

Increased Post-Operative Complications With Methylene Blue Versus Lymphazurin in Sentinel Lymph Node Biopsies for Skin Cancers

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Background: Sentinel lymph node biopsy (SLNB) is the standard of care in staging of melanoma and other skin cancers. Early studies used lymphazurin (LZ) for SLNB. A national shortage of LZ promoted methylene blue (MB) as an alternate stain.

Objective: This study compares complication rates between LZ and MB in SLNB patients, and the reliability dyes in locating sentinel lymph nodes.

Methods: Charts were reviewed for patients who underwent SLNB for skin cancers between September 2006 and November 2008 at Penn State Hershey Medical Center. The type and quantity of dye, the presence of blue stained sentinel nodes, and any complications were recorded.

Results: Ninety-three patients underwent SLNB (46 LZ, 47 MB). The amount of dye used was similar (avg. 0.93 ml LZ, 1.24 ml MB). The complication rate of LZ was 8.7%, and MB was 25.5%. There was significantly more ($P = 0.003$) skin graft complications in the MB patients (6/12) LZ (0/15).

Conclusions: There is a higher rate of complications with MB, especially with skin grafting. The reported risk of anaphylaxis with LZ has not been reported in volumes < 2 ml. The sentinel lymph node identification rate is historically similar between dyes, and in this study was noted to be higher with LZ.

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KEY WORDS: sentinel lymph node biopsy; lymphazurin; skin cancers; blue dye; melanoma

INTRODUCTION

Sentinel lymph node biopsies (SLNB) are standard for the staging of multiple cancer types such as breast, head and neck, colorectal cancer, and skin cancers such as melanoma, and Merkel cell carcinoma [1]. Sentinel lymph nodes are located via preoperative lymphoscintigraphy. This involves peritumoral, or peritumoral defect, injection of a radioisotope such as Technetium-99m. The radioisotope is carried in the lymphatics and filtered by the first lymph nodes encountered. This enables the physician to create a scintigraphic image, and also locate the node intraoperatively with the aid of a gamma probe. Additionally, intraoperative dye is injected intradermally around the cancer or scar and massaged for 5 min. The dye, much like the radioisotope, is taken up by the lymphatics in order to stain the first nodes encountered blue [2]. The combination of these two labeling techniques allows for a sentinel lymph node identification rate of approximately 95%, depending on the anatomic location [3].

SLNB was popularized in the early 1990s for the detection of metastatic melanoma to the regional nodal basin. The first large-scale randomized international trial defining SLNB in melanoma was the MSLT-1 (Multicenter Selective Lymphadenectomy Trial). The researchers in this trial used 2 ml or less of vital blue dye (Lymphazurin or Patent Blue dye) injected intradermally at the site of the malignancy [3]. The vital blue dyes used were Lymphazurin and Patent Blue which are isomers of each other [4]. For simplicity sake in this study we refer to both of these dyes as lymphazurin.

The choice of isomer generally depends on the manufacturing country, as Patent Blue is obtained from Therapex in Canada, while isosulfan blue (Lymphazurin) is a product of U.S. Surgical Corporation in the United States [4]. Hypersensitivity reactions for these dyes are typically cross-reactive, as with most other triphenylmethane dyes [5,6]. Triphenylmethane dyes are commonly found in our everyday environment in dyes for fabrics, cosmetics, paper, and leather. Since the public is in everyday contact with triphenylmethane dyes, there is the possibility of sensitization and subsequent allergic reactions. When vital blue dyes were used for lymphatic mapping in the 1960s–1970s there

was a high rate of allergic reactions (2.5%); however this was believed to be secondary to impurities in manufacturing as the rate has dropped since lymphazurin blue was produced for FDA approved pharmaceutical uses [7].

Multiple studies have shown that lymphazurin effectively stains the sentinel node for identification around 60% of the time when used without a radiolabeled marker such as Technetium-99m [8]. The safety of lymphazurin was confirmed in the MSLT-I study, with an extremely low systemic complication rate (2/1,173). The only two reactions in the study consisted of blue urticarial reactions without any anaphylactic-type symptoms [3].

Since the introduction of lymphazurin as the standard for SLNB, further studies have been published proposing the use of methylene blue as an alternative stain for SLNBs. These studies have shown methylene blue to be equally efficacious in locating sentinel nodes, and it is notably cheaper than Lymphazurin. Methylene blue is in the phenothiazine family, and chemically dissimilar to lymphazurin. These studies surfaced at an opportune time, secondary to nationwide shortages of lymphazurin as well as some case reports arising describing anaphylactic type reactions with lymphazurin. The incidence of these reactions was reportedly in the range of 1–2% of the studied populations [7,9–11]. Dr. Ian Komenaka at Columbia-Presbyterian Medical Center reviewed the literature of systemic reactions with lymphazurin and compiled their data with three other large institutional studies to determine the rate of allergic reactions with lymphazurin. They found a systemic complication rate of 18/1,663, 13 of which were anaphylactic

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shock type reactions and 5 blue urticarial reactions. These reports led to a nationwide trend of substituting methylene blue for lymphazurin in SLNB for malignant melanomas.

During the shortage, this institution was likewise unable to consistently obtain lymphazurin to use in SLNB for skin malignancies. Thus, methylene blue was used as a substitute for SLNBs whenever lymphazurin was unavailable.

As the scarceness of lymphazurin has mostly resolved, it is an appropriate time to reassess our use of methylene blue in SLNB for skin cancer patients. The goal of this study is to compare the complication rates between lymphazurin and methylene blue, as well as their efficacy in sentinel lymph node identification in skin cancer patients.

MATERIALS AND METHODS

In order to better study the applicability of methylene blue for use in skin cancer SLNB, a retrospective review of all skin cancer patients who underwent SLNB at Hershey Medical Center between September 2006 and December 2008, was completed following IRB approval (#29955EM). This specific time frame was chosen to encompass the time during which Lymphazurin was not readily available. As mentioned previously, whenever Lymphazurin was not available due to manufacture supply issues methylene blue was substituted.

Any patients whose operative record did not indicate the type of dye used for the biopsy were excluded from the data set.

The Lymphazurin used at M. S. Hershey Medical Center was obtained from U.S. Surgical/Tyco International (now known as Covidien) as a 1% solution, and the methylene blue from American Regent Laboratories as a 1% solution. The dye was injected intradermally surrounding the skin cancer or excisional biopsy site in a volume ranging from 0.2 to 2 ml depending on the size of the previous scar or primary tumor.

All intraoperative records were reviewed for anaphylactic type complications, type and volume of dye used, as well as presence of a blue stained sentinel lymph node. The sentinel lymph node was confirmed with preoperative lymphoscintigraphy along with intraoperative measurement of Technetium-99m levels by gamma probe. The type of reconstruction (i.e., skin graft, primary closure, local random/axial flaps) was documented. The post-operative records were also analyzed for complications or adverse events within 90 days of surgery.

RESULTS

There were 46 patients who received Lymphazurin and 47 who received methylene blue during the studied time period. These patients included 87 cases of melanoma, 1 porocarcinoma, 3 Merkel cell cancers, and 2 squamous cell cancers. The comorbidities of the patients in this study were not controlled or compared. The use of lymphazurin or methylene blue was random, depending on the availability of

lymphazurin, which was used preferably. The study was undertaken with the assumption that the patient populations in the two groups would be similar due random selection from the same patient pool with moderately large patient groups.

The average amount of dye used was similar between the Lymphazurin and methylene blue (0.93 ml LZ vs. 1.24 ml MB; two-tailed Student's *t*-test $P = 0.08$). The identification rates were comparable (49% [34/69] LZ vs. 45% [43/96] MB; two-tailed Fischer's exact test $P = 0.64$). There was a significantly higher complication rate with methylene blue (25.5% [12/47] MB vs. 8.7% [4/46] LZ; two-tailed Fischer's exact test $P = 0.05$). The average volume of methylene blue used in skin patients with complications was not statistically different than the volume of dye used in patients without complications (1.08 ml vs. 1.29 ml, $P = 0.67$, two-tailed Student's *t*-test); this was also the same when comparing the average volume of lymphazurin used in patients with and without complications (0.675 ml vs. 0.96 ml, $P = 0.15$, two-tailed Student's *t*-test; Table I).

All of the complications occurred in the acute post-operative period (<4 weeks). The types of complications noted with Lymphazurin involved infections (four patients) and minor skin slough (two), but importantly no skin graft complications out of 15 patients (six full thickness skin grafts, nine split thickness skin grafts). All four of the lymphazurin patients with infections required a course of oral antibiotics and one was admitted overnight for a single dose of IV antibiotics. One of the patients who had minor skin slough needed partial debridement of the wound in clinic. All of the wound complications were completely healed by 2 months post-operatively. There were more variable skin complications following use of methylene blue, including: tattooing (three; see Fig. 1), infections (three—one of which required debridement in the OR and one debrided in clinic), blistering (two), dehiscence (one), and partial or complete loss of skin grafts (six). Of the patients with infections, one was admitted for IV antibiotics and the rest were treated with oral antibiotics. All of the wounds were healed by 2 months post-operation. There was significantly more (50% [6/12]; $P = 0.003$, two-tailed Fisher's Exact Test) partial or complete loss of skin grafts in the methylene blue group of patients treated with skin grafts, consisting of 6 full thickness skin grafts and 6 split thickness skin grafts.

The anatomic site and associated failure rate of the skin grafts were analyzed between the two dyes. While all of the skin grafts placed over methylene blue injected beds in the lower extremities had complications (5/5), only one in four skin grafts had complications when lymphazurin was used. In fact, all of the regions of the body that were skin grafted following use of methylene blue (lower extremity, upper extremity and head/neck) had some complication (see Fig. 2). The patients in whom lymphazurin was used prior to grafting had only a single site with a complication (lower extremity; see Fig. 3). The lower extremity skin graft complication in the lymphazurin group was 10% graft loss, and the wound healed by secondary intention within 2 months.

TABLE I. Comparison of LZ and MB for SLNB

Sentinel lymph node biopsy	Lymphazurin	Methylene Blue	<i>P</i> -value
Number of patients	46	47	—
Avg. amount of dye used	0.93 ml	1.24 ml	0.08
Staining rate	49%	45%	0.64
Complication rate	8.7%	25.5%	0.05
Complete or partial skin graft loss	0%	50%	0.003
Volume dye used with complications	0.675 ml	1.08 ml	—
Volume dye used without complications	0.96 ml	1.29 ml	—
	$P = 0.15^*$	$P = 0.67^*$	

**P* values comparing the volume of dyes used in patients with and without complications, within the lymphazurin group and the methylene blue groups separately.



Fig. 1. This is a representative picture of tattooing as well as complete loss of a full thickness skin graft following a SLNB using 2 ml of methylene blue dye. The loss of graft occurred spontaneously 10 days post-operatively in a 45-year-old otherwise healthy female.

DISCUSSION

Many past studies have promoted the use of methylene blue [8,12–15]. Much of this literature has been based on the risk of anaphylactic reactions with the use of lymphazurin. There has also been a financial incentive to replace lymphazurin with methylene blue. Previous publications have noted a large price discrepancy between the two medications (\$0.33/ml methylene blue versus \$12.20/ml Lymphazurin) with a potential national savings of millions of dollars per year [12].

In reviewing the past studies on this topic, there is a discrepancy between the complication rate seen in the MSLT-1 study and subsequent studies and reviews, such as the one done by Komenaka. Furthermore, in

Methylene Blue Distribution of Skin Graft Site Complications

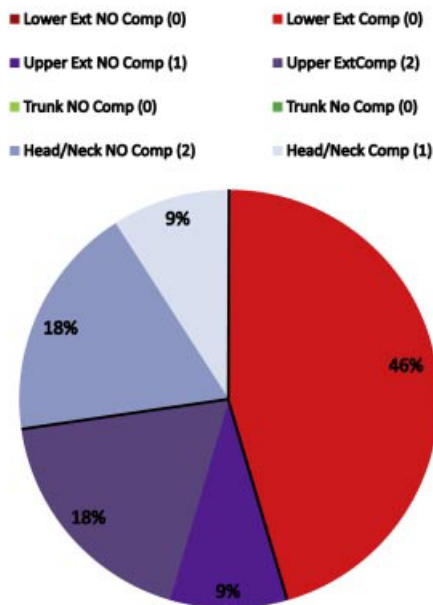


Fig. 2. MB distribution of skin graft site complications.

LZ Distribution of Skin Graft Site Complications

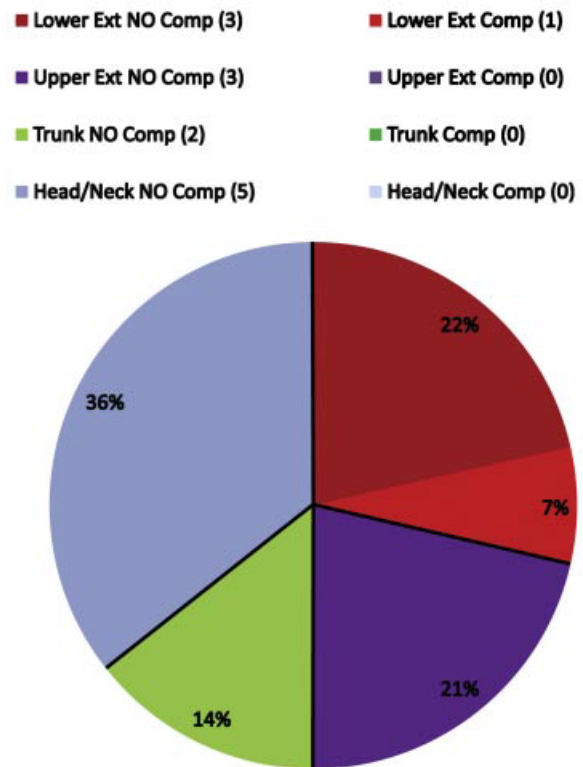


Fig. 3. LZ distribution of skin graft site complications.

our experience at HMC we found no anaphylactic reactions with lymphazurin for SLNB in skin cancers patients. However we began to notice an unusually high local complication rate with methylene blue.

Upon further examination of these studies it is evident that their application, to SLNB in skin cancer patients is inherently flawed. The majority of these studies reporting anaphylactic reactions were based on breast cancer populations [6,9,10,13,16]. There are important differences in the techniques used in SLNB for breast cancer versus skin cancer. In fact, the pioneering study describing SLNB for breast cancer specifically mentioned modifying the techniques detailed by Morton in the MSLT-1 trial. Instead of injecting the dye intradermally, it is injected more diffusely into the breast mass and surrounding parenchyma, subareolarly, or into the biopsy cavity if done after a previous biopsy [17,18]. The blood supply to subcutaneous fat and density of the tissue in this plane is much less than that found in the dermis. Therefore intra-dermally injected dye is possibly subjected to higher tissue pressure and greater absorption into the vasculature. Another difference in technique used for breast cancer is the use of larger volumes. Many of the reviews describing SLNB in this population recommend larger volumes of dye, 3–5 ml [17,19].

Breast cancer patients also have different reconstructive needs following cancer resection. The excision is usually done via lumpectomy or mastectomy resulting in a defect, which can either be primarily closed (with or without an expander), or reconstructed using large vascularized myocutaneous pedicled or free flaps. Conversely, defects following resection of skin cancers often require undermining with primary closure, skin grafting for coverage or small local random or axial flaps. The blood supply to small local random flaps, and skin grafts can be much more tenuous than that of mastectomy flaps or large myocutaneous flaps.

The most notable difference between past large studies like those done at MD Anderson, University of Michigan, and Mt. Zion Hospital versus the MSLT-1 trial as well as our study is the volume of dye used. A consistently higher volume of dye was used in all of the patients with anaphylactic reactions. Dr. Vincent Cimmino at University of Michigan noted anaphylactic complications in five patients, all of whom received between 3 and 5 ml. These patients included two melanoma patients and three breast cancer patients [7]. Dr. Daniel Albo at MD Anderson reported using 3–5 ml of lymphazurin in their seven breast cancer patients whom had anaphylactic reactions to lymphazurin [10]. And similarly, Dr. Stanley Leong and colleges at Mt. Zion hospital used between 4.7 and 4.8 ml of lymphazurin in their three melanoma patients who had anaphylactic reactions [11]. The MSLT-1 trial used only between 1 and 2 ml of lymphazurin, and at our hospital we used between 0.7 and 2 ml [3].

The combination of a different injection site and smaller volume of injected dye may very well account for the paucity of complications with the use of lymphazurin in the MSLT-1 trial.

The high rate of superficial complications noted with methylene blue raises the question of whether methylene blue is truly a benign agent, which should be used in SLNB for skin cancer patients.

There are several mechanisms by which methylene blue may cause the local tissue destruction. Methylene blue has been shown to inhibit nitric oxide (NO) thus promoting vasospasm which can worsen tissue ischemia [20]. Methylene blue may also be oxidized in tissues, resulting in the formation of formaldehyde and deaminized oxidized products, which in large quantities can be phagocytosed by macrophages causing a local inflammatory reaction [21].

In a review of the literature there have been many reports of cytotoxicity with methylene blue. Ophthalmologists have noted post-operative follicular and papillary conjunctivitis, as well as direct cytotoxicity to corneal endothelial cells when using methylene blue as a staining agent in cataract surgery [22,23]. There have been studies demonstrating adverse skin lesions including necrosis and ulceration at injection sites of methylene blue when it is injected intradermally in breast cancer patient for SLNB, which prompted the authors to recommend that methylene blue should not be injected intradermally when used in SLNB for breast cancer patients [24–26]. Methylene blue has even been proposed as a possible chemotherapeutic agent due to its erythroleukemic cytotoxicity [27].

There was residual dye noted in three patients seen in the post-operative period. This was cited as tattooing of the skin in the list of patient complications. Considering the possible toxicity of methylene blue, the question may arise as to whether there is a higher complication rate in patients with residual dye in the tumor bed following the excision of the skin cancer. This study was unable to evaluate for deleterious effects of residual dye at the graft site, as operative records failed to describe whether there was residual dye in the excised tumor bed prior to grafting. Certainly, this data and observation would be useful in any future studies.

CONCLUSION

Our study, resembling many studies in the past, demonstrated similar nodal staining rates between lymphazurin and methylene blue. Unique to this study, there were significantly more skin complications with methylene blue noted in the post-operative period. This was especially evident when skin grafts were used to cover the resultant skin cancer defect.

In view of the utility of skin grafts in skin cancer resection reconstructions and cytotoxicity of methylene blue, lymphazurin is the dye of choice for use in SLNB for skin cancer. Our study had no allergic reactions to lymphazurin. The absence of anaphylactic symptoms seen in our patients could be due to small study population size, with an expected incidence of around 1–2% [5]. However, it may

also be attributable to the use of volumes less than or equal to 2 ml, which is consistent with the MSLT-1 trial. This data suggests that Lymphazurin or Patent Blue should remain the dye of choice for SLNB in skin cancer patients until larger population studies better delineate the complications associated with the use of an appropriate volume of lymphazurin versus methylene blue in skin cancer patients.

REFERENCES

1. Wagman LD: Principles of surgical oncology. In: Pazdur R, Wagman LD, Camphausen KA, Hoskins WJ, editors. Cancer management: A multidisciplinary approach. New York, NY: CMP United Business Media; 2008.
2. Tanis PJ, Nieweg OE, Valdes Olmos RA, et al.: History of sentinel node and validation of the technique. *Breast Cancer Res* 2001;3: 109–112.
3. Morton DL, Cochran AJ, Thompson JF, et al.: Sentinel node biopsy for early-stage melanoma: Accuracy and morbidity in MSLT-1, an international multicenter trial. *Ann Surg* 2005;242:302–311; discussion 311–303.
4. Tsopelas C, Sutton R: Why certain dyes are useful for localizing the sentinel lymph node. *J Nucl Med* 2002;43:1377–1382.
5. Scherer K, Studer W, Figueiredo V, et al. Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: Case report and review of the nomenclature of vital blue dyes. *Ann Allergy Asthma Immunol* 2006;96:497–500.
6. Efron P, Knudsen E, Hirshorn S, et al. Anaphylactic reaction to isosulfan blue used for sentinel node biopsy: Case report and literature review. *Breast J* 2002;8:396–399.
7. Cimmino VM, Brown AC, Szocik JF, et al.: Allergic reactions to isosulfan blue during sentinel node biopsy—a common event. *Surgery* 2001;130:439–442.
8. Liu Y, Truini C, Ariyan S: A randomized study comparing the effectiveness of methylene blue dye with lymphazurin blue dye in sentinel lymph node biopsy for the treatment of cutaneous melanoma. *Ann Surg Oncol* 2008;15:2412–2417.
9. Komenaka IK, Bauer VP, Schnabel FR, et al.: Allergic reactions to isosulfan blue in sentinel lymph node mapping. *Breast J* 2005;11:70–72.
10. Albo D, Wayne JD, Hunt KK, et al.: Anaphylactic reactions to isosulfan blue dye during sentinel lymph node biopsy for breast cancer. *Am J Surg* 2001;182:393–398.
11. Leong SP, Donegan E, Heffernon W, et al.: Adverse reactions to isosulfan blue during selective sentinel lymph node dissection in melanoma. *Ann Surg Oncol* 2000;7:361–366.
12. Simmons RM, Smith SM, Osborne MP: Methylene blue dye as an alternative to isosulfan blue dye for sentinel lymph node localization. *Breast J* 2001;7:181–183.
13. Thevarajah S, Huston TL, Simmons RM: A comparison of the adverse reactions associated with isosulfan blue versus methylene blue dye in sentinel lymph node biopsy for breast cancer. *Am J Surg* 2005;189:236–239.
14. Varghese P, Abdel-Rahman AT, Akberali S, et al.: Methylene blue dye—A safe and effective alternative for sentinel lymph node localization. *Breast J* 2008;14:61–67.
15. Zakaria S, Hoskin TL, Degnim AC: Safety and, technical success of methylene blue dye for lymphatic mapping in breast cancer. *Am J Surg* 2008;196:228–233.
16. Wilke LG, McCall LM, Posther KE, et al.: Surgical complications associated with sentinel lymph node biopsy: Results from a prospective international cooperative group trial. *Ann Surg Oncol* 2006;13:491–500.
17. Giuliano AE, Kirgan DM, Guenther JM, et al. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–398; discussion 398–401.
18. Bauer TW, Spitz FR, Callans LS, et al.: Subareolar and peritumoral injection identify similar sentinel nodes for breast cancer. *Ann Surg Oncol* 2002;9:169–176.
19. Cox CE, Salud CJ, Harrington MA: The role of selective sentinel lymph node dissection in breast cancer. *Surg Clin North Am* 2000;80:1759–1777.

20. Johnson PC, Sheppeck R: The dark side of methylene blue. *Plast Reconstr Surg* 1989;83:1076–1077.
21. Perry PM, Meinhard E: Nectotic subcutaneous abscesses following injections of methylene blue. *Br J Clin Pract* 1974;28:289–291.
22. Brownstein S, Liszauer AD, Jackson WB: Ocular complications of a topical methylene blue-vasoconstrictor-anesthetic preparation. *Can J Ophthalmol* 1989;24:317–324.
23. Chang YS, Tseng SY, Tseng SH, et al.: Comparison of dyes for cataract surgery. Part I. Cytotoxicity to corneal endothelial cells in a rabbit model. *J Cataract Refract Surg* 2005;31:792–798.
24. Stradling B, Aranha G, Gabram S: Adverse skin lesions after methylene blue injections for sentinel lymph node localization. *Am J Surg* 2002;184:350–352.
25. Bleicher RJ, Kloth DD, Robinson D, et al. Inflammatory cutaneous adverse effects of methylene blue dye injection for lymphatic mapping/sentinel lymphadenectomy. *J Surg Oncol* 2009;99:356–360.
26. Salhab M, Al Sarakbi W, Mokbel K: Skin and, fat necrosis of the breast following methylene blue dye injection for sentinel node biopsy in a patient with breast cancer. *Int Semin Surg Oncol* 2005;2:26.
27. Kirszberg C, Rumjanek VM, Capella MA: Methylene blue is more toxic to erythroleukemic cells than to normal peripheral blood mononuclear cells: A possible use in chemotherapy. *Cancer Chemother Pharmacol* 2005;56:659–665.