Background and Objectives: Although serratus plane block reportedly provides satisfactory analgesia for breast and thoracic surgeries, the optimal technique for consistent success has not been studied. The goal of this anatomical study was to evaluate the impact of volume, level, and site of injection on the extent of injectate spread that can influence anesthetic coverage.

Methods: Ultrasound-guided dye injection and subsequent dissection were performed in 39 cadaveric hemithoraces. Methylene blue was injected according to 1 of 4 injection protocols as follows: one 20-mL bolus, either superficial or deep to the serratus anterior muscle (SAM), at the fifth rib level (groups SUP-20 and DEEP-20, respectively), or two 20-mL boluses, either superior or deep to the SAM, one at the third rib and one at the fifth rib level (group SUP-40 and group DEEP-40, respectively). Following injection, dissection and 3-dimensional digitization were performed to map the area of dye spread.

Results: We found that the extent of dye spread was mostly influenced by the volume of injection rather than the plane of injection (superficial vs deep to SAM). Increasing the volume from 20 to 40 mL doubled the area of injectate spread and promoted dye spread preferentially to the anterior chest wall, with some impact on cephalad-to-caudad spread and no impact on posterior spread. Dye was found most consistently in the axilla when a separate injection was performed at the third rib level.

Conclusions: Our data showed that a high-volume double-injection technique provides extensive and consistent dye spread in the anterior chest wall and axilla, regardless of the plane of injection relative to the SAM. This technique likely provides more reliable analgesic coverage for breast and thoracic wall and axilla, regardless of the plane of injection relative to the SAM.

METHODS

The project was approved by the University of Toronto, Health Research Ethics Board (no. 27210). Serratus plane dye injection and dissection were performed in the hemithoraces of 20 lightly embalmed cadaveric specimens (a total of 39 hemithoraces) with a mean age of 82 ± 12 years. Specimens were excluded if there was any visible sign of injury, pathology, pacemaker insertion, or previous surgery of the trunk or upper limb. Each 20-mL dye injection consisted of 0.5 mL of methylene blue mixed in 19.5 mL of water. A linear high-frequency 8- to 13-MHz probe (GE Venue 40, Wauwatosa, WI) was used for all injections. Each hemithorax was injected using 1 of 4 protocols per randomization:

1. group SUP-20 = one 20-mL injection superficial to SAM at the level of the fifth rib at the midaxillary line (MAL) (n = 11);
2. group DEEP-20 = one 20-mL injection deep to SAM at the level of the fifth rib at the MAL (n = 10);
3. group SUP-40 = two 20-mL injections (total 40 mL), superficial to SAM, one at the level of the third rib at the anterior axillary line (AAL) and the other at the level of the fifth rib at the MAL (n = 8); and
4. group DEEP-40 = two 20-mL injections (total 40 mL), deep to SAM, one at the level of the third rib level at the AAL and the other at the level of the fifth rib at the MAL (n = 10).

The cadavers were placed in the lateral decubitus position with the nondependent arm flexed, adducted, and raised over the head as much as possible (mimicking clinical practice). The differences in injectate spread were mostly influenced by the volume of injection rather than by the plane of injection (superficial vs deep to SAM). The optimal technique for consistent success has not been studied. The goal of this study was to compare the area of injectate spread between a single-injection and two-injection protocols. The project was approved by the University of Toronto, Health Research Ethics Board (no. 27210). Serratus plane dye injection and dissection were performed in the hemithoraces of 20 lightly embalmed cadaveric specimens (a total of 39 hemithoraces) with a mean age of 82 ± 12 years. Specimens were excluded if there was any visible sign of injury, pathology, pacemaker insertion, or previous surgery of the trunk or upper limb. Each 20-mL dye injection consisted of 0.5 mL of methylene blue mixed in 19.5 mL of water. A linear high-frequency 8- to 13-MHz probe (GE Venue 40, Wauwatosa, WI) was used for all injections. Each hemithorax was injected using 1 of 4 protocols per randomization:

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Dissection, Digitization and 3-Dimensional Modeling

Dissection of the specimen commenced 30 minutes after injection. With the cadaver in the supine position and the upper limb abducted to 45 degrees, the skin and superficial fascia were dissected to expose the pectoralis major muscle, the SAM, the latissimus dorsi muscle, and the interdigitating fiber bundles of the external oblique muscle. To establish a reference frame for digitization, 3 screws were drilled into bony landmarks including the left and right clavicles and the body of the sternum. Digitized data were collected using a Microscribe G2X Digitizer (Immersion Corporation, San Jose, CA) along with data collection software developed in our laboratory. Photographs were taken throughout the dissection process.

In specimens that received injections superficial to SAM (group SUP-20 and group SUP-40), the injectate spread was carefully exposed by removing any remaining overlying tissue. The perimeter and surface of the injectate spread were digitized at 5- to 7-mm intervals in a grid pattern. To map the location of the area of injectate spread, the ribs, costal cartilages, sternum, and SAM were exposed and digitized.

In specimens that received injections deep to the SAM (group DEEP-20 and group DEEP-40), the SAM was reflected following digitization of its external surface. Once completely exposed, the injectate spread was digitized. For localization of injectate spread, the ribs, costal cartilages, and sternum were fully exposed and digitized. The digitized data of each specimen were imported into Autodesk Maya (Autodesk, San Rafael, CA) for 3-dimensional (3D) reconstruction of the ribs, sternum, SAM, and the injectate spread.

Data Analysis

The location, level, and area of injectate spread were documented from the photographs and 3D models as follows: (1) the area of injectate spread was quantified from the 3D models using Autodesk Maya; (2) the location of injectate spread was defined relative to the midclavicular and anterior axillary, midaxillary, and posterior axillary lines (PALs); (3) the level of injectate spread was demarcated by the superior and inferior rib; (4) the lateral cutaneous branches of the intercostal nerves within the area of injectate spread were documented using the MAL as the reference point (Fig. 1). Comparison between the 4 groups was performed to determine which technique provided the most extensive injectate spread. Descriptive statistics, a 1-way analysis of variance, and independent-samples t tests were used to determine significant differences between groups.

RESULTS

We found that the volume of injection had the most impact on the extent of injectate spread as compared with the plane of

![FIGURE 1. Dissection showing anterior terminal branches of the lateral cutaneous branches of the intercostal nerves.](image)

TABLE 1. Location and Area of Dye Spread for Each Injection Protocol

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Superficial to SAM Single Injection (Group SUP-20)</th>
<th>Deep to SAM Single Injection (Group DEEP-20)</th>
<th>Superficial to SAM Double Injection (Group SUP-40)</th>
<th>Deep to SAM Double Injection (Group DEEP-40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>11 (5/6)</td>
<td>10 (4/6)</td>
<td>8 (3/5)</td>
<td>10 (4/6)</td>
</tr>
<tr>
<td>Mean area of dye spread, cm²</td>
<td>41.4 ± 16.4</td>
<td>42.1 ± 30.6</td>
<td>93.1 ± 31.1</td>
<td>103.5 ± 36.6</td>
</tr>
<tr>
<td>Location of dye spread</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axilla</td>
<td>18.2% (2/11)</td>
<td>None</td>
<td>62.5% (5/8)</td>
<td>70.0% (7/10)</td>
</tr>
<tr>
<td>Anterior to MCL</td>
<td>None</td>
<td>None</td>
<td>12.5% (1/8)</td>
<td>50.0% (5/10)</td>
</tr>
<tr>
<td>Between MCL and AAL</td>
<td>63.6% (7/11)</td>
<td>50.0% (5/10)</td>
<td>75.0% (6/10)</td>
<td>100.0% (10/10)</td>
</tr>
<tr>
<td>Between AAL to MAL</td>
<td>100.0% (11/11)</td>
<td>100.0% (10/10)</td>
<td>100.0% (8/8)</td>
<td>100.0% (10/10)</td>
</tr>
<tr>
<td>Between MAL and PAL</td>
<td>27.3% (3/11)</td>
<td>20.0% (2/10)</td>
<td>50.0% (4/8)</td>
<td>50.0% (5/10)</td>
</tr>
<tr>
<td>Posterior to PAL</td>
<td>9.1% (1/11)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Superior rib*</td>
<td>3 (3–4)</td>
<td>3 (3–5)</td>
<td>1 (1–3)</td>
<td>2 (1–2)</td>
</tr>
<tr>
<td>Inferior rib*</td>
<td>7 (6–8)</td>
<td>7 (5–9)</td>
<td>8 (6–9)</td>
<td>7 (6–8)</td>
</tr>
</tbody>
</table>

*Rib with greatest frequency (range).

Sup indicates superficial.
injection. With 2 injections (40 mL total), the greatest mean area of injectate spread in groups SUP-40 and DEEP-40 was more than double that for a single 20-mL injection (Table 1), and the difference was statistically significant (SUP-40 vs SUP-20: $t_{16} = -4.896 [P < 0.001]$; SUP-40 vs DEEP-20: $t_{15} = -3.678 [P = 0.002]$; DEEP-40 vs SUP-20: $t_{18} = -4.769 [P < 0.001]$; DEEP-40 vs DEEP-20: $t_{15} = -3.678 [P = 0.002]$). In contrast, the plane of injection, whether superficial or deep to SAM, had minimal impact on the extent of injectate spread irrespective of the injection volume, 41.4 versus 42.1 cm² for single injection ($t_{10} = 0.114, P = 910$) and 93.1 and 103.5 cm² for double injection($t_{14} = 0.570, P = 0.578$).

Dye stain was located most consistently at the location of dye injection (third and fifth ribs) along the anterior and MAL. In all specimens, injectate spread was visualized most reliably (~100%) between the MAL and AAL irrespective of the number or plane of injections (superficial or deep to SAM). Our injection technique (longitudinal scan, in-plane, caudad-to-cephalad needle advancement) seemed to favor anterior dye spread to reach the MCL in more than 50% of the cases with single injection and 75% to 100% with the double-injection technique. Posterior injectate spread was limited, however, with dye located between the MAL and PAL in 50% of the specimens following double injections and only 20% to 27.3% following single injection. Dye spread posterior to the PAL rarely happened (1/39 injection).

With respect to cephalad to caudad spread, our study found that a single 20-mL bolus injection achieved dye spread over 4 intercostal levels in the majority of cases, most often from the third to seventh rib after injection at the fifth rib level. However, there was wide variability in dye spread from 2 to 6 intercostal levels after a 20-mL injection and from 4 to 7 levels after a 40-mL injection. The most frequent inferior level of injectate spread was the seventh rib for all injection conditions. Superior injectate spread reached as high as the first and second ribs following a third rib level injection but only reached the third rib after a fifth rib level injection (Table 1). Similarly, dye spread to the axilla was more pronounced with double injections (62%–70% vs 0%–18% with single injection, Table 1 and Fig. 3) independent of the plane of injection (superficial or deep to SAM). Dye spread most consistently stained the T2 to T7 lateral cutaneous branches of the intercostal nerves. The anterior terminal branches of the lateral cutaneous branches of the intercostal nerves were stained at varying lengths over the anterior chest wall.

Throughout our study, we found that the fascial plane deep to SAM was easier to identify during hydrodissection under ultrasound guidance. This intermuscular plane was anatomically well defined to allow dye spread as noted in open dissection. In contrast, the plane superficial to SAM was less distinct and more difficult to establish during hydrodissection. On open dissection, patchy dye spread was frequently observed extending into subcutaneous fatty tissue away from the terminal intercostal nerve branches when injection was superficial to SAM (Fig. 2A).

**DISCUSSION**

Our anatomic study shows that of the 4 injection conditions studied the double-injection technique (one at the third rib and one at the fifth rib) resulted in the most extensive and consistent spread. The plane of injection, whether superficial or deep to the SAM, did not influence the total surface area of injectate spread or the anteroposterior and cephalad-to-caudad spread. In contrast, the number and volume of injections seemed to be more important determinants. The mean area of dye spread was doubled when the injectate volume was increased from 20 to 40 mL. The level of injection is also important. A more cephalad injection at the third rib level resulted in dye spread to the axilla and the first and second ribs.

Recently, Blanco et al. described 2 interfascial plane block techniques, PECS II and serratus plane blocks, for pain relief following breast surgery. With both techniques, local anesthetic may be injected either superficial or deep to the SAM. Whether a deep or superficial injection is preferred in the clinical setting is unknown. In a preliminary volunteer study, a greater extent of sensory anesthesia over the anterior chest wall and a longer duration were noted with a superficial injection (T2–T9 vs T2–T6 with deep injection). On the other hand, some argue that a deep injection is preferred to avoid local anesthetic spread to the long thoracic nerve and to promote greater caudad local anesthetic spread.

Our findings demonstrated that dye spread to the anterior and lateral chest wall and in the cephalad-to-caudad direction was not different between the deep and superficial injection techniques. However, we did note that dye injected deep to the SAM stayed in the SAM intercostal fascial compartment with minimal back diffusion to the superficial plane, supporting the observation that spread to the long thoracic nerve would be avoided.

Injection deep to the SAM has some other technical and theoretical advantages. For example, the injection plane between the SAM and external intercostal muscle/rib is well defined anatomically and easier to recognize sonographically than the plane superficial to SAM. This could result in greater needling precision and a more consistent injectate spread and analgesic response. A deep

**FIGURE 2.** Dye spread following a 40-mL injection. A, Injection in the fascial plane superficial to serratus anterior muscle. B, Injection deep to serratus anterior muscle. Ant, anterior; arrows, dye spread margins; Inf, inferior; PM, pectoralis major muscle; Post, posterior; SA, serratus anterior muscle; SC, subcutaneous tissue; Sup, superior.
injection likely prevents injectate spread into superficial subcutaneous fatty tissue away from the intercostal nerve branches. A deep injection targets the lateral cutaneous branches of the intercostal nerves before they emerge from the SAM and branch into anterior and posterior terminal branches. Local anesthetic injected superficial to the SAM may be “washed out” during a mastectomy procedure, whereas a deep injection is less likely affected by surgical dissection. Finally, the catheter dislodgement rate may be lower.3

Conceivably, success of PECS and SPBs is volume dependent as sufficient quantity of local anesthetic must reach the terminal branches of the thoracic intercostal nerves along the MAL to take effect.16 However, little is known about the optimal volume of injection to achieve the desired dermalomaternal spread and duration of sensory anesthesia in the clinical setting. Kunigo et al11 studied the impact of injectate volume (20 vs 40 mL) on anesthetic effect of SPB with a single 0.375% ropivacaine bolus injection at the fourth rib level. As expected, the 40-mL injection achieved more sensory levels (6 vs 4 dermatomes for cold and 4 vs 2 dermatomes for pinprick anesthesia). Anesthesia was most consistent and successful at the location of injection (T4 dermatome) but least detected at T1 and T8 dermatomes even with a 40-mL injection. This is not surprising. In our study, we also observed a high degree of variability in the extent of cephalad-to-caudad spread (from 2 to 6 intercostal levels) with a 20-mL bolus. When the volume was increased to 40 mL, spread was preferentially anterior and not in the cephalad-to-caudad direction. Contrary to Blanco and colleagues’ findings and consistent with Kunigo et al,11 we did not observe reliable spread over 5 to 6 levels after every 20-mL injection at the fifth rib level. Rather, a separate injection at the third rib level more reliably achieved dye spread to the first and second intercostal spaces than a fifth rib level injection. These observations suggest that it is time to rethink our injection strategy and evaluate if a double injection (eg, 20 mL each) to 3 intercostal spaces apart may be clinically more efficacious than a single 40-mL injection. Such practice is common with thoracic paravertebral and intercostal blocks where multilevel injections are preferred over a single large-volume injection to achieve consistent success. Also, it may be time to evaluate the minimum effective local anesthetic volume required for each SPB injection. If the goal is to anesthetize the lateral cutaneous branches of the thoracic intercostal nerves, perhaps a 10-mL injection is just as effective as 20 mL, pending future evaluation.

To relieve postmastectomy pain, PECS II and SPB aim to provide analgesic coverage by blocking the T2–T6 intercostal nerves along the MAL. In the past few years, a number of clinical SPB studies have reported variable success following a single-bolus superficial injection at the fourth rib,11 fifth rib,1,17,18 and sixth rib,9 as well as single-bolus deep injection at the fifth rib19 and sixth rib.20 Similarly for PECS II block, there were reports of a single-bolus superficial injection at the second/third rib,14 third rib,2,22 and fourth rib23 as well as a single-bolus deep injection at the second rib.13,24 Whether PECS II block or SPB is more suited for mastectomy and which rib level is the optimal level to inject remain a subject of debate at present, pending future comparative studies. Our study suggests that dye spread often reaches 1 to 2 levels cephalad and 1 to 2 levels caudad from the location of injection. If the goal is to provide T2 to T6 analgesic coverage, the fourth rib might be the most ideal level for a single-bolus injection, and the third and fifth rib for a double injection. Whether 2 separate 10-mL injections produce more reliable spread and equivalent analgesia as a single 20-mL injection is currently unknown, pending future study data. Also, if axillary dissection is required during mastectomy surgery, our study suggests a separate third rib level injection is recommended for cephalad local anesthetic spread.

Our study differs from past cadaver studies in several ways. Daga et al,9 Mayes et al,10 and Torre et al13 examined injectate spread following methylene blue dye or saline/air mixture injection deep to the SAM, whereas Varghese et al12 injected superficial to SAM. All past anatomical studies evaluated the spread pattern of a 20-mL injection. The effect of a larger-volume bolus injection has not been examined. Also, past cadaver studies have not compared the impact of a superficial injection with a deep injection using a standardized injection technique. Furthermore, using digitization and 3D reconstruction, we have documented injectate spread in situ and its spatial relationship with other
structures of interest including bone, muscle, nerves, and vessels. The 3D models represent a permanent record of the entire serial dissection carried out on each specimen. In previous anatomic studies, dye spread was documented using manual measurements, photographs, and/or line illustrations and mostly described the superior-inferior and anterior-posterior margins of spread. With unique digitization technology, the total area of spread is mapped, facilitating comparison between specimens as shown in Figure 3.

Study Limitations
In this study, we have examined the combined effect of the volume and number of SPB injections on the extent of spread. That is, 2 injections were made at 2 different rib levels when the injectate volume was increased from 20 to 40 mL. To assess the volume effect independently, both low and high volumes should have been administered at the same rib level. Likewise, to assess the impact of the number of injections independently, the total volume should have been kept to 20 mL, and two 10-mL injections would be necessary. One may argue that the spread pattern observed with 40 mL in this study was the result of a combined third rib and fifth rib injection. While this is true, we believe a high-volume injection technique we described in this study is clinically relevant and worthy of examination.

CONCLUSIONS
Our findings suggest that the number and total volume of SPB injections have a greater influence on the extent of injectate spread than the plane of injection (superficial or deep) relative to the SAM. A high-volume injection technique (40 mL divided between 2 injection levels) provides more extensive spread than a lower-volume injection (20 mL at a single level) to reach the anterior chest wall and the axilla. Future studies are required to validate these anatomical findings against clinical block success and the extent of analgesic coverage.

REFERENCES