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.1,3

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.4,6 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-
chopathy, 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 S5) 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 anesthesiologist (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 determination 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. The infrared light emission of between 660 and 910 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).

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.
*Concentration conversion: To convert creatinine to micromoles per liter, multiply by 88.4.
*Data are given as mean ± SD.

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).
incorrect measurement of this saturation, which has gen-
terally for the patent blue V dye, there is no real alteration
node detection in patients with breast cancer include a
lymphatic system.20 This might explain the findings of
these mechanisms explain the retention capacity of the
methylation blue, via conjugation to ammonium groups;
transportation reactions, allows the dye to be accumulated
while interfering with similar wave-
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, meth-
eylene 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 "desatu-
ration" of hemoglobin.9,18

Besides the interference by both dyes in the satu-
ration 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 ade-
quately large molecular size and reach the lymphatic system from
the injection site.19 Furthermore, binding with endog-

When differences by time in oxygen saturation values
are considered for each group only, differences between values
for isosulfan blue at 15 and 60 minutes were signifi-
cant ($P<.001$), although a clear return toward baseline by
time was registered in isosulfan blue readings (Figure 1).
The differences between the figures for gasometric satu-
ratio and gasometric partial oxygen pressure between
the 2 groups and with regard to the controls were not
significant (Figures 2 and 3).

Determination of the plasma dye levels shows that the
methylene blue values are at all times significantly lower than the isosulfan blue values (Figure 4).

The effects reported when using dyes for sentinel lymph
detection in patients with breast cancer include a
desaturation in the pulse-oximetric reading taken by the
anesthetist as part of the patient’s monitoring proto-
col.7,8 This desaturation is not a real problem but the re-
sult of the interference that dilution of the dye in the pa-
tient’s plasma causes in the photometric measurement of peripheral blood oxygen saturation. In other words, it
does not reflect a decrease in oxygen saturation but an
incorrect measurement of this saturation, which has gen-

Some previous series had already shown that, particu-
larly for the patent blue V dye, there is no real alteration
in oxygenation when the peripheral saturation reading
is compared with that obtained in the blood-gas analy-
sis.9 Our results corroborate these findings by showing
that although there is desaturation in the pulse-

 oximetric reading, there are no significant differences in
the figures for partial oxygen pressure and arterial satu-
ratio in the blood-gas analysis. To our knowledge, it has
not been determined whether this factitious desaturation
process occurring with the dyes occurred to the same
extent with dyes that, while interfering with similar wave-
lengths 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, meth-
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Besides the interference by both dyes in the satu-
ration 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 ade-
quately large molecular size and reach the lymphatic system from
the injection site.19 Furthermore, binding with endog-
nous proteins in the lymphatic system, generally via sul-
fonation reactions, allows the dye to be accumulated
and transported through the lymphatic system.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 migra-
tion that differences in molecular structure may explain
the different behavior of dyes in the pulse-oximetric read-
ing. Isosulfan blue is a widely used dye in selective sen-
tinel 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 bind-
ing in lymph and plasma.20,21 Conversely, methylene blue
shows no affinity to proteins at a temperature of 37°C20;
its circulation in dissolved form, diffuses passively, di-
rectly into lymphatic and blood capillaries, and is theo-
retically retained less and cleared more from the blood-
stream 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.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 blood-
stream, and would at all times register lower serum lev-
els than isosulfan blue.

The potential relationship between (factitious) hy-
poxia and plasma dye levels is suggested by their re-
versed curves (Figures 1 and 4, respectively). The iso-

Figure 3. Evolution of arterial oxygen saturation values recorded by
blood-gas analysis. Data are given as mean ± SE. For the control group vs
the methylene blue group, $P=.18$; for the control group vs the isosulfan blue
group, $P=.18$; and for the methylene blue group vs the isosulfan blue
group, $P=.14$.

Figure 4. Evolution of plasma dye content in the 2 groups. Data are given as
mean ± SE. $P=.01$ for the difference between the 2 groups.
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,22-24 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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REFERENCES