Lymphatic Mapping/ Lymph Node Biopsy Update

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Learning Objectives

• Define/describe lymphatic mapping/sentinel lymph node biopsy
• List five disease states where lymphatic mapping/sentinel lymph node biopsy has proven useful
• Compare/contrast lymphatic mapping/sentinel lymph node biopsy procedures with conventional surgical approaches for treating malignant melanoma
• List the radiopharmaceuticals used for lymphatic mapping/sentinel lymph node biopsy and compare/contrast their different properties
• Describe the methods for preparing Tc-99m sulfur colloid for use in lymphatic mapping/sentinel lymph node biopsy procedures
• Describe the nuclear medicine clinical procedure involving lymphatic mapping/sentinel lymph node biopsy
• Discuss developments in radiopharmaceuticals and instrumentation associated with lymphatic mapping/sentinel lymph node biopsy

Self-Assessment Questions

1. Which of the following IS NOT a function of the lymphatic system?
   b. Maintenance of fluid balance.
   c. Removal of invasive foreign materials such as microorganisms.
   d. Secretion of clotting factors in response to a cut.

2. Which of the following types of solid tumors represents the most likely candidates for the sentinel lymph node biopsy procedure?
   a. breast cancer
   b. colorectal cancer
   c. prostate cancer
   d. lung cancer

3. Which of the following clinical procedures is used for the final determination of metastatic spread into lymph nodes?
   a. Intraoperative lymphatic mapping of radioactivity in lymph nodes
   b. Appearance of lymph nodes on lymphoscintigraphy images
   c. Appearance of blue color in lymph nodes at sentinel lymph node biopsy
   d. Pathological examination of excised lymph nodes
Self-Assessment Questions

4. Which of the following is the only FDA-approved drug for visualization of lymphatic vessels and lymph nodes during surgery?
   a. isosulfan blue
   b. methylene blue
   c. indocyanine green
   d. lidocaine hydrochlo ride for injection

5. Which of the following is critical when considering the patient dosage of technetium Tc-99m tilmanocept?
   a. quantity (µg) of tilmanocept
   b. volume (mL) of injection
   c. quantity (mCi) of radioactivity
   d. all the above

The Lymph System

Network of fluid, nodes and nodules, organs, ducts, glands and vessels that continuously and aggressively cleanse the body of waste matter. Except for cartilage, nails and hair, the entire body is bathed in lymph.

Lymph Node Metastasis is Common

- Subcapsular space contains dendritic cells
- Outer cortex - filled with lymph follicles
  - Outer edge of follicle contains more T cells
  - Inner germinal center is the site of B-cell proliferation
- Inner medulla - Medullary cords of lymphocytes, macrophages, plasma cells (activated B cells)
Sentinel Lymph Node Biopsy
• Gold Standard for evaluation of LN mets?
• Complete LN dissection
  • Only 1/3 of patients have metastatic disease to the LN's and will benefit from surgery
  • Morbidity/post-op complications are significant
  • Sensitivity of pathologic evaluation
• Sentinel Lymph Node Biopsy
  • Accurately predicts status of regional LN's
  • Spares 2/3's of patients unnecessary surgery
  • Increased sensitivity of pathologic evaluation


In most cases, more is better…

Post-Op Lymphedema
In the case of lymph node removal, less is better....... as long as it is the correct lymph node.
Lymph Node Biopsy: The Need

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  • Increased sensitivity of pathologic evaluation


Lymph Node Biopsy Indications

• Primary and Secondary Lymphedema
  • Evaluation of lymphatic patency

• Evaluation of Lymph Node Metastasis
  • Melanoma
  • Breast Cancer
  • Metastasis in regional lymph nodes decreases 5 yr survival by 30-40% in melanoma, breast cancer and colon cancer.


Lymph Node Biopsy Clinical Significance

• Sentinel Lymph Node(s)-first lymph node(s) draining from the tumor.

• Evaluation of Lymph Node Metastasis
  • Less than 2% of histologically negative SLN subsequently develop metastatic disease.
  • Metastasis in regional lymph nodes decreases 5 yr survival by 30-40%

Sentinel Node Mapping: Node Selection is Critical

Lymph Nodes of the Upper Limb and Breast
1. Cubital axillary lymph nodes
2. Apical axillary
3 & 4. Lateral (surface) axillary
5. Central axillary
6. Brachial axillary
7. Interpectoral
8. Paramammary
9. Parasternal (internal mammary)

Radioguided SLN Biopsy
SLN concept first described by Gould in parotid gland carcinoma in 1960.
Krag et al first reported on the use of radioguided SLN biopsy in breast cancer patients in 1993. Over 3,500 publications involving SLN and breast cancer patients have appeared in the literature since then.
Morton et al first reported on the use of radioguided SLN biopsy in cutaneous malignant melanoma in 1992. Since then, there have been nearly 4,000 articles associated with melanoma and SLN biopsy.

Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomized phase 3 trial
The Lancet Oncology, Early Online Publication, 21 September 2010
doi:10.1016/S1470-2045(10)70207-2 Cite or Link Using DOI

- 5,611 early stage breast cancer patients with no indication the disease had spread to lymph nodes on preliminary work-up
- Half underwent axillary L.N. dissections and half only SLN biopsy with other treatments (radiation, chemotherapy, etc) based on the findings.
- Patients were followed for eight years with no difference in survival rates.
The Gamma Detection System

- Intended Use – medical device for detecting and quantifying gamma radiation
- Indications – used externally and intraoperatively to detect radioactive emissions from body tissues or organs where radiopharmaceuticals localize

RadioNUCLIDES used in surgery

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Physical half-life</th>
<th>Principal gamma photon emissions</th>
<th>Percent photon yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt-57 (57Co)</td>
<td>271.8 days</td>
<td>122, 136 km</td>
<td>85.6, 10.6%</td>
</tr>
<tr>
<td>Fluorine-18 (18F)</td>
<td>110 minutes</td>
<td>511 km*</td>
<td>200%</td>
</tr>
<tr>
<td>Gallium-67 (67Ga)</td>
<td>78.5 hours (3.26 days)</td>
<td>171, 207 km</td>
<td>90.5, 9.5%</td>
</tr>
<tr>
<td>Indium-111 (111In)</td>
<td>72.2 hours</td>
<td>188 km</td>
<td>92.6%</td>
</tr>
<tr>
<td>Iodine-123 (123I)</td>
<td>58.3 hours (2.4 days)</td>
<td>364 km</td>
<td>89.8%</td>
</tr>
<tr>
<td>Iodine-124 (124I)</td>
<td>2.5 days (1.5 days)</td>
<td>364 km</td>
<td>83.5%</td>
</tr>
<tr>
<td>Iodine-125 (125I)</td>
<td>100.3 hours (4.18 days)</td>
<td>364 km</td>
<td>83.1%</td>
</tr>
<tr>
<td>Iodine-131 (131I)</td>
<td>13.2 hours</td>
<td>159 keV</td>
<td>83.4%</td>
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<tr>
<td>Technetium-99m (99mTc)</td>
<td>2.02 hours</td>
<td>140, 142 keV</td>
<td>89.9, 11.1%</td>
</tr>
<tr>
<td>Thallium-201 (201Tl)</td>
<td>73.0 hours (3.33 days)</td>
<td>159 keV</td>
<td>89.3, 10.6%</td>
</tr>
</tbody>
</table>

* The 511 keV gamma photons are generated from positron-electron annihilation.


Radioactive Compounds for Radioguided SLN Biopsy

- Tc-99m agents -sulfur colloid -nanocolloid -human albumin -tin colloid -dextran -hydroxyethyl starch -stannous phytate -antimony trisulfide -blue liposomes -tilmanocept

Early imaging

Design Features

- Radiation Detector
  - Scintillation detectors (NaI, CsI, LSO, BGO)
  - Semiconductor detectors (CdTe, CZT, HgI2)
- Display Unit
  - System controls
  - Scaler
  - Sound assembly

Probe Assembly
Intraoperative Applications

- Sentinel lymph node identification
  - breast, melanoma, head & neck, prostate, gastrointestinal, oral, genitourinary cancers
- Radioguided surgery (over 50 clinical applications identified)
  - RIGS
  - Minimally invasive parathyroidectomy
  - Evaluation of ischemic bowel
  - Localization/identification of ureters
  - Localization of osteoid osteomas
  - Evaluation of anastomosis blood flow

Intraoperative Lymphatic Mapping

ILM Protocol

- Radioactive imaging agent is injected at tumor site
- Agent follows drainage path of tumor to nearest lymph node(s)
- Draining lymph node(s) imaged with gamma detection device and/or gamma camera in nuclear medicine
- Lymph nodes show signs of malignancy
  - Physician removes additional regional lymph nodes
- Lymph nodes show NO signs of malignancy
  - Remaining regional lymph nodes are not removed

Lymphatic Mapping/SLN Biopsy

Clinical Incidence

150,000 patients undergo radioguided sentinel lymph node biopsy procedures each year in the U.S.

-Millennium Research Group
Technetium Tc-99m Sulfur Colloid

Prepared according to the package insert, the average particle size range reported to be 0.3-0.35 μm

Approximately 5% greater than 10 μm


Technetium-99m Sulfur Colloid for Lymphoscintigraphy: Compounding Parameters

Pharmacokinetics

- Particle Size Considerations
  - Lymphatic Uptake
    - Interstitial diffusion from the site of injection
  - Nodal Retention
    - Simple filtration by nodal meshwork
    - Macrophage endocytosis
- Ideal Size: 100-300nm (0.1-0.3μm)*

* Majority of published reports on clinical experience.
Preparation of Filtered Tc-99m Sulfur Colloid

• Heat 80mCi (2mL, old) Na\textsubscript{99m}TcO\textsubscript{4} + Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} + HCl for 3 minutes, cool for 3 minutes
• Buffered to pH 4.5 to 7.5 with Vial B
• Remove 15mCi/1mL q.s. to 3mL with NSS
• Filter through a 0.22 \(\mu\text{m}\) sterile filter into a sterile, pyrogen-free, empty vial.
• Provides 6-10mCi/3mL (7-12% recovery)

Technetium Tc-99m Sulfur Colloid
Clinical Experience

• 50+ years of clinical use in nuclear medicine for evaluating diseases of the reticuloendothelial and gastrointestinal systems
• An understanding of the physico-chemical properties and biodistribution/kinetics after interstitial administration of SC led to acceptance of the off-label use of this radiopharmaceutical for ILM procedures.
• An extensive collection of clinical reports on the safe use of SC for lymphatic mapping led the FDA to approve the added indication for localization of lymph nodes draining a primary tumor in patients with breast cancer and melanoma when used with a hand-held gamma counter.

Technetium Tc-99m Sulfur Colloid
Clinical Procedure

• Particulate Radiopharmaceutical
  – Lymphatic mapping (pre-surgical or patency)
  – Intraoperative gamma-probe guided sentinel node localization for biopsy
  – Dosage administration
    • 0.1 - 4.0 mCi in 1-4 syringes
    • 4 divided subcutaneous injections
    • 2-4 hours prior to surgery
**Lymphoseek® Navidea Biopharmaceuticals**
- A novel, synthetic, soluble solution of tilmanocept radiolabeled with Tc-99m
- Tilmanocept molecules are relatively small, 7nm in diameter, each one carries multiple units of mannose
- Selectively targets and binds to mannose binding receptor (CD206) proteins found on the surface of macrophages.
- Allows for timely and precise identification of tumor draining lymph nodes
- Designed to clear the injection site rapidly


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**Lymphoseek: A Timeline**
- 2001 – Lymphoseek synthesis first described.
- 2003 – First clinical studies using Lymphoseek
- March 13, 2013 – FDA approval of Lymphoseek
- June, 2014 – sNDA-1 approval
- October, 2014 – sNDA-2 approval

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**Lymphoseek Indications and Safety Information**

**Lymphoseek is a radioactive diagnostic agent indicated with or without imaging for:**
- Lymphatic mapping using a handheld gamma counter to locate lymph nodes draining a primary tumor site in patients with solid tumors for which this procedure is a component of intraoperative management.
- Guiding sentinel lymph node biopsy using a handheld gamma counter in patients with clinically node negative squamous cell carcinoma of the oral cavity, breast cancer or melanoma.

**Important Safety Information**
- In clinical trials with Lymphoseek, no serious hypersensitivity reactions were reported. However, local hypersensitivity reactions due to its chemical similarity to dextran. Serious hypersensitivity reactions have been associated with dextran and modified forms of dextran (such as iron dextran drugs).
- Prior to the administration of Lymphoseek, patients should be asked about previous hypersensitivity reactions to drugs, in particular dextran and modified forms of dextran. Resuscitation equipment and trained personnel should be available at the time of Lymphoseek administration, and patients observed for signs or symptoms of hypersensitivity following injection.
- Any radiation-emitting product may increase the risk for cancer. Adhere to dose recommendations and ensure safe handling to minimize the risk for excessive radiation exposure to patients or healthcare workers.

In clinical trials, no patients experienced serious adverse reactions and the most common adverse reactions were injection site irritation and/or pain (<1%).

FULL LYMPHOSEEK PRESCRIBING INFORMATION CAN BE FOUND AT: [www.lymphoseek.com](http://www.lymphoseek.com)
Technetium Tc-99m Tilmanocept

**Chemical name:** \(^{99m}\text{Tc}-\text{Diethylenetriaminepentaacetic acid-mannosyl-dextran} \) (Dextran10 core)

**Mr and Size:** ~18,000; ~7nm

**Target:** mannose binding receptors (CD206)

**Mechanism:** Ligand-receptor binding

**Method of detection:** *Preoperatively:* Gamma planar imaging, single-photon emission computed tomography (SPECT);

*Intraoperatively:* Hand-held gamma-detecting probe.


Tc-99m Tilmanocept

**Mechanism of Action**

- Selective binding to mannose receptor
  - Lymph node uptake/binding is specific
  - Binds to mannose binding receptor (CD206) proteins found on the surface of macrophages
  - Nodal Retention
- Rapid clearance from injection site
- Cleared slowly from lymph nodes (Teff = 1.75-3.05 hours)

- A pre-formed, 7nm synthetic molecule

Lymphoseek: Clinical Procedure

- Total injection volume and number of sites – physician preference
- Recommended dose is 18.5 MBq (0.5 mCi) radioactivity dose and 50 µg mass dose
- Recommended total injection volumes
  - 0.1mL in 1 syringe
  - 0.5mL in 1 or multiple syringes (0.1 or 0.25mL each)
  - 1mL in multiple syringes (0.2, 0.25 or 0.5mL each)
- Recommended injection routes include subcutaneous, intradermal, subareolar or peritumoral
- Recommended time from administration to lymphatic mapping is 15 minutes up to 15 hours post-injection

Vital Blue Dye Clinical Procedure

- Low Molecular Weight Blue Dyes
  - Intraoperative visual confirmation of lymphatic anatomy
  - Isosulfan Blue or Methylene Blue
    - 4 - 5 cc
    - 4 divided subcutaneous injections
    - Immediately prior to surgery
Lymphoscintigraphy of a Melanoma Patient with Tc 99m Tilmanocept – Day Before Surgery

Patient with dorsal midline melanoma.

Image reveals bilateral hot lymph nodes.

Preoperative imaging approximately one hour after injection:
- 2.0 mCi of labeled Tc 99m tilmanocept intradermally to the mid-line upper back;

The value of rapid complementary imaging is depicted here such that both the left and right axillae produced a hot spot.

In a case where such complimentary images were less efficient, one of the drainage paths may have been missed.
Clinical Procedure

- Low Molecular Weight Blue Dyes
  - Intraoperative visual confirmation of lymphatic anatomy
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Tilmanocept Clinical Studies

<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>Indications</th>
<th># Patients</th>
<th>Status</th>
<th>Primary Endpoint / Outcome</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1B</td>
<td>Breast Cancer or Melanoma</td>
<td>48</td>
<td>Completed</td>
<td>Safety</td>
</tr>
<tr>
<td>1</td>
<td>1P</td>
<td>Prostate, Colon, Breast Cancer</td>
<td>70</td>
<td>Ongoing</td>
<td>To support lifecycle management</td>
</tr>
<tr>
<td>2</td>
<td>1P</td>
<td>Breast Cancer or Melanoma</td>
<td>80</td>
<td>Completed 2008</td>
<td>99% successful in identifying lymphatic tissue</td>
</tr>
<tr>
<td>3</td>
<td>2B</td>
<td>Breast Cancer or Melanoma</td>
<td>179</td>
<td>Completed 2009</td>
<td>Concordance with vital blue dye (VBD)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Breast Cancer or Melanoma</td>
<td>153</td>
<td>Completed 2010</td>
<td>Concordance/reverse concordance with VBD</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Head &amp; Neck Squamous Cell Carcinoma</td>
<td>ongoing</td>
<td>Post-marketing study - underway</td>
<td>Label extension</td>
</tr>
</tbody>
</table>

Radiation Safety Issues

- Surgeon hand exposure of approximately 10 mrem per operation
- Surgical specimens fell below the radiologic control level (0.002µCi/g) at 72h for primary tumor, 46h for melanoma nodes and 33h for breast cancer nodes

Deep Blue Liposomes

Can be used by the pathologist handling the surgical specimens.

Can be used by the surgeon during his/her exploration.

May be used by veterinarians when searching for fractures.

Thermin et al.

Radiation Resistant Medical Instrument

Inventors: Mark D. Thomas, Columbus, Ohio
Mr. G. W. Washington, Bothell, WA
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