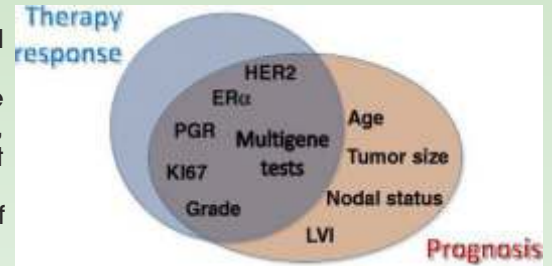


# RECEPTOR POSITIVITY IN 4500 INDIAN BREAST CANCER PATIENTS – DID STRINGENT QUALITY ASSURANCE ACHIEVE OPTIMAL PERCENTAGE OF POSITIVITY?

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## INTRODUCTION

- The use of biomarkers ensures breast cancer patients receive optimal treatment.
- The standard method for evaluation of ER and PR has been IHC. It must be recognized, however, that preanalytical variables such as time to fixation, methods used for tissue processing, and antigen retrieval significantly impact the analysis
- Improvement in quality over the last decade has led to a higher number of patients being candidates for targeted therapy.



## AIMS & OBJECTIVES

To analyze the effects of stringent Quality Assurance protocols in pre-analytical, analytical & post-analytical phases of ER, PgR & Her2/neu testing by IHC in an Indian accredited referral laboratory setting.

### OPTIMIZING IHC

- 1) Preanalytic variables (collection, fixation, and storage of samples)
- 2) Analytic variables (the method of testing itself)
- 3) Thresholds to define results (Interpretive i.e. scoring, cut off)
- 4) Postanalytic variables (handling of the results, such as reporting)

## MATERIALS & METHODS

- Retrospective analysis of 4500 breast cancer patients for ER, PR and Her2/neu receptor expression for the last 4 years
- SOPs were circulated amongst all surgeons & laboratories for ideal fixation especially with special respect to
  1. Cold Ischemic time
  2. Total fixation time
  3. Choice of fixative.
- IHC was carried out using manual & automated staining with standard clones.
- The ASCO/CAP guidelines for reporting ER, PgR & Her2/neu were applied & complimented with a Quick score.
- An analysis of 522 equivocal cases received for Her2/neu by FISH was also carried out.

## RESULTS

- Duration of study : January 2012 to August 2015
- Total number of patients : 4500
- Age range : 21 to 99 years ; Average 51.5 yr

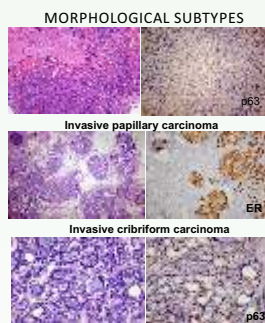
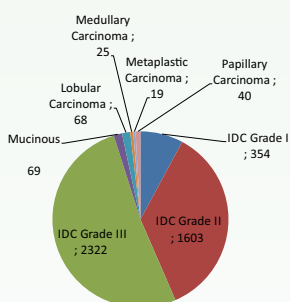
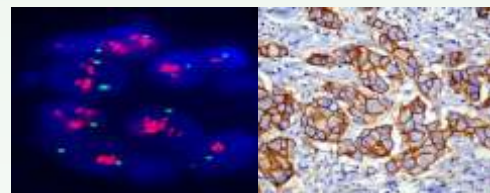
Receptor	Positive cases	Percentage of positivity
ER ( n =4274)	2874	67 %
PgR (n=4259)	2458	58 %

Her2neu (n= 3896)	No of cases	Percentage of positivity
Positive 996	26%	
Negative	2134	54.77%
Equivocal	766	19.66%

• Triple negative: 573 cases ; 13 %

### Result Her2Neu by FISH

- IHC was equivocal in 522 cases
- Stratification into 45.7% positive & 49.7% negative.
- 4.4% cases remained equivocal even on FISH.
- Rather of these, in 1% cases IHC could help categorize.



## DISCUSSION

- It has been previously observed that Indian patients with breast cancer have a higher tendency to have these tumours.
- As a result of more than a decade intensive research in breast cancer, apparent that testing results can be crucially influenced by pre-analytical and interpretation issues.
- This is probably one of the largest Indian cohort validating that quality control impacts receptor status positively.

## ANALYTICAL VARIABLES

### Antigen Retrieval

- Microwave should be avoided to prevent drying of sections
- The most commonly used buffer is 10Mm Sodium Citrate pH6
- Pressure cooker/ Decloaking chamber/PT-Module– Consistent

### Preferred Antibody clones

- ER :SP1 has 8-fold higher affinity compared to previously used 1D5
- PgR: clones 1294, 1A6, and 312 have been shown to be sensitive. Her 2 Neu : 4B5, A0485, CB11, Hercep Test, Sp3

## Interpretation

There are 2 primary parameters for scoring:

Proportion score (PS, percentage of positive neoplastic cells) and intensity score (IS)

The ASCO/CAP guidelines lowered the IHC cut off point to 1%.

## Quality Control

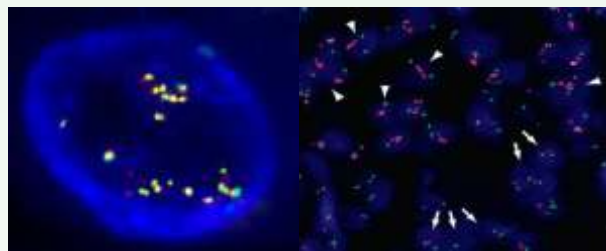
Batch controls, both positive and negative control tissues & control tissues should be subjected to the same fixation and processing conditions as the patient tissue.

	Nisaet et al 2008	Faheem et al 2012 7 Pak	Shet et al 2009-8	Navani et al 2005-9 Automated	Muddu wa 2009-10 Sri Lanka	Shetty et al 2013-11	Onitilo et al 2009-12	Gautam et al 2014	Our data
Mean Age	48.3	48	31-50	24-ve	52.5	41-50	62.7	50	51.5
Cohort	50	1226	11780		151	126	1134	450	4500
Predom morphology	IDC II	IDC NOS	IDC III	40% +ve	IDC III	IDC NOS	IDC	IDC	IDC III
ER Pos %	32.7	62.2	50.5		45.7	36.5	77.9	52	67%
PgR Pos %	25.3	60.1	42		48.3	31.7	59.1	41.5	58
Her2Neu	24.7	38.9	NA		19	2.4	17.7	-	26
Triple Neg	NA	-	NA		-	-	13.4	-	13

Diagnosis	ER+, PR+, Her2neu - Luminal A	ER+, PR-, Her2neu + Luminal B	ER-, PR-, Her2neu + Her 2 type	ER-, PR-, Her2neu - Triple Negative Basal type
IDC Grade I	95%	1%	3.7%	1%
IDC Grade II	49%	4%	8%	9%
IDC Grade III	23%	6%	16%	24%
Papillary Carcinoma	91%	3%	0	6%
Mucinous Carcinoma	98%	0	0	0
Medullary Carcinoma	0%	0	0	100%
Metaplastic Carcinoma	0%	0	0	100%
Lobular Carcinoma	94%	0	0	3.5%
Reported	50-70%		15-20%	10-15 35% Post(General) menopausal

## HER2 FISH :Polysomy 17: Masking Her2 Amplification

- Our data : 25% Polysomy Observed equally between Positive and negative cases.
- As per 2013 guidelines even if the ratio is not met the Her2 copy number should be more than 6 and hence 6 of our cases according to 2013 guidelines were reported as Positive.
- Prevalence of intratumoral heterogeneity was noted in 30% of our cases; reported incidence rates being 1.3%–54%.
- The concordance observed between IHC and FISH for positive and negative results was 96% and 97% in 213 cases.



## CONCLUSION

- Stringent Quality Assurance protocols for IHC including optimal fixation, methodology & interpretation yield excellent receptor positivity making more patients amenable to hormonal & targeted therapy.
- All laboratories performing Biomarkers & involved in Theranostics must constantly analyze their data, validate against biology and be aware of reported variations.
- Participation in Proficiency Testing programs ensures monitoring of achieved quality as a continuum.

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