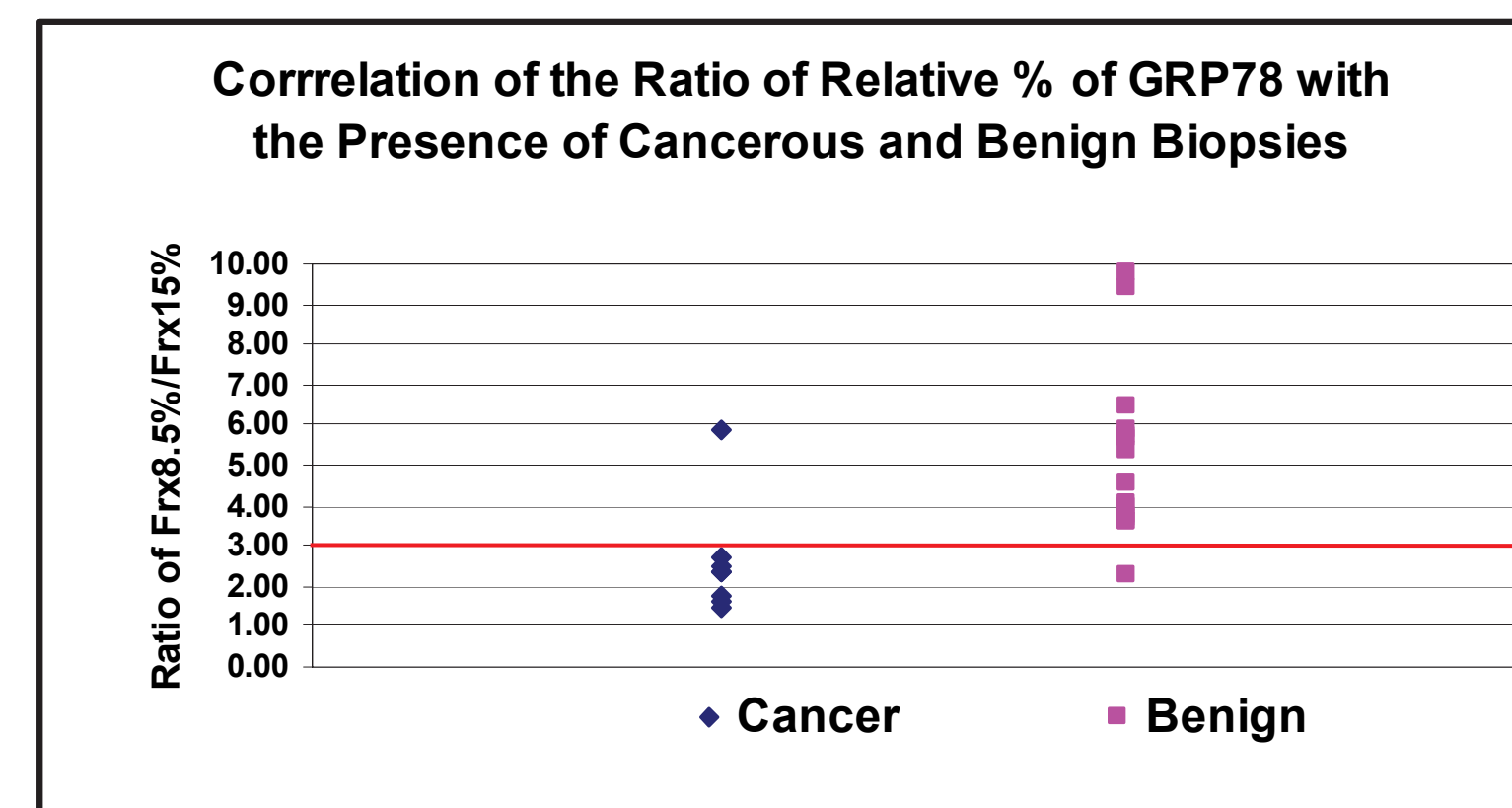
Clinical Data and Pathological Results

Patient #	DRE*	PSA ** ng/ml	PCA-3 Plus**	Diagnosis based on Biopsy Histology	Number of Cancerous Cores	Gleason Score	% Cancer/ core
1	Abnormal	4.80	42.40	Benign			
2	Abnormal	1.54	49.60	Benign			
3	Normal	5.40	39.80	Benign			
4	Normal	4.58	97.00	Benign			
5	Normal	4.90	60.50	Benign			
6	Abnormal	2.46	2.50	Benign			
7	Normal	2.41	25.10	Benign			
8	Normal	5.64	10.20	Benign			
9	Normal	4.46	5.10	Benign			
10	Normal	4.20	22.30	Benign			
11	Normal	4.56	9.30	Benign			
12	Normal	12.10	4.00	Benign			
13	Normal	5.70	3.10	Benign			
14	Normal	5.34	9.70	Benign			
15	Normal	5.10	8.20	Benign			
16	Normal	8.13	9.90	Benign			
17	Normal	5.80	14.30	PCa	2	6	5-10
18	Abnormal	>1000	56.60	PCa	12	7	5-90
19	Abnormal	24.70	91.70	PCa	11	7, 9	20-90
20	Abnormal	3.50	128.40	PCa	8	6, 7	6-80
21	Normal	6.20	104.10	PCa	7	6	2-85
22	Abnormal	2.30	15.00	PCa	3	6	10-60
23	Normal	5.10	9.50	PCa	3	6	2-10
24	Abnormal	6.10	51.40	PCa	3	6, 7	30-90

Yellow area above corresponds to those samples judged by pathological analysis to contain varying amounts of cancerous tissue.

EDGE*TEST Results

Correlation with Pathological Results

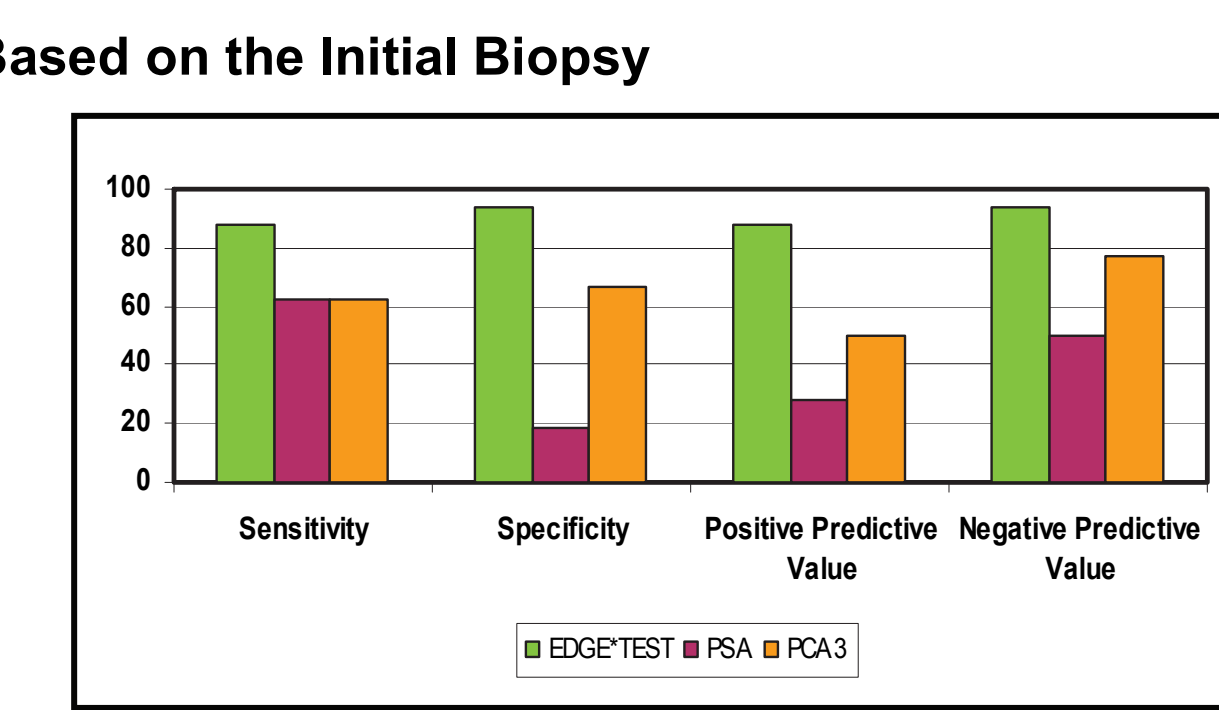


Graph shows the clustered distribution of the ratios of relative percent distribution of GRP78 in fraction 8.5% vs. fraction 15% in a group of patients with cancerous and benign biopsies. The ratio of 3.00 indicated by the red arrow is an arbitrary cut off; a ratio less than 3.00 indicates cancer and a ratio above 3.00 indicates benign.

Comparison of EDGE*TEST with PSA and PCA-3 plus Within the Given Sample

EDGE*TEST vs. Traditional Tests	EDGE*TEST	PSA	PCA3
Sensitivity	87.50	62.50	62.50
Specificity	93.75	18.75	66.66
Positive Predictive Value	87.50	27.77	50.00
Negative Predictive Value	93.75	50.00	76.92
Likelihood Ratio Positive Test	14.00	0.76	1.87
Likelihood Ratio Negative Test	0.133	2.000	0.562

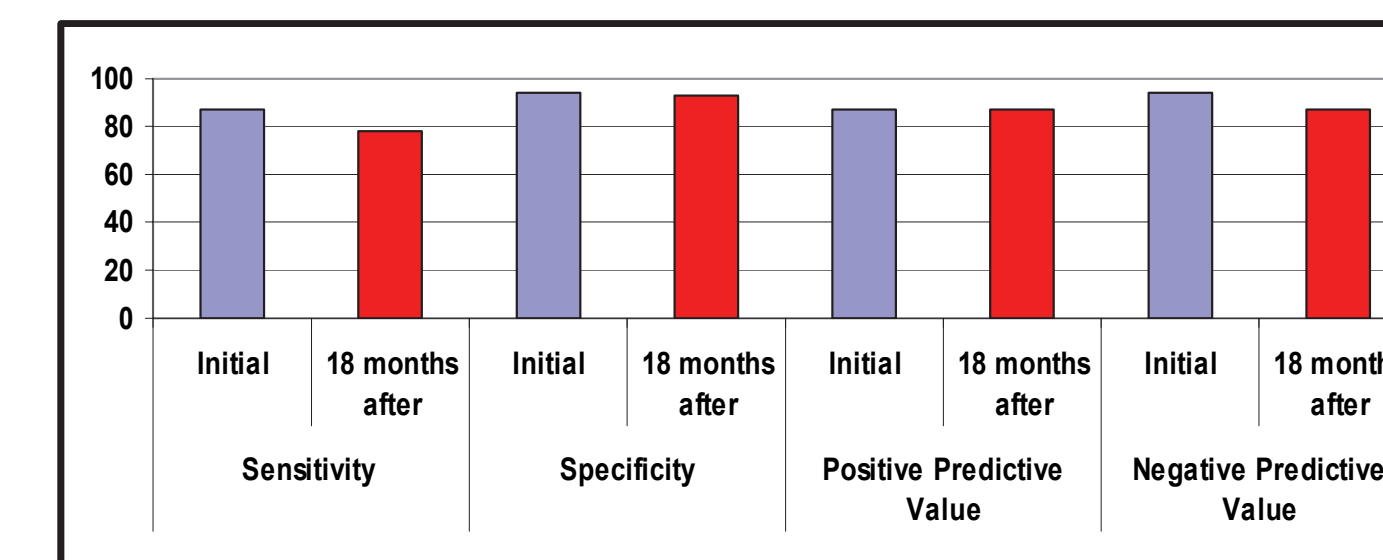
Diagnostic Review Guideline: EDGE*TEST <3.00, cancer
PSA: >4, cancer
PCA3: >35, cancer



Sensitivity = TP/(TP+FN); Specificity = TN/(TN+FP); Positive Predictive Value = TP/(TP+FP); Negative Predictive Value = TN/(TN+FN); Likelihood Ratio Positive Test = Sensitivity/(1 - Specificity); Likelihood Ratio Negative Test = (1 - Sensitivity)/Specificity
TP = True Positive; FP = False Positive; TN = True Negative; FN = False Negative

Comparison of EDGE*TEST Predictability at 18 Months vs. at Initial Biopsy

	Initial	18 months after
Sensitivity	87.50	77.78
Specificity	93.75	93.33
Positive Predictive Value	87.50	87.50
Negative Predictive Value	93.75	87.50



Conclusion:

- The EDGE*TEST predictability based on the initial biopsy is significantly superior to traditional tests.
- After 18 months follow-up, EDGE*TEST still shows high predictive data of benign disease and not cancer.
- Changes in subcellular relative distribution of GRP78 in prostate tissue may be used as a predictor for prostate cancer.
- Changes in relative levels of apoptosis in prostate tissue may be used as a predictor for prostate cancer.
- GRP78 could be used as a potential biomarker of tissue based prostate cancer diagnostics.
- The EDGE*TEST method only needs two needle biopsies, and provides more sensitivity and more specificity than the traditional PSA test and the genetic PCA3 test.
- The EDGE*TEST data arising from the process is internally referenced and DOES NOT need any internal or external standards.
- Since the method relies on the ratio between internal fractions of the sample, the method is independent of sample amount.
- The EDGE*TEST deserves further evaluation as a complimentary predictor of PCa and has been shown to be dramatically more effective than DRE, PSA or PCA3+.