SF 06 GU-03 Update in prostate imaging

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LN imaging using US contrast

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The first route of spread of most cancers is through the lymphatics, prompting the direct assessment of draining lymph nodes as the first requirement to define prognosis and to triage patients into the most appropriate therapy. Resection and direct inspection of the sentinel lymph node (SLN)-the first node that receives growth factors, byproducts, and escaping cancer cells from a tumor focus-has revolutionized the approach to breast cancer and melanoma patients and is being expanded to other cancers. SLN resection in lieu of standard lymphadenectomy to decrease lymphatic bed injury began over 15 years ago to stage melanoma and breast tumors because the tumor and its drainage field are both superficial (1). Nearly 240,000 SLN resections are now performed in the USA yearly in breast cancer patients alone (2), and if the SLN is normal, as is the case in 60–70% of patients, the downstream nodes will not contain metastases (3, 4).

Since it was first introduced in 1994 (1), a great deal of data has been generated on the positive impact of SLN resection on the care of breast cancer patients. The intent of limiting resection to the SLN rather than all axillary nodes is to minimize the disruption of lymphatics that cripples patients for life with lymphedema and other complications (5). It is now established that when the SLN has no tumor deposits, it carries > 96% predictive value that the downstream nodes are negative (3, 6). The 4% failure is likely due to incorrectly labeling a node as the SLN. Since patients with positive SLNs require removal of all axillary nodes for proper staging, and since only 30-40% of SLNs are found malignant (3, 4), SLN resection has eliminated 2/3 of axillary resections, decreasing cost and morbidity. The rate of malignant SLNs will likely continue to decrease as effective screening identifies more patients with early-stage disease. It is also important to note that when the SLN contains macrometastases ($\geq$ 3 mm), which we should detect, 2/3 of patients will have positive downstream nodes. In contrast, when the SLN contains micrometastases (< 3 mm), which we may miss, 5% of patients will have at most one positive downstream node (6).

While SLN resection in breast cancer is mature, significant challenges still remain: 1) SLN detection and recognition remains an intra-operative procedure; 2) both the radiocolloid and the blue dye mark the SLN and also downstream nodes, increasing dissection and the opportunity to mislabel a downstream node as the SLN; and 3) SLN resection is needed to determine if patients require a 2nd operation to remove all axillary nodes.

Lymph node filling can be achieved by direct cannulation of the lymph vessel (not practical) or by injecting ultra-small particles of iron oxide (20–50 nm) intravenously (IV) that fill all nodes in the body. While neither of these methods can be used for SLN recognition, clinical data show that when nodes are filled with contrast, metastases are detected as filling defects (7, 8). SLN filling can also be achieved when contrast is injected subcutaneously (SQ) in the tissue drained by the SLN. Water-soluble agents-iodinated agents, Gd-chelates, and blue dyes injected SQ-pass freely into both the blood and lymph capillaries to be cleared rapidly. As molecular weight (9) or particle size (10) increase, entry into blood vessels slows and ceases at about 10–20 nm and lymphatic clearance slows. When particle size was increased from 40 to 400 nm, particles remaining at the injection site increased from 25 to 95% (11). When particles exceed 150–200 nm, clearance is dominated by the

very slow cellular uptake and transport (12). While non-targeted, small molecular weight agents travel through the lymph unimpeded, small molecules targeted to phagocytes and particles are trapped in the SLN (9, 10).

SLN resection begins with the injection of a radiocolloid near the tumor up to a day before surgery. The radioactive particles slowly enter the lymph vessel to be captured by lymph nodes. Using a pencil probe, the surgeon attempts to locate the radioactive nodes that can be masked by the large radiation field emitted from the adjacent SQ injection site. Once likely SLN sites are identified, a blue dye is injected SQ near the tumor to stain the draining lymph duct that is tracked surgically to the draining node, which is removed, its radioactivity measured ex-vivo, and then sent for detailed histological analysis. A resected node is considered a SLN if it is blue, hot, or both. Consensus in breast cancer is that the lowest false-negative rate (missed SLN) is achieved when the combination of blue dye and radiocolloid are injected SQ near the tumor to see and remove the hot and/or blue nodes (4). Neither the radiocolloid nor the blue dye are ideal since both mark the SLN as well as downstream nodes, resulting in greater dissection. The major challenges in SLN resection are the inaccuracy in recognizing the true SLN that has to be done intra-operatively and the need to remove the node to determine its malignant status.

MBs have been approved as US contrast agents for cardiac imaging in the USA and for the whole body in the remainder of the world for over a decade. They are 1- to 5-μm fluorocarbon gas cores encapsulated within a single lipid layer. A clinical IV dose is ~1–5 $\times$ 10^8 MBs that is delivered in 0.1 to 1 mL total volume depending on the agent's formulation. This miniscule dose is sufficient to fill the heart and all vessels with echoes and enhance all perfused tissues on real time US because US is extremely sensitive to MBs (13). US contrast has had a major impact in cardiac and liver imaging, with new indications being added as clinical experience is increasing. MBs are elastic because of their gas core and thin lipid shell, allowing them to deform and to expand and contract when exposed to US pressure. These result in two unique characteristics that are important for SLN imaging: 1) when exposed to US pressure, they behave non-linearly as compared to tissues to generate unique signals; with appropriate signal processing, complete tissue subtraction is possible (Fig. 1d), allowing the recognition of a single MB (13). 2) MB compression and expansion at the appropriate US frequency and pressure disrupts the MB shell, resulting in MB destruction. Analogous to photo-bleaching, MB destruction sequences have been used to alter image contrast to be flow- or blood volume-weighted (14), as well as to estimate relative blood flow (15), or simply clear the field to watch it refill in real-time.

Because MBs are deformable, and given the extreme sensitivity of US to MBs, we hypothesized that injecting MBs SQ and massaging the site could push enough MBs into the lymph-particularly the smaller MBs-to allow sufficient filling of the 1st draining node, by definition the SLN, to the injection site, allowing its detection on US. Using the clinical MB formulation, we showed that not only did the SLN fill, but the lymph vessel could be followed from the injection site to the node analogous to the blue dye used intra-operatively (Fig. 1; from Figs. 1 and 3 of Ref [16]). The number of times this could be repeated is significantly affected by MB formulation (17). We also showed that completely filling the SLN allowed the detection of nodal metastases as filling defects (Fig. 1) (16). More important, because MBs in the node could be eliminated by US, re-massaging the injection site refilled the lymph vessels and node again. Since this could be repeated many times, all lymph vessels draining the injection site and their associated SLNs can be identified (16), and, the true

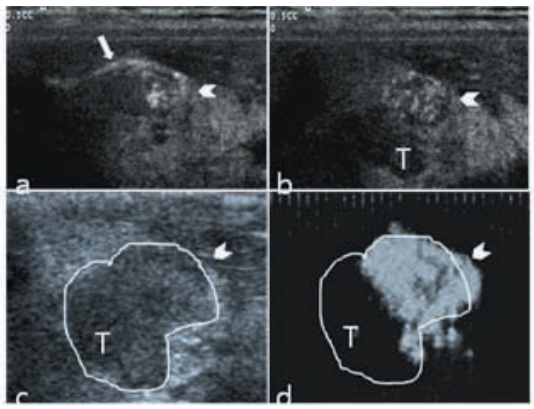

Figure 1. Images from the first proof of concept study show MBs filling the lymph duct (arrow in (**a**)) leading to and filling the draining node (arrowhead in (**a**) & (**b**)). Standard US of the node shows a large tumor (T) within the outline of the node (**c**), the normal portion of which begins to fill (arrowhead in (**b**)) and completely fills (arrowhead in (**d**)) while the tumor (T) appeared as a filling defect. (From Fig 1 and 3 of Ref 16).

SLN more accurately localized. We were issued a patent (18), the data were first reported at the Radiological Society of North America in 1999, and the first publication appeared in 2002 (16). We called the technique indirect lymphosonography (ILS) and then optimized the formulation and showed that the SLN could be refilled over 12 times following a single 1 mL injection (17).

Since our original reports, several investigators have duplicated our results (19-26), and advanced the technique to the clinic in Europe and Japan (22–24), where MBs are clinically approved. They confirmed in a pig model that lymph ducts are visible and that tumors within the node appear as filling defects (20). More important, ILS performed pre-operatively with a clinical formulation in 54 breast cancer patients was as accurate in marking the SLN as the intra-operative radiocolloid-blue dye technique (24). While we are proceeding toward a Phase I trial in the USA for this indication, our ultimate goal is to preoperatively: 1) recognize and mark the SLN(s) to simplify resection, minimize injury to the lymphatic bed and decrease operative time; 2) triage patients into those at high vs. low risk for lymphatic spread to improve upon the 30-40% yield and to decrease unnecessary SLN resections; and 3) detect tumor deposits preoperatively to eliminate one operation by proceeding directly to standard lymphadenectomy.

We continue to improve upon the MB formulation to increase the detection and recognition of the true SLN and to stage the SLN pre-operatively. More important we are developing targeted MB formulation to identify patients at high risk for nodal metastases.

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