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Determining Accurate Dye Combinations for Sentinel Lymph Node Detection: A Systematic Review

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Background: Lymphatic dyes are commonly used to map the drainage path from tumor to lymphatics, which are biopsied to determine if spread has occurred. A blue dye in combination with technetium-99 is considered the gold standard for mapping, although many other dyes and dye combinations are used. Not all of these substances have the same detection efficacy.
Methods: A systematic review of PubMed, SCOPUS, Web of Science, and Medline was performed. The predefined search terms were (indocyanine green OR isosulfan blue OR lymphazurin OR patent blue OR methylene blue OR fluorescein OR technetium-99) AND combination AND dye AND (sentinel lymph node biopsy OR lymphedema OR lymphatics OR lymph OR microsurgery OR cancer OR tumor OR melanoma OR carcinoma OR sarcoma).
Results: The initial search returned 4267 articles. From these studies, 37 were selected as candidates that met inclusion criteria. After a full-text review, 34 studies were selected for inclusion. Eighty-nine methods of sentinel lymph node (SLN) detection were trialed using 22 unique dyes, dye combinations, or other tracers. In total, 12,157 SLNs of 12,801 SLNs were identified. Dye accuracy ranged from 100% to 69.8% detection. Five dye combinations had 100% accuracy. Dye combinations were more accurate than single dyes.
Conclusions: Combining lymphatic dyes improves SLN detection results. Replacing technetium-99 with ICG may allow for increased access to SLN procedures with comparable results. The ideal SLN tracer is a low-cost molecule with a high affinity for lymphatic vessels due to size and chemical composition, visualization without specialized equipment, and no adverse effects. *(Plast Reconstr Surg Glob Open 2024; 12:e5598; doi: 10.1097/GOX.0000000000005598; Published online 8 February 2024.)*

INTRODUCTION

Cancer is the second leading cause of death in the United States and a major cause of morbidity and mortality worldwide. Detection and treatment before metastasis underlie the basis of screening tests and improve long-term outcomes. Many cancers, such as breast, melanoma, gynecological, and urological, spread through the lymphatic system with the first site of dissemination being the immediate draining lymph nodes, the sentinel lymph nodes (SLNs), then the second and third tier nodes, and beyond.[1,2] This predictable method of spread allows for the use of lymph nodes in cancer staging and prognosis with their involvement often being the most significant prognostic factor.[3]

Although lymph nodes play a vital role in cancer staging, many options currently exist for the detection of lymphatic spread.[4,5] Previously, if there was risk of metastatic spread, all regional lymph nodes were dissected, leading to increased morbidity from lymphedema, nerve injury, chronic shoulder pain, or joint dysfunction.[6,7] In 1992, Morton and Cochran introduced intraoperative SLN biopsies and dynamic early nodal metastases individualized to each patient.[8,9] Their method of intraoperative lymphatic mapping, using dye to map the drainage path from tumor to lymphatics and SLN biopsies to confirm spread, reserved the need for complete lymph node dissection only in cases where tumor spread is identified. Since then, many different dyes and radioactive tracers

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have been used to determine if tumor cells have drained to the adjacent lymph node basin, as well as radioactive tracers. However, not all of these substances have the same sensitivity and specificity when it comes to identifying the sentinel lymph node(s).

Ideally, the oncologic surgeon seeks a method that yields high sensitivity and specificity, thus perfectly identifying all the SLNs associated with a given tumor, mitigating the chances of false negatives and false positives. This will allow the surgeon to avoid unnecessary lymph node dissection and decrease both the morbidity associated with the procedure and the likelihood of potentially leaving cancerous lymph nodes in situ. Although some individual dyes have achieved high-detection accuracy, combinations of dyes have shown to improve results. The purpose of this study was to review which dye, combination of dyes, or dyes in combination with other substances such as heavy isotopes provide the greatest accuracy in SLN mapping.

METHODS

A systematic review of PubMed, SCOPUS, Web of Science, and Medline was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines on December 15, 2022. The predefined search terms were (indocyanine green OR isosulfan blue OR lymphazurin OR patent blue OR methylene blue OR fluorescein OR technetium-99) AND combination AND dye AND (sentinel lymph node biopsy OR lymphedema OR lymphatics OR lymph OR microsurgery OR cancer OR tumor OR melanoma OR carcinoma OR sarcoma). All articles that used a combination of lymphatic dyes, lymphatic dye and radiotracer, or lymphatic dye and other substance for SLN detection in the setting of cancer were included. We excluded studies that used animal models, in vitro studies, literature reviews and meta-analyses, and articles not written in English. The latter criterion was chosen for accurate methodology assessment, which affects the risk of bias determination. Titles and abstracts were screened by two independent reviewers and duplicates were removed. Full texts were then reviewed for eligibility. All conflicts were resolved by a third review author. Data were extracted and entered into a collection form. Risk of bias was assessed using a guide published by Elsevier.[10] Further details about the reviewing process are given in Figure 1.

Data Collection

Data from each study were extracted into a form with the following parameters: primary author, publication year, dye or dye combination studied, type of cancer, study design, number of patients, patient demographics, SLN detection rates, important study takeaways, and patient exclusion criteria. Studies were evaluated for their risk of bias based on the methods of participant selection, method of dye delivery, determination of node ground truth, study design, and control for confounding variables.

Takeaways

Question: Which combination of lymphatic tracers provides the greatest accuracy for SLN detection, in general and among specific cancer types?

Findings: A systematic review of four databases was conducted. The search returned 4267 articles, and 34 were selected for inclusion. In total, 89 methods of sentinel lymph node (SLN) detection were trialed using 22 unique combinations. Accuracy ranged from 100% (five combinations) to 69.8% detection. Dye combinations were more accurate than single dyes.

Meaning: Combining lymphatic tracers improves SLN detection results; replacing Tc-99 with ICG may allow for increased access to SLN procedures with comparable results.

RESULTS

The initial search for published articles in PubMed, SCOPUS, Web of Science, and Medline returned 4267 articles. After removing 1955 duplicates, 2312 unique studies remained. Based on titles and abstracts, 37 were selected as candidates that met inclusion criteria. After a full-text review of the candidates, 34 studies were selected for inclusion.[11–44] (**See table, Supplemental Digital Content 1,** which displays papers reviewed. **http://links.lww.com/PRSGO/D60**.) Three studies were excluded for not reporting relevant SLN detection rates.[45–47] The remaining 34 articles were analyzed for the efficacy of the dye or dye combinations in SLN detection accuracy.

Figure 1 and Supplemental Digital Content 1 show results from the electronic search. In total, the 34 papers trialed 89 methods of SLN detection using 22 unique dyes, dye combinations, or other tracer. Twenty-three (67.6%) articles investigated dye combinations in breast cancer, six (17.6%) investigated melanoma, oral, or penile cancer, and five (6.1%) investigated cervical, vulvar, or endometrial cancer. Blue dye (vital, isosulfan, methylene, patent) was used in 52 (58.4%) dye tests, indocyanine green (ICG) was used in 50 (56.2%) tests, technetium-99m (Tc-99) in 32 (36.0%) tests, indigo carmine in two (2.2%) tests, and carbon nanoparticles (CNs) in one (1.1%) test.

Dye Accuracy

Of the 12,801 SLNs, 12,157 SLNs were identified by all possible dye or dye combinations. Dye accuracy ranged from 100% to 69.8% detection. Five dye combinations had 100% accuracy; all of these used either ICG or Tc-99 or both. Dye combinations were more accurate than single dyes; the least accurate dye combination, ICG + indigo carmine, detected 96.4% of SLNs, whereas the least accurate dye, isosulfan blue, detected 69.8% of SLNs. However, the strength of the combination dyes is in part due to their mixture with highly accurate ICG or Tc-99; the ICG + isosulfan blue combination had an accuracy of 100%. Importantly, no dye combination did worse than any of its component dyes, suggesting that dye combinations only improve accuracy and do not have deleterious effects when combined (Table 1).

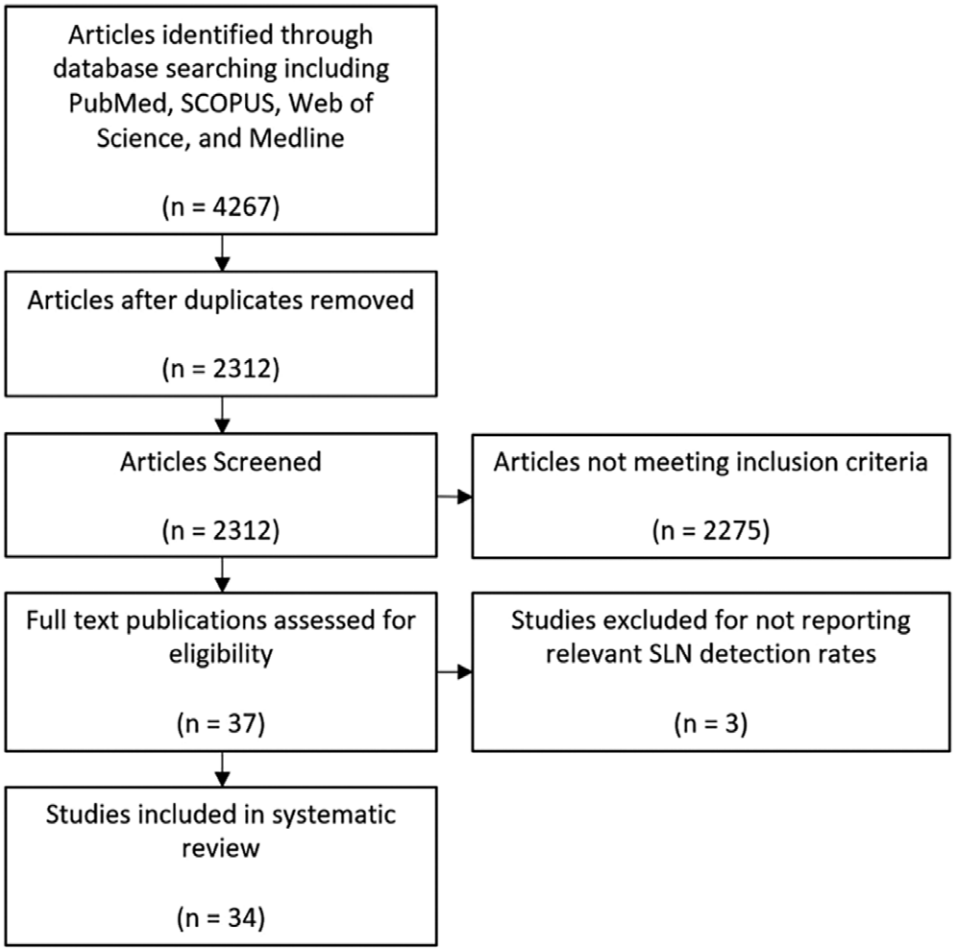

Fig. 1. PRISMA flowchart.

Table 1. Tracers Reviewed and Their SLN Identification Accuracy

Dye Combination	Identification Fraction	Identification Percent	Median (Range)	No. Articles
ICG + Tc-99 + patent blue	273/273	100	100.0 (100.0–100.0)	4
Methylene or patent blue + ICG + Tc-99	92/92	100		1
Isosulfan blue + ICG	75/75	100		1
Isosulfan blue + ICG + Tc-99	68/68	100		1
Vital blue + Tc-99	10/10	100		1
ICG + Tc-99	944/949	99.47	99.47 (98.41–100.0)	9
ICG + patent blue	1089/1096	99.36	99.36 (98.95–100.0)	5
ICG + methylene blue	2567/2593	99	99.02 (98.46–99.21)	11
CEUS + methylene blue	125/127	98.43		1
Carbon nanoparticles	59/60	98.33		1
Patent blue + Tc-99	515/526	97.91		1
Indigo carmine + Tc-99	35/36	97.22		1
Methylene blue + Tc-99	542/560	96.79	97.15 (91.67–98.7)	5
ICG + blue dye	86/89	96.63		1
ICG + indigo carmine	106/110	96.36		1
ICG	1886/1958	96.32	95.8 (83.7–96.32)	16
Methylene blue	1716/1889	90.84	84.49 (56.94–90.95)	7
Tc-99	884/978	90.39	90.12 (86.09–93.67)	9
Patent blue	740/831	89.05	89.63 (81.82–91.3)	7
Methylene or patent blue	70/92	76.09		1
Blue dye	155/217	71.43	71.94 (71.43–72.45)	2
Isosulfan blue	120/172	69.77	69.77 (53.61–86.21)	3

Dye by Cancer Type

The trends for most accurate dye combinations continue when analyzing dye combination accuracy specifically for type of cancer, with the highest accuracy combination for each type of cancer containing ICG, Tc-99, or both. Breast cancer had four possible combinations (ICG + Tc-99, ICG + isosulfan blue, Tc-99 + vital blue, ICG + Tc-99 + patent blue) with perfect SLN detection accuracy. Melanoma was most accurate with Tc-99 + patent blue, cervical cancer with Tc-99 + methylene blue, penile cancer with ICG + Tc-99, vulvar cancer with Tc-99 or any combination that included it, endometrial cancer with Tc-99 + methylene blue, and oral cancer with ICG + methylene blue. (**See table, Supplemental Digital Content 2,** which displays tracers reviewed and their SLN identification accuracy by cancer type. **http://links.lww.com/PRSGO/D61**.)

Other common cancers that drain to the lymphatic system were not excluded, but dye combination articles did not investigate them. Thus, they are not included in this review.

Risk of Bias within Studies

The most concerning factor affecting studies was variability in accounting for false-positive or false-negative results. Variations in dye detection method, patient characteristics (not reported in many cases), and comorbidities may have affected results. However, these concerns reflect real-world applications. Finally, some studies in this review are affected by a small number of nodes tested for a specific dye combination.

REVIEW OF DYE AND RADIOTRACER CHARACTERISTICS

Indocyanine Green

ICG is a water soluble but hydrophobic cyanine molecule that quickly binds to albumin and lipoproteins, especially HDL.[48,49] Its excitation (765–800 nm) and emission (830–840 nm) wavelengths reside in the near infrared (NIR) spectrum, thus requiring an NIR laser and NIR camera to excite and visualize.[50] ICG travels to the SLN in approximately 10 minutes after injection and remains visible for around 60 minutes.[48,51] It is quickly eliminated by the liver into the biliary tree and has a half-life of approximately 3 minutes.[52]

Technetium-99 Sulfur Colloid

Tc-99 is a homogenous noncrystalline substance with microparticles that contain a radioactive technetium isotope. The ideal particle size is 15–100 nm; particles too small will penetrate the capillary membrane and not migrate within the lymphatics, whereas particles too large migrate through the lymphatics at a slow rate that unnecessarily increases procedure time.[53,54] Tc-99 SLN detection can be done in two ways. Ten to 15 minutes after injection, the surgeon uses a gamma probe to identify the "hottest" node.[7] More commonly, patients are injected with Tc-99 hours before surgery, and SLNs are marked using a gamma camera or the lymphatic system is mapped using SPECT.[55] The half-life of Tc-99 is 6 hours, and overall radioactivity from an SLN procedure is low, on the order of a few dozen chest x-rays.[56]

Fluorescein Dye

Fluorescein is an organic, lymphophilic dye that has an isothiocyanate group that binds with the amine groups of intracellular proteins to act as an effective cell marker.[57] It is excited by blue light (465–490 nm) and emits a yellow-green fluorescence (510–530 nm).[57,58] Fluorescence microlymphography with fluorescein uses a fluorescence excitation filter in the former range on the microscope.[59] Whether injected subcutaneously or intradermally it reaches the SLN within minutes.[57,60] Fluorescein is primarily excreted renally, with a half-life of 23.5 minutes; its first pass metabolism is converted to fluorescein glucuronide, with a half-life of 264 minutes.[61] In rare cases, fluorescein administration may cause hives, hypotension, or anaphylaxis reactions.[58]

Blue Dyes

A number of blue dyes are commonly used in combination with Tc-99 as the gold standard for SLN detection. The choice of blue dye is often institution or region dependent. The plasma protein binding affinity of these dyes is related to the spacing of their sulfonic acid groups, with the highest affinity dyes having two sulfonate groups spaced two to six atoms apart.[62] However, this does not seem to have strong bearing on SLN identification accuracy, as our results show accuracy in decreasing order of methylene (no sulfonate groups), patent (one atom), isosulfan (two atoms). The absorption and emission spectra for these dyes fall within the visible light wavelengths, with the latter in the blue range (450–495 nm), giving their characteristic color. Although there are many blue dyes, we briefly review the three mentioned because they are the most popular.

Isosulfan Blue

Isosulfan blue's two sulfonate groups bind strongly to plasma proteins; its size allows it to travel in the lymphatic vessel but become trapped, leading to delineation of lymphatic vessels. Its half-life is on the order of hours and it is slowly excreted via the renal system. Allergic reactions are uncommon but can be serious, with approximately 1% of patients experiencing anaphylaxis.[63]

Patent Blue

Patent blue is an aniline dye and isomer of isosulfan blue. It behaves in a similar manner by binding to plasma protein and delineating the lymphatic vessels. It has a half-life of 1–2 days. It is poorly metabolized and excreted primarily renally with some biliary elimination. Adverse events include prolonged blue staining at the injection site and similar hypersensitivity reactions, including anaphylaxis, as isosulfan blue.[64]

Methylene Blue

Methylene blue (methylthioninium chloride) is a thiazine dye commonly used to treat methemoglobinemia. It

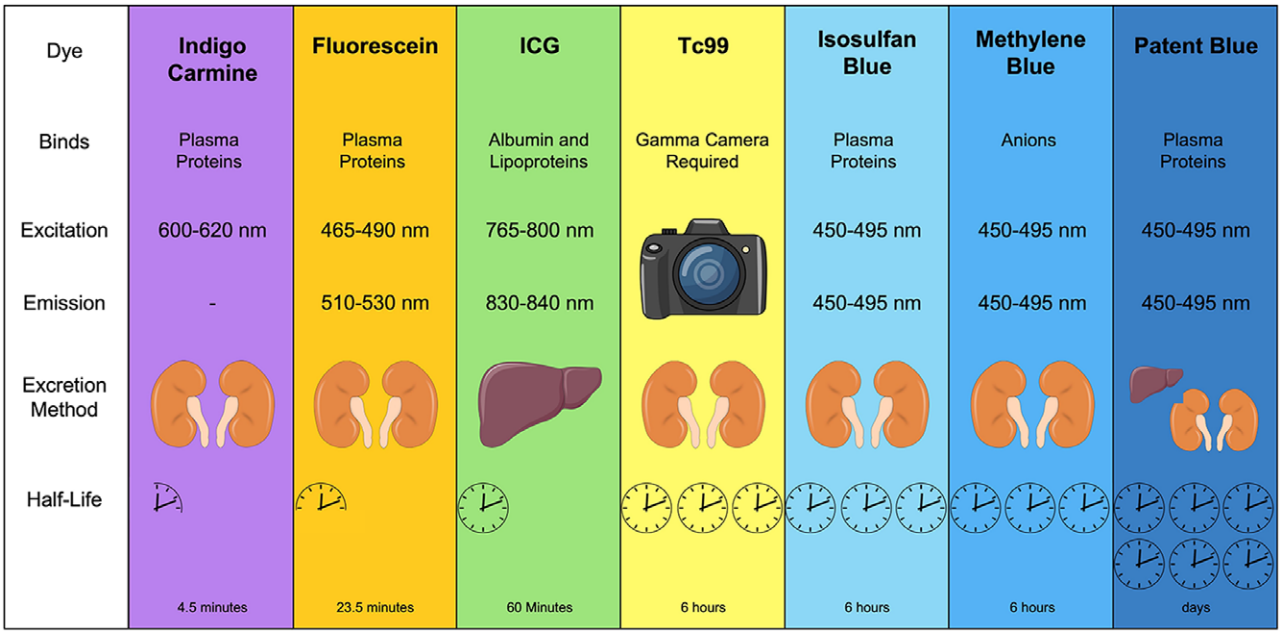

Fig. 2. Lymphatic dye infographic.

is a smaller molecule than isosulfan and patent blue and does not bind plasma proteins; its mechanism of lymphatic delineation is unclear and may be limited to nondiscriminatory mechanisms such as diffusion and anionic binding. Skin staining and injection site necrosis have been reported.[65]

Indigo Carmine Dye

Indigo carmine is a basic, organic sodium salt that rapidly binds to plasma proteins to travel to the lymphatics.[66,67] Commonly used in Japan, it was approved for medical use in the United States in July 2022. It is excited by orange light (600–620 nm) and rapidly excreted by the kidneys, which makes it especially useful for urologic procedures. Transient hypertension and bradycardia are known adverse reactions; hypotension and hypersensitivity reactions have also been reported[66] (Fig. 2).

DISCUSSION

The SLN biopsy technique, introduced by Morton and Cochran, uses the patient's variable lymphatic drainage to retain cosmesis and function and aligns with the modern trend to personalize healthcare.[68] Lymphatic dyes with high sensitivity and specificity for SLN detection will prevent morbidity and mortality, during sentinel lymph node biopsy and improve the efficacy of the surgery. Irrespective of the dye used, the practitioner should be on the lookout for secondary false positives that occur when the dye spreads to a higher tier node and false negatives that occur when stenotic vessels or flow abnormalities prevent dye drainage. One way to mitigate this problem is the use of a dye with a radiotracer.

Each type of dye behaves differently in vivo due to its biochemical composition, which informs its molecular interactions and optical properties. ICG stays intralymphatically by binding rapidly to plasma protein but requires special equipment to visualize and undergoes rapid hepatic excretion. Tc-99 particles are taken up by the lymphatics in a size-dependent manner and require a radiation detection method.[55] The blue dyes can be visualized without special equipment. Isosulfan blue and patent blue bind lymphatic proteins and become trapped in the vessels; methylene blue is a smaller molecule and does not bind plasma proteins but has comparable results to the other blue dyes.[69] As a result of these mechanistic differences, each dye or tracer leads to different levels of accuracy with lymphatic mapping and SLN detection.

As expected from current practices, studies that investigated ICG and Tc-99 demonstrated high levels of identification accuracy individually and better results when in combination with each other or other dyes. Presently, the gold standard for SLN biopsy in breast and other types of cancer is Tc-99 with a blue dye. However, ICG may function as a replacement for Tc-99 due to their comparable detection rates with blue dye. Studies directly comparing the two have found similar detection rates, and the results of our review are in line with this thinking.[38] This paradigm shift could increase global access to SLN biopsy due to ICG's increased availability and decreased logistic, training, and cost requirements compared with Tc-99.[44] The increased requirements of Tc-99 reduce the ability to perform the current gold standard method to approximately 60% of eligible patients in developed countries and sparsely for patients in resource-poor countries.[70]

Two SLN biopsy techniques that did not use ICG or Tc-99 had levels of accuracy comparable to the ICG or Tc-99 combinations. Zhou et al compared ICG + methylene

blue dye with contrast enhanced ultrasound (CEUS) and methylene blue dye and showed that the two methods had equivalent SLN detection rates and recurrence rates at a median of 4 years.[30] SLNs were marked on patient skin during CEUS 30 minutes before surgery, and methylene blue tracing was performed in the operating room. This procedure has a relatively low cost and equipment barrier and increases detection rates of methylene blue to those of methylene blue + ICG or methylene blue + Tc-99.[71,72]

Qin et al compared ICG + methylene blue to CNs. CNs have a strong affinity for the lymphatic system and not blood capillaries, and after peritumoral tissue injection, they accumulate in the SLN, turning the lymph node black.[73] Importantly, they are less likely than dye to migrate beyond the SLN to a higher tier node.[73] Qin showed that CNs have a slightly inferior identification rate to ICG + methylene blue, but other studies have shown that CNs are superior to the gold standard Tc-99 + blue dye.[40,73] CNs work in the same time frame as dyes (10–15 minutes) and have been shown to be safe in limited studies.[74]

Although not investigated in any studies in this review, in recent years nondye, superparamagnetic iron oxide (SPIO) nanoparticles, which have conventionally been used as a contrast agent for magnetic resonance imaging, have demonstrated a high accuracy for SLN detection. SPIO nanoparticles are injected into the patient 3–15 days before surgery and detected with a magnetometer in the operating room.[73] Their SLN detection results have been noninferior to the gold standard, with comparable identification and false-negative rates.[75] Injections of SPIO are slightly less expensive than Tc-99 and reduce operating time due to the injection being given preoperatively.[76]

One of the main limitations of this review is comparing studies that investigated different numbers of nodes for each tracer. Our smallest number of lymph nodes for a tracer combination was 10, whereas other studies investigated hundreds of lymph nodes. Additionally, we combined results for individual or combination tracers that were the same across studies, although the methods for dye application varied, albeit acceptably, between groups in some cases. Additionally, we were limited to reporting SLN identification rates because of the variability in reporting of false-negative and recurrence rates among included studies.

Although lymphatic tracers, dyes, and other substances have created a new paradigm for cancer staging and morbidity prevention, questions remain around methods to optimize this technique. This review shows that while ICG and Tc-99 work well individually, combinations with these tracers improve results. However, both of these tracers have their drawbacks.

CONCLUSIONS

The ideal SLN tracer is a low-cost molecule that requires no specialized visualization equipment, has a high affinity for binding lymphatic proteins, and is between 100 and 200 nm. It needs to be small enough to quickly migrate to the SLN but large enough to remain localized for at least the procedure duration. It should also have few to no adverse effects. These features would allow the tracer to have a high SLN detection rate, sensitivity, specificity, and availability in cancer centers globally.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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