Cytokeratin Staining to Detect Residual Disease in Patients Undergoing Neoadjuvant Chemoradiation followed by Esophagectomy for Locally Advanced Esophageal Cancer

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Citation

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Abstract

Thirty percent of patients undergoing neoadjuvant chemoradiation therapy (neo-CRT) for locally advanced esophageal cancer achieve a complete pathologic response (CPR) based on standard Hematoxylin and Eosin staining (CPR-HE). There is controversy regarding the prognostic value of CPR-HE. Immunohistochemical staining may be useful to detect subtle microscopic residual disease in such patients. We undertook this present study to test this hypothesis. Eighteen consecutive patients who achieved CPR-HE were identified. Seven patients had positive cytokeratin staining; three had microscopic residual disease that was not identified on original hematoxylin-eosin sections. Three of the seven patients (43%) with positive cytokeratin staining had systemic relapse (60 % of patients with systemic relapse).Cytokeratin staining could be a useful adjunct to routine hemaoxylin-eosin staining to identify residual tumor in such patients. The prognostic significance of CPR-CK, however, needs to be validated in a large cohort.

Abbreviations used in alphabetical order- AC-adenocarcinnoma; AWD- advanced; CK- cytokeratin; CPR-complete pathological response; CPR-CK- complete pathologic response on cytokeratin staining; CPR-HE-complete pathologic response on hematoxylin and eosin staining; EC- esophageal cancer; HE- hematoxylin and eosin; IHC-immunohistochemistry; NA- not applicable; NED- no evidence of disease; neo-CRT- neoadjuvant chemoradiation therapy; PFS- progression free survival; OS-overall survival; RPCI- Roswell Park Cancer Institute; SCC-squamous cell carcinoma

INTRODUCTION

Esophageal cancer is the ninth most common malignancy in the world with an estimated annual incidence of 15,560 and an annual mortality of 13,940 in the United States in 2007 [1]. Even for localized esophageal cancer (T2-3, N1 or M1a), the 5-year survival rate is a meager 20 % [2,3]. The most favorable outcome is noted in patients without any evidence of residual cancer in the resected specimen (complete pathologic response-CPR) [4,5,6,7]. Neoadjuvant chemoradiation (neo-CRT) is commonly used to treat patients with localized carcinoma of the esophagus and has several theoretical advantages including a higher rate of margin negative resection (R0 resection), better patient tolerability compared to adjuvant therapy, early treatment of micrometastases and assessment of in vivo therapeutic response. Despite neo-CRT, overall long term prognosis remains dismal in certain patients. Hammond et al [7] showed that patients who demonstrate a CPR following neo-CRT have excellent long-term survival. In contrast another trial reported no statistically significant difference in overall survival or disease free survival between those who had a CPR versus no CPR after neo-CRT in a cohort of 118 patients [8]. This raises the possibility that there may be heterogeneity in population with CPR that may in part be explained by the inadequacy of traditional histopathological assessment by hematoxylin and eosin (HE) staining.

Another potential use of immunohistochemistry (IHC) may be to predict residual tumor in patients undergoing neo-CRT to detect residual tumor prior to surgery. Thirty percent of such patients achieve CPR and may not need to undergo esophagectomy. The positive predictive value of post-chemoradiation esophageal biopsy was found to be 92%, negative predictive value 23%, sensitivity 23% and specificity 92%, by Yang et al in a cohort of 52 patients [9]. These numbers may be improved further by use of IHC.

The use of immunochemistry to aid the detection of residual disease is an area of special interest [10,11,12,13,14]. The cytokeratins (CK) are intermediate filaments found in the cytoplasm of epithelial cells. Immunohistochemical staining directed against cytokeratins may be used to identify microscopic residual disease that was missed on traditional HE staining in esophageal cancer. We hypothesize that accuracy of the 'CPR' after neo-CRT can be aided by the use of IHC. Based on this hypothesis, we undertook this pilot study to assess the usefulness of CK staining to detect residual disease in the resected esophagectomy specimen. Correlation with clinical outcome was done as a secondary and purely exploratory goal.

METHODS

PATIENT SELECTION

Medical records of patients with locally advanced esophageal cancer treated at Roswell Park Cancer Institute (RPCI) between January 1, 1996 and December 31, 2006 with neo-CRT followed by esophagectomy were reviewed and analyzed retrospectively. All who attained CPR-HE after neo-CRT (n=18) were studied further with CK staining to detect any microscopic residual disease to determine a CPR-CK for the purpose of our pilot study. Patients were followed till the last available follow up in the medical records or till they progressed, whichever was earlier.

STAINING

Standard HE staining was performed on pathology specimens (which included the entire gastro-esophageal junction) from these 18 patients and reviewed by a surgical pathologist with special interest in gastro-intestinal malignancies (C.L.). One tissue block per patient with representative tissue was selected for immunohistochemical staining after review of all available blocks. In this study, the same block as examined by HE was used for immunohistochemical stains. To perform immunohistochemical stains, deeper sections were taken. The formalin fixed, paraffin wax embedded sections were dewaxed. To enhance immunostaining, these sections were subjected to enzyme digestion for epitope retrieval using protease 1 (Ventana Medical Systems, Tucson, Arizona, USA). Endogenous peroxidase and non-specific binding were blocked before addition of the primary antibody. Sections were then immunostained using the DAKO autostainer (Carpentaria California) immunohistochemistry system; using antibodies to CK5/6 for SCC (DAKO, 1:100 for 30 minutes) and AE1/3 for AC (DAKO, 1:100 for 30 minutes). Monoclonal mouse (IgG) antihuman antibodies to specific cytokeratins were used. The appropriate biotinylated secondary antibodies were detected using an iVIEW DAB (3, 3-diaminobenzidine tetrahydrochloride) conjugate. The complex was visualised with DAKO Mouse Envision Kit.

Patients with no evidence of CK positive residual cancer cells were defined as CPR-CK. ‘True CPR' was defined as HE negative and CK negative for evidence of residual disease (HE- and CK-).

STATISTICAL ANALYSIS

To study the statistical relationship between pairs of nominal variables, Fisher's exact test was used. The exact Wilcoxon test was used to study the statistical relationship between nominal and ordinal variables. A 0.05 nominal significance level was used in all testing. Survival analysis was done for purely exploratory purpose. Overall survival (OS) and progression free survival (PFS) distributions were calculated by the Kaplan-Meier method [15]. Due to small sample sizes, a permutation test based on the log-rank statistic was used to test the significance with respect to the survival distributions [16]. All statistical analysis were done as a secondary goal and on a purely exploratory basis.

RESULTS

PATIENT DEMOGRAPHICS

Eighteen patients were found to have CPR-HE after neo-CRT followed by Ivor-Lewis esophagectomy for locally advanced EC between January 1, 1996 and December 31, 2006 at RPCI. Sixteen patients had AC and two had SCC. All patients were males. Median age at diagnosis was 58.5 years (range- 50 to 74 years) while mean age was 59.4 years. Patients received protracted infusion of 5-fluorouracil with oxaliplatin or cisplatin along with a total of 50.4 Gy of radiation in the neoadjuvant setting during this time period. One patient received 3 cycles of MIMIC (mitomycin, ifosamide and cisplatin) at an outside facility prior to transfer of care at RPCI. Two other patients received capecitabine along with oxaliplatin while another two received cisplatin along with irinotecan as neoadjuvant therapy. Table 1 summarizes baseline patient demographics.

Figure 1

Table 1: Patient Demographics and Treatment Received

Patient number	Age	Sex	Histology	Clinical stage	Tumor location	Neoadjuvant chemotherapy	Lymph node status after surgery (positive for residual disease/number removed)
1	50	M	Poor AC	II	42 cm	MIMIC	0/7
2	59	M	Poor AC	IVa	37 cm	5-FU and cisplatin	0/18
3	74	M	Mod AC	IIa	33 cm	5-FU and OXA	0/5
4	50	M	Poor AC	III	35 cm	5-FU and OXA	0/22
5	61	M	Mod AC	IVa	30 cm	5-FU and cisplatin	0/8
6	58	M	Poor AC	III	35 cm	5-FU and CIS	0/15
7	67	M	Poor AC*	II	35 cm	5-FU and cisplatin	0/11
8	56	M	Well AC	III	34 cm	5-FU and OXA	0/7
9	55	M	Mod AC	III	34 cm	5-FU and OXA	0/33
10	53	M	Mod AC	III	34 cm	5-FU and OXA	0/14
11	71	M	Mod AC	IIb	38 cm	5-FU and OXA	0/19
12	53	M	Mod AC	III	25 cm	5-FU and OXA	0/16
13	67	M	Mod AC	III	31	XELOX	0/9
14	54	M	Poor AC*	IIb	33 cm	CIS and CPT-11	0/14
15	66	M	SCC	III	19 cm	CIS and CPT-11	0/8
16	50	M	Mod AC	III	34 cm	XELOX	0/19
17	62	M	Mod AC	IIa	33 cm	CIS and CPT-11	0/28
18	63	M	Poor AC*	III	Distal 1-2cm	CIS and CPT-11	0/20

Asterisk * denotes patients with signet ring cell feature on pathology. Abbreviations used: AC- adenocarcinoma; Poor AC- poorly differentiated AC; mod AC- moderately differentiated AC.

HISTOPATHOLOGY

Results of CK staining of the pathological slides of esophagectomy specimens are shown in Table 2.

Figure 2

Table 2: Staining pattern and survival data (based on last follow up at RPCI).

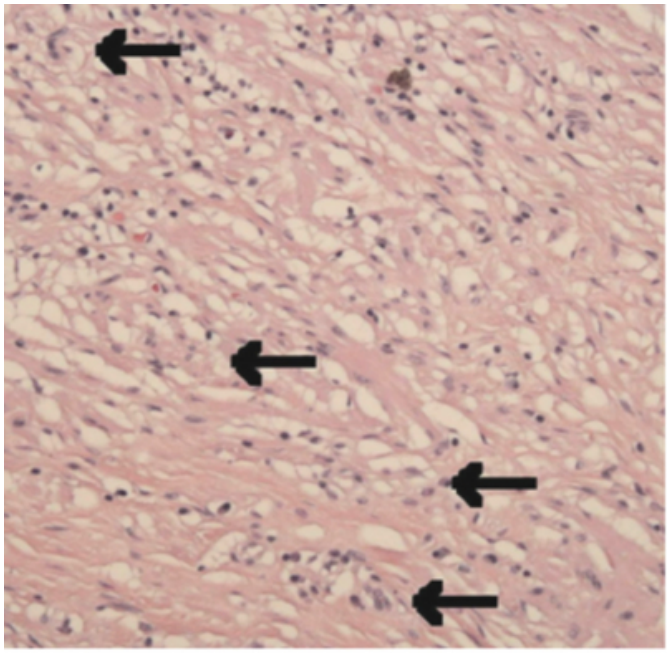
Patient number	CK staining	HE-repeat	Disease Status On Last follow up	Site of recurrence	Survival* (months)	PFS (months)
1	-	-	NED	NA	107.2	107.2
2	-	-	NED	NA	76.3	76.3
3	+	+	NED	NA	50.9	50.9
4	-	-	NED	NA	62.1	62.1
5	+	+	AWD	Liver	16.7	16.7
6	+	-	NED	NA	46.4	46.4
7	-	-	AWD	Mediastinal lymph nodes	10.4	10.0
8	-	-	NED	NA	23.2	23.2
9	+	-	AWD	Lungs	20.4	11.7
10	+	+	NED	NA	37	37
11	-	-	AWD	Liver	9.4	7.4
12	-	-	NED	NA	7.8	7.8
13	-	-	NED	NA	24	24
14	+	-	NED	NA	23	23
15	-	-	NED	NA	18.7	18.7
16	-	-	NED	NA	16.3	16.3
17	-	-	NED	NA	12.5	12.5
18	+	-	AWD	Peritoneum	11.9	11.5

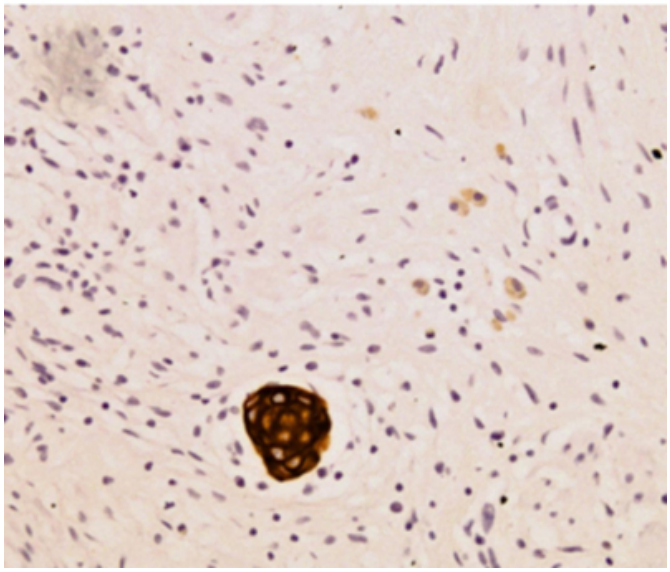
All patients had CPR-HE. Abbreviations used- NA- not applicable, NED- no evidence of disease, AWD- advanced.

Seven patients (39 %) had CK+ staining. On second look HE examination of these cases, 3 out of the seven (43 %) were found to have malignant cells. CK staining aided in detection of subtle residual disease missed on original HE in these patients (Figure 1).

Figure 3

Figure 1: Resection specimens deemed to have a complete pathological response were stained with either cytokeratin AE 1/3 (for cases with a biopsy diagnosis of adenocarcinoma) or cytokeratin 5/6 (for cases with a biopsy diagnosis of squamous cell carcinoma). A 100X magnification of a hematoxylin and eosin stained section with arrows pointing to groups of atypical cells. These cells are not diagnostic of residual carcinoma by H&E stain alone. A 100X magnification of a cytokeratin AE 1/3 stain of the same area shown in panel A, which demonstrates residual adenocarcinoma.





CLINICAL OUTCOMES

After a median follow up of 21.7 months (range 7.8 to 107.2 months), there were 5 recurrences (patients # 6, 7, 9, 12, 13). Four had distant metastasis and one had nodal recurrence (#7). Two patients died of advanced disease. The remaining 16 patients were still alive with follow-up ranging from 7.8 to 107.2 months. The estimated PFS for all 18 patients was 69.7% at 12 months.

CK+ GROUP

Seven patients had residual disease detected on CK staining (# 3, 5, 6, 9, 10, 14 and 18). All had an initial diagnosis of AC. Median age of this group was 58 years (Mean = 59.7 years, range- 53 to 74 years). Of the seven CK+ patients, three (43%) had systemic relapse. This represented 60 % of the patients with systemic relapse (# 6, 9 and 13). The other four CK+ patients remained NED at 37, 51, 46, and 23 months, respectively. Median PFS was at least 11.7 months in the CK+ group.

CK- GROUP

The remaining 11 patients had negative CK staining. Second look HE did not show any evidence of residual disease in this subset. Median age of this population was 59 years (Mean= 59.2 years, range-50 to 71 years). All but two patients had NED status at last follow-up. One patient progressed at 10.4 months with nodal recurrence (# 7) and the other had systemic disease (# 11). Median PFS was at least 10 months in the CK- group (sample size inadequate for statistical analysis).

There was no significant difference in the PFS and OS distributions between the CK+ and CK- groups. There was no statistically significant association between CK staining and clinical stage, age, histology, or lymph node status. On further analyses, there was no difference between patients with CK-/second look HE + (residual disease on repeat HE staining), CK-/HE- (true CPR) and CK+/HE- (patients with residual disease on IHC but not on repeat HE). Again, the study is limited by small sample size.

DISCUSSION

Our study of 18 patients who achieved CPR-HE showed that 39 % of these patients had positive CK staining for residual tumor cells in resected esophagectomy specimen. Three of the 7 patients actually had microscopic residual disease on a second look light microscopic examination of the original HE slides. This suggests that CK staining can be a sensitive adjunct to routine light microscopic examination to detect residual disease. Three of the 7 patients who had positive CK staining had systemic relapse. Though theoretically, there may be survival benefit in patients with ‘true CPR', our

present study failed to show that. This may be because of the small sample size.

Various studies looking at IHC staining to look for residual tumor in lymph nodes have shown a trend towards better disease free survival in patients with negative IHC staining. Table 3 depicts studies utilizing immunohistochemical staining to detect microscopic residual disease in patients originally thought to have CPR on traditional HE. These trials looked at IHC staining of the lymph nodes. The percentage of patients detected to be CK+ for AE1/3 in our study (39 %) was similar to that seen in these studies (ranging from 30-40%). There appears to be a trend towards better disease free survival in patients with negative IHC staining in lymph nodes.

Figure 4

Table 3: Use of immunohistochemistry to aid in detection of residual disease in resected lymph nodes in patients with esophageal cancer

Study	IHC	Number of patients (HE negative)	IHC positive	5-year relapse free Survival (IHC- vs IHC +)	P value
Izbicki et al[10]	Ber-EP4	68	42	68% vs 25%(OS)	<0.001
Waterman et al[11]	AE1 and CAM 5.2	20*	14	OS-77% vs 14%	0.03
Komukai et al[12]	AE1/AE3	37	14	91% vs 50%	0.042
Heeren et al[13]	AE1/AE3	60	18	Not attained vs 36 months**	<0.001
Sato et al[14]	AE1/AE3	50	20	86 % vs 73%	0.34

*Patients with less than 10% nodal involvement by traditional HE

** Median disease-free survival

Izbicki et al [10], reported similar findings using Ber-EP4 antibody (directed against two glycopolypeptides on the surface and in the cytoplasm of most epithelial cells).There was a statistically significant difference in overall survival in Ber-EP4 positive versus negative cases (68 % versus 25%; p<0.001). In contrast to the studies listed in table 3, our investigation was geared towards CK staining in the resected primary tumor and not the involved lymph nodes. There is paucity of data for the former to compare our results to. However, it is clear from these trials that traditional HE staining alone may miss residual disease and pathological staging based on standard HE alone is inadequate.

These data suggest that immunohistochemical assessment of resected esophageal specimen can be used to refine the staging system for esophageal cancer, particularly after neo-CRT. Adjuvant therapy may be beneficial for the subset of patients with CK+ residual disease [17,18]. IHC may also be used to increase sensitivity of post-chemoradiation biopsy to select patients who do not require esophagectomy [9]. The prognostic significance of CK staining, however, needs to be validated in a future trial with a larger patient cohort.

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