Effect on Oximetry of Dyes Used for Sentinel Lymph Node Biopsy

Are There Differences?

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Hypothesis: There are differences between readings of peripheral blood oxygen saturation when the effect on saturation values of methylene blue is compared with that of isosulfan blue when used in sentinel lymph node biopsy in patients with breast cancer.

Design: Prospective randomized study.

Setting: University tertiary care center.

Patients: Thirty-two women undergoing surgery for breast cancer using sentinel lymph node biopsy.

Interventions: Sentinel lymph node biopsy using methylene blue (16 patients) and isosulfan blue (16 patients); there was also a control group of 6 surgical patients in whom dyes were not used.

Main Outcome Measures: Peripheral saturation of blood using pulse oximetry, oxygen saturation by blood-gas analysis, partial oxygen pressure by blood-gas analysis, and plasma dye levels, recorded before dye injection and 15, 30, and 60 minutes afterward.

Results: The 2 dyes interfered with the peripheral saturation reading, but only isosulfan blue showed significant differences. The differences in blood-gas analysis values between the 2 groups and with regard to the controls were not significant.

Conclusions: Methylene blue interferes less than isosulfan blue in the peripheral saturation reading. Desaturation is factitious in both cases, and does not correspond to alterations in blood-gas analysis values.

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Selective sentinel lymph node biopsy represents a major milestone in the treatment of patients with breast cancer. This method can identify lymph nodes with a greater likelihood of being involved in the event of lymphatic spread of the disease and has the advantages of being able to avoid unnecessary lymphadenectomies and staging the process correctly; it has been regarded as even more accurate than standard lymphadenectomy, because it carries out a much more exhaustive study of the isolated lymph node or lymph nodes by also using immunohistochemical techniques.

The agreed method for performing this technique combines the use of isotopic tracers and vital dyes, which yields a higher detection rate than the 2 methods used separately.

Several vital dyes have been used, notably methylene blue, isosulfan blue, and patent blue. Each of them has its specific advantages and disadvantages, but generally one of the most common drawbacks is interference with the oxygen saturation readings taken intraoperatively by the anesthetist using photospectrometric techniques. The staining caused when these substances enter the bloodstream, and before they are eliminated by the kidneys, interferes with the data collected via these techniques; even if it is a factitious desaturation, it may cause monitoring problems in these patients.

This study compares the effect on saturation values of 2 of the vital dyes (methylene blue and isosulfan blue) most commonly used for sentinel lymph node biopsy in patients with breast cancer.

Methods

A prospective randomized study was conducted on 32 women undergoing consecutive operations for breast cancer in whom sentinel lymph node biopsy was indicated. Excluded were patients with anemia, cardiopathy, bron-
chopath, and renal or hepatic insufficiency. The indications for sentinel lymph node biopsy included patients with single palpable tumors measuring less than 3 cm and those without clinically palpable adenopathies. All the patients were informed of the technique and gave their written consent. All of them underwent the mixed technique, with simultaneous use of an isotopic tracer (Nanocoll; Nicomed-Italia, Saluggia, Italy), injected around the tumor 2 to 12 hours before the operation, and a dye, injected around the areola after intubation of the patient and with a breast massage for at least 10 minutes. The patients were distributed consecutively in a 1:1 ratio to include 16 women in each of the following 2 groups: (1) 5 mL of 1% methylene blue and (2) 5 mL of 1% isosulfan blue (1% Lymphazurin; Ben Venue Laboratories Inc, Bedford, Ohio).

Six patients undergoing surgery under general anesthesia, without the use of dyes, were used as a control group; the same variables were recorded in these patients.

Monitoring was done in all the patients by pulse oximetry using a monitor (model S/5) and an oxygen transducer (Oxisensor II D25; Datex-Ohmeda Inc, Andover, Mass), with a red and infrared light emission of between 660 and 910 nm. The radial artery was cannulated during the operation to obtain samples for determining oxygen saturation via blood-gas analysis, partial oxygen pressure, and serum dye levels before dye injection and 15, 30, and 60 minutes afterward. The anesthetist (C.G.-P., V.C., or E.D.) recording the data was unaware of which dye had been injected.

Eight patients from each dye group underwent determinations of their serum dye levels at 15, 30, and 60 minutes after injection. The plasma was separated immediately by centrifugation (3000 rpm for 15 minutes), and the methylene blue and isosulfan blue levels were determined by the increase in absorbency at 666 and 638 nm, respectively, using a spectrophotometric sweep (Lambda 20; Perkin-Elmer, Torrance, Calif) between 570 and 720 nm, via the Allen method, correcting the absorbency of the plasma with the preoperative dye-free sample from each patient. The increase in absorbency was converted into concentration (milligrams per liter) using a standard curve for each dye.

All the patients maintained stable arterial tension values during surgery, with no significant fluctuations. There were no differences between the groups for age, serum creatinine levels, or fraction of inspired oxygen used during the operation (Table).

Comparison of the between-group quantitative variables was done using a means comparison with the t test, regarding a value of P < .05 as significant. To compare the figures for oxygen saturation (in blood-gas analysis and pulse oximetry), partial oxygen pressure at each time between the 2 dye groups, and plasma dye level in each group, an analysis of variance was used of repeated measurements corresponding to a hierarchical factorial design; the between-group analysis, represented by the graphs, was done by comparison of measurements using the minimum significant difference criterion. Statistical calculations were performed using a software application (BMDP; Statistical Software Inc, Los Angeles, Calif).

### RESULTS

As can be seen in Figures 1, 2, and 3, pulse oximetry showed a desaturation process with both dyes, but it was not accompanied by a real desaturation in the blood-gas analysis readings.

Differences were found in the pulse-oximetric saturation values 15 minutes after injection of the dye, the values being significantly lower when isosulfan blue was used (P < .001 at 15 minutes). Although the methylene blue group values were also lower than the control values, these differences were not statistically significant (Figure 1).

#### Table. Comparison of Groups With Regard to Age, Fio2, and Creatinine Levels*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group (n = 6)</th>
<th>Methylene Blue Group (n = 16)</th>
<th>Isosulfan Blue Group (n = 16)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.6 ± 0.8</td>
<td>56.5 ± 2.6</td>
<td>53.4 ± 2.1</td>
<td>.32</td>
</tr>
<tr>
<td>Fio2</td>
<td>35.8 ± 1.5</td>
<td>35.5 ± 0.8</td>
<td>35.0 ± 0.6</td>
<td>.62</td>
</tr>
<tr>
<td>Creatinine level, mg/dL</td>
<td>0.83 ± 0.03</td>
<td>0.80 ± 0.03</td>
<td>0.79 ± 0.04</td>
<td>.85</td>
</tr>
</tbody>
</table>

Abbreviation: Fio2, fraction of inspired oxygen.

SI conversion factor: To convert creatinine to micromoles per liter, multiply by 88.4.

*Data are given as mean ± SD.
those studied herein.\textsuperscript{7,10-17} Incorrect measurement of this saturation, which has generated much interest, results from the interference that dilution of the dye in the patient's plasma causes in the photometric measurement of peripheral blood oxygen saturation. In other words, this desaturation is not a real problem but the result of procedures occurring with the dyes occurred to the same extent with dyes that, while interfering with similar wavelengths in the photometric process, present differences in absorption and transport. This is the case of the 2 dyes studied herein.

In pulse oximetry, a sensor emits red and infrared light at a wavelength of between 660 and 910 nm. This light is partially absorbed by the blood, and the reflected part is picked up by the other sensor, which calculates peripheral arterial saturation based on the difference obtained. In physiological conditions, methylene blue and isosulfan blue have peak absorption at wavelengths of 666 and 638 nm, respectively. This implies that in the range of wavelengths used by the pulse oximeter, the dyes absorb much light and cause interference, leading to overestimation of the “desaturation” of hemoglobin.\textsuperscript{9,18}

Besides the interference by both dyes in the saturation measurement, the differences between them must also be considered. For a dye—regardless of which—to enter and mark the lymph node, it must have an adequate molecular size and reach the lymphatic system from the injection site.\textsuperscript{19} Furthermore, binding with endogenous proteins in the lymphatic system, generally via sulfonation reactions, allows the dye to be accumulated and transported through the lymphatic system.\textsuperscript{20}

Dyes also pass into the bloodstream, directly or after their migration through the lymphatic system, from where they are eliminated by renal clearing. It is in this migration that differences in molecular structure may explain the different behavior of dyes in the pulse-oximetric reading. Isosulfan blue is a widely used dye in sentinel lymph node biopsy. It is a patent blue isomer and, as such, has 2 sulfonic groups in its chemical structure, which, although to a small extent, allow protein binding in lymph and plasma.\textsuperscript{20,21} Conversely, methylene blue shows no affinity to proteins at a temperature of 37°C.\textsuperscript{20} It circulates in dissolved form, diffuses passively, directly into lymphatic and blood capillaries, and is theoretically retained less and cleared more from the bloodstream through the kidneys. All the same, molecular reaction mechanisms have been reported, independent of protein sulfhydryl groups, for compounds such as methylene blue, via conjugation to ammonium groups; these mechanisms explain the retention capacity of the lymphatic system.\textsuperscript{20} This might explain the findings of our study: although the 2 dyes interfere with the pulse-oximetry readings, methylene blue would do so to a lesser extent because of a smaller accumulation in the bloodstream, and would at all times register lower serum levels than isosulfan blue.

The potential relationship between (factitious) hypoxia and plasma dye levels is suggested by their reversed curves (Figures 1 and 4, respectively). The iso-
sulfan blue reading's return toward baseline (Figure 1) could be explained by differences between the dyes' renal clearance.

The difference observed between methylene blue and isosulfan blue is because of the plasma dye level and its interference with pulse oximetry due to different peak absorptions.

We see, therefore, that although both dyes are similarly useful for detecting the sentinel lymph node, methylene blue interferes less in the peripheral monitoring of saturation by pulse oximetry. If to this we add the greater availability of methylene blue and its lower cost, we may consider that methylene blue is a valid and even advantageous alternative to isosulfan blue in selective sentinel lymph node biopsy.

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