Automated Analysis of p53 Immunohistochemistry in Patients with Barrett's Esophagus Predicts Disease Progression to Advanced Neoplasia

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BACKGROUND

- There are currently no validated markers to predict Barrett's esophagus (BE) patients at a high risk of progressing to advanced neoplasia.
- *TP53* mutation is highly recurrent in esophageal adenocarcinomas and is also detected in patients with non-dysplastic BE, predominantly those that progress to advanced neoplasia.

OBJECTIVES

- We investigated the utility of p53 immunohistochemistry in predicting advanced neoplasia in patients with BE.
- To circumvent the challenges associated with manual assessment we performed automated image analysis.

METHODS

- The progressor cohort was comprised of 92 patients, with total 144 non-dysplastic biopsies and the control cohort (non-progressor) was comprised of 148 patients.
- Clinical details of both groups are described in table 1.
- The absence of dysplasia in both the progressor and non-progressor cohorts was confirmed by 3 experienced gastrointestinal pathologists that were blinded to the immunohistochemistry results.
- p53 immunohistochemistry slides were scanned and regions of interest were annotated.
- The nuclear staining was categorized using the Visiopharm automated image analysis platform into 3 classes: 1+, 2+ and 3+ (Figure 1).
- Receiver Operating Characteristics (ROC) curve was used to identify optimal cut-points between the progressor and non-progressor cohort.

RESULTS

- p53 nuclear reactivity was noted in both the progressor and non-progressor groups, however, there were significant differences in numbers of 3+ and 2+ p53 positive epithelial cells between the two cohorts (p <0.0001) (table 2).
- At a cut point of ≥10 cells 3+ cells, p53 immunohistochemistry predicted progression to advanced neoplasia with a sensitivity and specificity of 44% and 93%, respectively (table 3).

Table 1: Summary of Progressor & Non progressor patients

Clinical Parameters (No of patients =246)	BE Non-progressor biopsies (No of patients & biopsies =148)	BE Progressor biopsies (No of patients= 92 & No of biopsies =144)	P value
Male/Female ratio	93:55	79:13	<0.001
Mean age in years at endoscopic and histologic diagnosis of BE	56.3±10.9,57, 24-81	62.4±11.5, 61.5, 29.5-87.1	<0.001
BMI	28.3±5.4, 28.3, 15.5-49.6	29.0±6.3	0.400
Mean Length of Barrett's epithelium in cm	3.6±2.8, 3.0, 0.5-15.0	5.3±3.9, 4, 0.5-21.0	0.014
Endoscopic type of BE (n=185) Irregular Z line Short BE Long BE	38.3% 27.7% 34.0%	0 25% 75%	<0.001
Highest grade of neoplasia	BE	HGD= 44 (71) ADCA= 48 (73)	-
Mean follow-up in (HGD/ADCA or BE) *	10.28±5.1, 11, 1-24	4.2±4.4, 2, 1-21	-

Table 2: Comparison of p53 expression in progressor and non-progressor groups

P53 staining Parameters (N=285)	BE Non-progressor biopsies (n=148)	BE Progressor biopsies (n=144)	P value
3+ p53 positive epithelial cells (Mean ± SD, Median, Range)	9.7± 65.7, 1, 0-767	266.8± 866.7, 7, 0-7189	0.001
3+ positive p53 epithelial cells < 10 cells ≥ 10 cells	 138 (93.3%) 10 (6.8%)	 80 (55.9%) 63 (44.1%)	<0.001
Presence of 3+ p53 positive glands (≥ 1 gland)	2.7%	30.8%	<0.001
3+ p53 staining of surface epithelium	0	12 (8.4%)	<0.001
2+ positive p53 epithelial cells (Mean ± SD, Median, Range)	151.0 ± 220.2, 81, 0-1608	653.8 ± 984.0, 322, 0-5092	<0.001
Diffuse 2+ p53 staining	14 (9.5%)	42 (29.4%)	<0.001
P53 3+ positive cells (≥ 10 cells) OR 2+ Diffuse staining OR Any 3+ p53 positive glands OR 3+ p53 surface epithelium	(20) 13.7%	(74) 51.7%	<0.001

Table 3: Sensitivity and specificity of p53 as a marker of Barrett's Esophagus associated advanced neoplasia

p53 staining parameters	Cut off points	Non-progressor vs Progressor BE			
		Sensitivity	Specificity	PPV	NPV
P53 3+ positive epithelial cells	(≥ 10 cells)	44.1%	93.2%	86.3%	63.3%
3+ P53 positive gland	(≥ 1 gland)	30.8%	97.3%	91.7%	59.3%
P53 2+ positive epithelial cells	(≥ 150 cells)	69.9%	68.9%	68.5%	70.3%
Diffuse 2+ positivity		29.4%	90.5%	75.0%	57.0%
Surface epithelial positivity		8.4%	100%	100%	53.0%
P53 3+ positive cells (≥ 10) OR 2+ Diffuse staining OR 3+ p53 positive glands (≥ 1) OR 3+ p53 surface epithelium		51.7%	86.5%	78.7%	65.0%

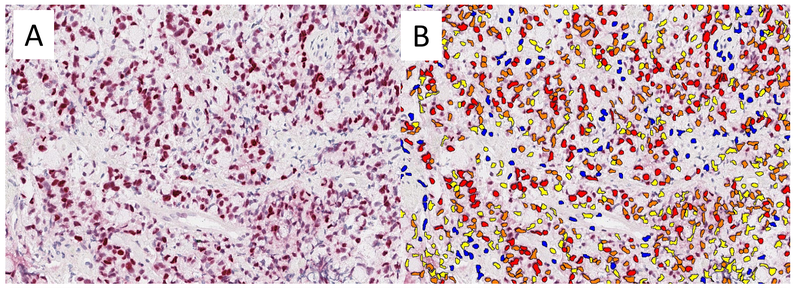

Figure 1: A. Representative image showing p53 positive cells, B. p53 Scoring on automated image analysis platform, 3+ Intensity (strong): Red, 2+ Intensity (moderate): Orange 1+ Intensity (weak): Yellow, Negative: Blue, (100x)

CONCLUSION

- Quantitative analysis of p53 immunohistochemistry in non-dysplastic BE biopsies can help identify patients at a high risk of advanced neoplasia.
- Patients with Barrett's esophagus showing strong reactivity for p53 in the glandular compartment should be placed in an accelerated screening program.