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ISOLATED TUMOUR CELLS IN OESOPHAGEAL CANCER: APPLYING THE SENTINEL LYMPH NODE CONCEPT

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THESIS ABSTRACT

INTRODUCTION: Accurate staging of oesophageal cancer is critical in predicting prognosis and tailoring therapy. However, the current TNM based staging system is suboptimal because it combines patients with very different outcomes into each disease stage. Our aims are to identify pathological factors or molecular markers that can significantly improve the accuracy of the oesophageal cancer staging system by both a retrospective database review as well as detailed analysis of oesophageal cancer specimens. The benefit of incorporating sentinel lymph node biopsy with oesophageal resection will also be determined.

METHODS: 240 patients (mean age, 62 yrs) were identified from an Oesophageal Cancer database between 1997 and 2007. We re-examined all pathology slides from the original resection to identify significant prognostic factors, and to determine suitable paraffin blocks for the remaining parts of the study. Tissue microarrays were constructed from 89 paraffin blocks for HER2 gene amplification by silver-enhanced *in situ* hybridization (SISH). Incidence of HER2 positivity, and correlation to clinicopathological variables were determined. Of the original 240 patients, we identified 119 patients who were classified as node-negative. Additional sections with immunohistochemistry (IHC) staining were performed on the relevant paraffin blocks. The yield of occult tumour deposits was determined along with their prognostic significance. Thirty-one consecutive oesophageal cancer patients underwent resection and sentinel lymph node retrieval. Endoscopic peritumoural injection of ^{99m}Tc antimony colloid was performed, and sentinel lymph nodes were identified and sent off separately for serial sections and IHC.

RESULTS: The 5-year overall survival rate was 36% (median, 24 months). Only histological grade and refined nodal status were found to be independent prognostic factors. True HER2 gene amplification was detected in 14 (16%) oesophageal cancer specimens. No significant associations were found among gene amplification, clinicopathological factors, or survival. Of 119 node negative patients, 31 patients (26%) were found to have occult tumour deposits with serial sections and IHC. Five-year survival rates were 60% for patients who remained node-negative, 33% for patients with isolated tumor cells, 40% for patients with micrometastases, and 0 for the patient with a metastasis (P=0.02). At least one sentinel lymph node (median, 3) was identified in 29 of 31 patients (success rate, 94%). In 28 of 29 patients, the sentinel lymph node accurately predicted findings in non-sentinel nodes (accuracy, 96%).

CONCLUSIONS: A staging model in oesophageal cancer which incorporates refined nodal status and histological grade appears to be more accurate than the current TNM staging system. While molecular targeting may be possible for approximately 16% of oesophageal adenocarcinoma patients, HER2 oncogene amplification was not associated with any affect on survival in this study. Almost one third of all node negative patients had occult tumour deposits in their nodes that were missed on their original pathology. Surprisingly, even those with isolated tumour cells had a signficiantly worse prognosis than those without. Sentinel lymph node biopsy seems to be feasible and accurate in predicting overall nodal status. It improves staging accuracy and should therefore become standard of care in the surgical treatment of patients with oesophageal cancer.

THESIS DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to ***Sarah K Thompson*** and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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PUBLISHED WORKS

Thompson SK, Ruszkiewicz AR, Jamieson GG, Esterman A, Wijnhoven BPL, Devitt PG, Watson DI. Improving the accuracy of TNM staging in esophageal cancer: a pathological review of resected specimens. *Annals of Surgical Oncology* 2008; 15(12): 3447-3458.

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Thompson SK, Sullivan TR, Davies R, Ruszkiewicz AR. HER-2/*neu* gene amplification in esophageal adenocarcinoma and its influence on survival. *Annals of Surgical Oncology* 2011; 18(7): 2010-2017.

- *Published by Springer Science + Business Media, LLC © 2011 The Society of Surgical Oncology, Inc. The original publication, DOI http://dx.doi.org/10.1245/s10434-011-1554-1, is available at www.springer.com.*

Thompson SK, Ruszkiewicz AR, Jamieson GG, Sullivan TR, Devitt PG. Isolated tumor cells in esophageal cancer: implications for the surgeon and the pathologist. *Annals of Surgery* 2010; 252(2); 299-306.

- *Published by Wolters Kluwer Health © 2010 by Lippincott Williams & Wilkins. The original publication, DOI http://dx.doi.org/10.1097/SLA.0b013e3181e61e15, is available at www.lww.com.*

Thompson SK, Bartholomeusz D, Devitt PG, Lamb PJ, Ruszkiewicz AR, Jamieson GG. Feasibility study of sentinel lymph node biopsy in esophageal cancer with conservative lymphadenectomy. *Surgical Endoscopy* 2011; 25(3); 817-825.

- *Published by Springer New York © Springer Science + Business Media LLC 2011. The original publication, DOI: http://dx.doi.org/10.1007/s00464-010-1265-x, is available at www.springerlink.com.*

Thompson SK, Bartholomeusz D, Jamieson GG. Sentinel lymph node biopsy in esophageal cancer: should it be standard of care? *Journal of Gastrointestinal Surgery* 2011; Accepted Paper.

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