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# SENTINEL LYMPH NODE ASSESSMENT IN OESOPHAGEAL CANCER: CREATION OF A MULTIMODALITY TRACER

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# GLOSSARY

| | |
|---|---|
| AJCC | American Joint Committee on Cancer |
| ATC | antimony trisulphide colloid |
| CT | computed tomography |
| DT | dye technique |
| EMR | endoscopic mucosal resection |
| ESD | endoscopic submucosal dissection |
| ESR | erythrocyte sedimentation rate |
| EUS | Endoscopic ultrasound |
| FDA | Food and Drug Administration |
| FNA | fine needle aspiration |
| FOV | field-of-view |
| FWHM | full width half maximum |
| Gd | gadolinium |
| GOJ | gastroesophageal junction |
| GORD | gastro-oesophageal reflux disease |
| H&E | haematoxylin and eosin |
| HCl | hydrogen chloride |
| ICG | indocyanine green |
| ID | injected dose |
| IHC | immunohistochemistry |
| ITC | isolated tumour cells |
| ITLC-SG | instant thin layer chromatography silica-gel |
| MRI | magnetic resonance imaging |
| MTJ | magnetic tunnelling junction |
| NIR | near infrared |
| NPS | nonpoint-source |
| PCB | printed circuit board |
| PET | Positron emission tomography |
| PS | point-source |

| | |
|---|---|
| QC | quality control |
| RCT | radiocolloid technique |
| RF | random forest |
| RT-PCR | reverse-transcriptase polymerase chain reaction |
| SLN | sentinel lymph node |
| SNR | signal to noise ratio |
| SPIONs | superparamagnetic iron oxide nanoparticles |
| TC | technetium |
| TNM | tumour node metastasis |
| UICC | Union for International Cancer Control |
| WECC | worldwide esophageal cancer collaboration |

# ABSTRACT


## Introduction

Early oesophageal cancer can now be treated endoscopically; however, more accurate lymph node assessment is required. The sentinel lymph node (SLN) is defined as the first lymph node to which the primary tumour drains. This node should reflect the status (i.e. benign or malignant) of the entire lymph node basin. Our aims were to identify current difficulties that limit the acceptance of the SLN concept in oesophageal cancer, as well as to create a multimodal (magnetic resonance imaging [MRI] and gamma) tracer, to enable more detailed perioperative SLN assessment.

## Methods

A literature review was undertaken, targeting studies assessing oesophageal cancer and SLN. MEDLINE, the Cochrane Database of Systematic Review, as well as PUBMED, were consulted. The keywords and medical subject headings (MeSH) used were '(o)esophageal cancer', '(o)esophageal adenocarcinoma', '(o)esophageal squamous cell carcinoma' and 'SLN' used in combination with AND or OR. Only studies in English were considered. $^{99m}$Tc-labelled magnetic nanoparticle formulations were then investigated for further use as a multimodality SLN contrast agent. Radiolabelling of the dextran-coated magnetic nanoparticles was undertaken, and instant thin layer chromatography developed to assess efficiency. Bio-

distribution was determined after intravenous and subdermal injection in the tails of Sprague-Dawley rats. Lymphoscintigraphy was performed on the subdermally injected rats to assess lymphatic mapping.

Following this, the multimodality SLN tracer was injected into the oesophagus of four swine. MRI images were then acquired, and then sentinel nodes were further assessed intraoperatively with a gamma probe. Bio-distribution was then assessed, comparing $^{99m}$Tc-antimony trisulphide colloid (ATC) and $^{99m}$Tc-superparamagnetic iron oxide nanoparticles (SPIONs). Lymph nodes and reticuloendothelial organs were then harvested and counted to determine the percentage of injected dose (%ID).

## Results

The literature review demonstrated at least four issues limiting the use of the SLN concept in oesophageal cancer. These included: timing of tracer – different radiocolloids overseas, with different rates of flow; the chest cavity – difficulty of access post-injection; blue dye – not useful, with proven false-negative rate; and shine-through effect.

The multimodality tracer formulation was optimised, with labelling efficiency confirmed at >99%. In the Sprague-Dawley rats, the tracer was injected subdermally, with lymphoscintigraphy demonstrating excellent delineation of the sentinel nodes. The reticuloendothelial organs were then removed and activity measured, with the liver showing 3.2% highest mean activity, followed by the kidneys with 2%, the spleen at 0.3% and the lungs at 0.1%.

The gamma probe detected all nodes identified on MRI imaging of the four swine injected with $^{99m}$Tc-SPIONs. Radiolabelling efficiency was >98%. $^{99m}$Tc-ATC and $^{99m}$Tc-SPIONs were taken up by swine liver and lungs with similar percentage ID values, and there was 7% ID of $^{99m}$Tc-SPIONs by the kidneys, compared to <1% ID of $^{99m}$Tc-ATC in the same organs.

## Conclusion

There are numerous issues that require evaluation and agreement in the international community to ensure greater use of the SLN concept in oesophageal cancer.

A multimodality, $^{99m}$Tc-SPIONs, tracer was validated using both MRI imaging and a gamma probe. This tracer requires further assessment, as it could allow pre-operative assessment of sentinel lymph nodes in early 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 ***George L Balalis*** 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide.

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# PUBLISHED WORKS AND PRESENTATIONS

## Publications

Balalis GL, Thompson SK. Sentinel lymph node biopsy in esophageal cancer: an essential step towards individualized care. *Annals of Surgical Innovation and Research* 2014, 8:2. Published by Biomed Central Ltd. The original publication, http://www.asir-journal.com/content/8/1/2 doi:10.1186/1750-1164-8-2

Balalis GL, Cousins A, Tsopelas C, Devitt P, Madigan D, Bartholomeusz D, Thierry B, Thompson SK. A multimodality $^{99m}$Tc-labelled iron oxide nanotracer to improve sentinel node identification in early oesophageal cancer. *Currently in review*

## Publications related to this thesis

Cousins A, Balalis GL, Thompson SK, Forero Morales D, Mohtar A, Wedding AB, Thierry B. Novel Handheld Magnetometer Probe Based on Magnetic Tunnelling Junction Sensors for Intraoperative Sentinel Lymph Node Identification. *Sci. Rep. 5, 10842; doi: 10.1038/srep10842 (2015)*

Cousins A, Balalis GL, Tsopelas C, Thompson SK, Bartholomeusz D, Wedding BA, Thierry B. Radiolabelling of $^{99m}$Tc-labelled iron oxide for use in sentinel node detection. *Currently in review*

## Oral presentations

Balalis GL. A novel multimodality nanotracer to improve sentinel node identification in early oesophageal cancer. *Australian and New Zealand Gastric and Oesophageal Surgeons Association* (*ANZGSOA) Annual Meeting 2014, Queenstown, New Zealand.*

Balalis GL. MRI imaging with nanoparticles to assess lymphatic drainage of the distal oesophagus in a swine model. *Royal Australasian College of Surgeons Annual Scientific Congress, 2014, Singapore*.

Balalis GL. Multimodality Nanotracer For Assessment Of Sentinel Lymph Nodes In Oesophageal Cancer. *Board in General Surgery (South Australia) Registrar's Paper Day, 2015, Adelaide, South Australia.*

## Oral presentations related to this thesis

Cousins A, Balalis GL, Wedding BA, Thompson SK, Thierry B. Oesophageal Sentinel Lymph Node Identification in a Swine Model using Magnetic Lymphotropic Contrast Agents. 2014 *International Conference on Nanoscience and Nanotechnology (ICONN), Adelaide, South Australia.*

## Poster presentations

Balalis GL. Reproducibility of sentinel lymph node drainage pathways in the lower oesophagus using a novel multimodality tracer. *5th Asia Pacific Gastroesophageal Cancer Congress (APGCC), Brisbane, Australia, 2015.*

Balalis GL. MRI imaging with nanoparticles to assess lymphatic drainage of the distal oesophagus. 2014 Florey International Postgraduate Research Conference, *Adelaide, Australia.*

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