Blue dye for identification of sentinel nodes in breast cancer and malignant melanoma: a systematic review

Abstract

The combined technique (radioisotope and blue dye) is the gold standard for sentinel lymph node biopsy (SLNB) and there is wide variation in techniques and blue dyes used. We performed a systematic review to assess the need for radioisotope and the optimal blue dye for SLNB. A total of 21 studies were included. The SLNB identification rates are high with all the commonly used blue dyes. Furthermore, methylene blue is superior to iso-sulphan blue and Patent Blue V with respect to false negative rates. The combined technique remains the most accurate and effective technique for SLNB. In order to standardise the SLNB technique, comparative trials to determine the most effective blue dye and national guidelines are required.

Keywords

Sentinel lymph node biopsy (SLNB); Combined technique; Radioisotope; Blue dye; Methylene blue; Patent Blue V; Iso-sulphan blue; Breast; Malignant melanoma.

Introduction

Sentinel lymph node biopsy (SLNB) has been used in breast cancer and malignant melanoma for surgical lymph nodal staging since the early 90's [1]. The standard technique for localising sentinel lymph nodes is the combined technique of radioisotope (99mTc) and blue dye injected into the breast or near the melanoma site, however there is wide variation in dyes and techniques used [2]. SLNB was first performed using a radioisotope injection in 1993 by Krag *et al*. [3]. Blue dye was first reported in 1992 when Morton *et al.* used iso-sulphan blue (Mylan Institutional LLC, United States) and Patent Blue V (Guerbet, France) for SLNB in 223 patients, obtaining an identification rate of 82% [1]. By using a combination of both techniques a higher identification rate is achievable [4, 5]. In experienced hands high identification rates of up to 96% are achieved with blue dye alone [6].

The use of radioisotopes creates logistical challenges for hospitals, including the handling and disposal of radioisotopes, training of staff, and legislative requirements. These factors in addition to lack of access to radioisotopes, have limited the uptake of SLNB worldwide. Although the incidence of cancer is rising, the use of the SLNB procedure has reached a plateau, with around 60% of an estimated 500,000 patients in developed countries having access to the procedure. This figure falls to 5% in China and is even lower in the rest of the world [7-9]. So for developed countries were radioisotopes are readily available, there is interest in eliminating blue dye and using radioisotope on its own but current evidence suggests SLN identification rate is significantly lower with radioisotope alone. Whereas worldwide and in developing countries, where there is limited or no access to radioisotopes, there is interest in using blue dye alone and in ascertaining which is the optimal blue dye to use.

The most common blue dyes used in SLNB are iso-sulphan blue 1%, Patent Blue V sodium 2.5% and methylene blue 1% (*figure 1*); other dyes such as indigocarmine or indocyanine have been used in the Far East due to lack of availability to other blue dyes [5, 10]. Iso-sulphan blue, otherwise known as lymphazurin blue, is an isomer of Patent Blue V which has two different constituents, a calcium and a sodium based salt, the latter being used in SLNB [5]. Iso-sulphan blue has been applied as a colouring agent in textiles, cosmetics and the paper and leather industry [11]. It binds to albumin and other local proteins and is absorbed

by the lymphatic system, which makes it suitable for SLNBs [12]. Adverse events such as interference with the pulse oxygen oxymetry, blue tattooing of the skin, discoloration of body fluids and anaphylactic and allergic reactions have been reported in the literature [11-18]. A skin test can be performed to detect any hypersensitivity but it lacks sensitivity [15-17].

Patent Blue V dye has been used for food colouring, cosmetics, textiles and in the paper industry [19]. It is recommended by the Association of Breast Surgery for use in SLNB in the United Kingdom [2, 20]. It shares similar mechanisms of action and adverse events with iso-sulphan blue [19, 21, 22]. Patent Blue V is also known by the names alphazurine, sulfan blue, sulphane blue, Patent blue violet and Patent blue pure [5].

Methylene blue (Akorn, United Kingdom; American Regent, United States; and Colonis Pharma, United Kingdom) is a dark green crystalline compound, which becomes dark blue in solution [5]. It has been commonly used in medicine in both diagnostic and therapeutic procedures [5]. It also has been used in resuscitation to improve the outcomes in patients with hypovolemic states [23]. Due to the wider availability and lower cost, many centres have changed their practice to methylene blue [24-26].

Kim *et al.* [27] published a systematic review in which 69 trials were evaluated performing SLNB followed by axillary lymph node clearance for early breast cancer and concluded that the combined technique has a better identification rate compared to the radioisotope or blue dye techniques alone. Furthermore, Valsecchi *et al.* [28] performed a meta-analysis of 71 studies which used SLNB for staging of malignant melanoma and found a mean identification rate of 98.1% and a mean false negative rate of 12.5% supporting the use of SLNB for staging patients with malignant melanoma. However, five-ten years later, there are still no national or international guidelines pertaining to standards of using the combined radioisotope and blue dye technique in SLNB.

We performed a systematic review to assess the need for radioisotope and the optimal blue dye for SLNB in breast cancer and malignant melanoma. This systematic review was performed to assess the need for radioisotope and the optimal blue dye for SLNB in breast cancer and malignant melanoma. There is currently no standardised technique for SLNB, as several blue dyes are used and some centres have stopped using radioisotope or blue dye

altogether, even though several systematic reviews have demonstrated the superiority of the combined technique.

Materials and Methods

Study selection

A systematic review of the literature was performed using PubMed and Medline databases to identify all studies published up to June 2015 evaluating the role of blue dyes for SLNB. The MESH terms used were sentinel AND node AND cancer AND any combination of blue dye, methylene blue (MBD), isosulph* blue (IBD), Patent Blue (PBD), indigocarmine (IDC), sulphan blue, sulphane blue, patent violet or patent pure. We required reports to be in the English language and the subjects to be human. To broaden the search the related articles function was used. References of included articles were searched by hand to broaden the search. The last search was conducted on June 26th, 2015.

Inclusion criteria

Studies were eligible if they met the following criteria: (1) studies performed on human subjects with breast cancer or malignant melanoma, (2) studies including radioisotope in a comparative arm (3) studies including blue dye in both comparative arms, (4) studies describing the identification rate and/or (5) studies describing the complication rates. For studies with overlapping study populations, only the most recent study was included.

Exclusion criteria

Studies were excluded if they failed to meet the inclusion criteria. Studies in which all patients had SLNB with the same technique, studies with inconsistent injection site and/or studies reporting on less than 50 patients were excluded. Studies using different types of radioisotope, indocyanine green or magnetic dye were excluded as lymph nodes localised with these technique cannot be identified solely by direct vision. Letters, editorials and case reports were also excluded from the study.

Data extraction

Each study was evaluated for either inclusion or exclusion. One reviewer, (M.P.) extracted data from all selected studies and a second reviewer (P.C.) verified the accuracy of the extracted data. In case of a disagreement, the senior author (M.D.) made the final decision.

Risk of bias in individual studies

To determine the suitability of randomised controlled trials (RCTs), the "Risk of bias" tool, as described in the Cochrane Handbook [29] was used. The quality of cohort studies was assessed according to the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [30]. Six items of the amended STROBE statement were considered relevant for quality evaluation. Studies with a score of less than four were excluded. Two reviewers (M.P. and P.C.) independently performed the assessment. In case of discordance, the senior author (M.D.) made the final decision.

Statistical analysis

All extracted data were tabulated, synthesized and presented as means and percentages. Numerators and denominators were provided to address outcomes of included studies. For continuous variables the mean ± standard deviation (SD), median and range were extracted and reported where available. The false negative rate was defined as the percentage of involved nodes missed with respectively the combined or the blue dye technique alone. Meta-analysis was performed using network analysis, with two random effects (correcting for blue dye and radioisotope) using maximum likelihood via a purpose written FORTRAN program. The probabilities were presented in probability ± standard error (95% confidence interval limits).

Results

Selected studies

A total of 1825 articles published up to June 2015 were identified from the literature search (figure 2). Searching through references of included articles identified four further articles. After reviewing the titles and abstracts, 1722 articles were excluded and 107 articles underwent full text examination. A total of 22 articles fulfilled the inclusion criteria.

However, one study [31] reported on less than 50 patients and therefore was excluded, resulting in a total of 21 studies [24, 32-51] deemed eligible for further analysis.

Study characteristics

A total of 21 studies with 6082 clinically node negative patients and 6133 SLNBs were included in this systematic review. Eligible studies encompassed 11 prospective studies [24, 33, 35, 37-39, 46-48, 50, 51], six retrospective studies [32, 34, 36, 40, 42, 49], three randomised studies [43-45] and one RCT [41]. The mean age was 55.7±2.2 (17-87 years, 12 studies (2082 patients)) [24, 34, 35, 37, 39-41, 45, 46, 48, 50, 51]. SLNB was performed for staging of the axilla in clinically node negative patients with breast cancer in 18 studies [24, 33-39, 41, 42, 44-51] and melanoma in three studies [32, 40, 43]. Studies on melanoma included 441 patients with melanomas located in the head and neck, upper and lower extremities and torso. Tumour characteristics and the type of surgery were not reported [32, 40, 43]. In studies on breast cancer, 1615 patients (28.6%) were diagnosed with invasive carcinoma, 92 patients (1.6%) with non-invasive carcinoma and the tumour type was not reported in 3934 patients (69.7%). Breast conserving surgery was performed in 633 patients (11.2%), mastectomy in 560 patients (9.9%) and the type of surgery was not reported in 4448 patients (78.9%). SLNBs in patients with breast recurrence were not included or not reported in the studies.

All studies used the radioisotope technique in a proportion of patients. The radioisotope was injected between 1-7 days before surgery in one study [43], on the day prior to surgery in ten studies [33-35, 37, 40, 42, 45, 48-50], on the morning of surgery in five studies [39, 41, 46, 47, 51] and the time of injection was not reported in five studies (table 1) [24, 32, 36, 38, 44]. The radioisotope was injected peri-tumorally in 11 studies [24, 33, 35-38, 42, 45, 48, 49, 51], intra-dermally in four studies [32, 40, 43, 47], subdermally in three studies [34, 41, 50], peri-areolarly in one study [39] and not reported in two studies [44, 46]. In 12 studies [32-34, 37, 38, 40, 43, 46-50] between 0-1mCi of radioisotope was injected, more than 1mCi was injected in four studies [35, 36, 45, 51], between 1-2ml in two studies [24, 42] and not reported in three studies.

Patent Blue V was used in ten studies [33, 35, 38-41, 45, 48, 49, 51], methylene blue in six studies [24, 34, 37, 39, 43, 50] and iso-sulphan blue in eight studies [24, 32, 36, 42-44, 46,

47]. The blue dye was injected on the morning of surgery in one study [49], just prior to surgery in 18 studies [24, 32-35, 37-43, 45-48, 50, 51] and the time of injection was not reported in two studies (table 1) [36, 44]. Blue dye was injected peri-tumorally in 12 studies [24, 33, 35, 36, 38, 42, 45-49, 51], sub-areolarly in three studies [37, 39, 50], intra-dermally in three studies [32, 40, 43], subdermally in two studies [34, 41] and was not reported in one study [44]. Between 0-1ml of blue dye was injected in five studies [32, 36, 40, 41, 50], 1-2 ml in six studies [35, 39, 43, 45, 48, 49], 2-5 ml in eight studies [24, 33, 37, 38, 42, 46, 47, 51], more than 5 ml in one study [34] and one study [44] did not report on the injected dose.

Patent Blue V alone was compared to the combined technique in nine studies [33, 35, 38, 40, 41, 45, 48, 49, 51], iso-sulphan blue alone was compared to the combined technique in six studies [32, 36, 42, 44, 46, 47] and methylene blue alone was compared to the combined technique in three studies [34, 37, 50].

Methylene blue was compared to iso-sulphan blue in two studies [24, 43] and was compared to Patent Blue V in one study [39]. All three studies [24, 39, 43] were performed with radioisotope injected in all patients.

Quality assessment

Six criteria of the amended STROBE statement [30] were used to perform a quality assessment of the included cohort studies (table 2a). All studies stated their study objectives and all but one study [36] reported on clear inclusion criteria. The SLNB technique was standardised in all but three studies [24, 32, 49] and standardised histopathology was not used in five studies [33, 34, 42, 45, 51]. Patients were followed-up after surgery in two studies [32, 40] and one study [40] reported on incomplete data which caused withdrawals from the study. The overall STROBE score was 4.6±0.5 (4.0-5.0). Three studies [35, 38, 44] used previously published information which described all relevant information.

The Cochrane checklist [29] was used to determine the quality of the RCT [41] (table 2b). Adequate sequence generation was present, patients were randomised and a power analysis was performed. Concealed allocation was not applied and blinding was not possible

due to different injection procedures. Incomplete data were not addressed and selective or other biases were not found. The study had a mean score of 5.0.

Outcomes

SLNB identification rate

The identification rate of the combined technique was 95.0±5.7% (82-100%) and with blue dye alone 86.2±10.0% (65-98%; tables 3 and 4). The identification rate by the type of blue dye was 83.2±10.3% (65-96%) [33, 35, 38, 40, 41, 45, 48, 49, 51] with Patent Blue V, 92.7±8.4% (83-98%) [34, 37, 50] with methylene blue and 86.7±9.3% (73-98%) [32, 36, 42, 44, 46, 47] with iso-sulphan blue. Combining blue dye with radioisotope showed an identification rate of 94.7±5.6% (83-100%) [33, 35, 38-41, 45, 48, 49, 51] with Patent Blue V, 97.7±2.3% (94-100%) [24, 34, 37, 39, 43, 50] with methylene blue and 93.4±7.0% (82-100%) [24, 32, 36, 42-44, 46, 47] with iso-sulphan blue.

Setting the random effects to zero, it appears that the blue dyes differ in probability of identifying a node, with methylene blue having a greater probability than Patent Blue V (p=0.0122). Including random effects for the radioisotope and the blue dye shows that there is no evidence that the three blue dyes differ in probability of identifying a node, with probabilities (± standard error (95% confidence interval)) of 0.945±0.0059 (0.933, 0.956), 0.946±0.0082 (0.929, 0.961) and 0.942±0.0082 (0.925, 0.958) for respectively iso-sulphan blue, methylene blue and Patent Blue V. The mean probability that the radioisotope will detect nodes which have not been detected by blue dye is 0.610±0.0352 (0.546, 0.684).

The identification rate when using blue dye alone was 85.3%±10.2% in the breast studies and 90.0%±0.0% in the melanoma studies. Adding radioisotope gives identification rates of 94.2%±5.7% and 99.0%±2.0% respectively. An additional term was added to the log-odds ratio to determine if a melanoma study caused any difference in the identification rate and this showed no significance (p=0.55). Hence we performed analysis on both breast and melanoma studies together.

Lymph node retrieval rate per patient

Mean lymph node retrieval rate per patient was 1.8±0.3 nodes (1.3-2.5 nodes) for the combined technique whereas for blue dye alone it was 1.6±0.3 nodes (1.1-2.1 nodes; table 3). By type of blue dye, mean lymph node retrieval rate per patient was 1.5±0.3 nodes (1.1-1.8 nodes) [33, 35, 40, 41, 48, 49] with Patent Blue V, 1.7±0.2 nodes (1.5-1.9 nodes) [34, 37, 50] with methylene blue and 1.8±0.4 nodes (1.4-2.1 nodes) [32, 42, 46] with iso-sulphan blue. When combined with radioisotope the mean lymph node retrieval rate per patient was 1.7±0.3 nodes (1.3-2.1 nodes) [33, 35, 40, 41, 48, 49] with Patent Blue V, 1.9±0.4 nodes (1.6-2.5 nodes) [24, 34, 37, 50] with methylene blue and 1.8±0.3 nodes (1.4-2.0 nodes) [24, 32, 42, 46] with iso-sulphan blue.

The node retrieval rates were 1.62±0.3 nodes for the breast studies and 1.40±0.0 with the melanoma studies with blue dye alone and 1.78±0.34 nodes versus 1.65±0.35 nodes with blue dye and radioisotope, respectively.

False negative rate

The mean false negative rate (missed involved nodes not detected during SLNB but with axillary node clearance) of the blue dye alone technique was 11.5±7.4% (0-23%) [33-37, 41, 42, 44, 45, 48-50]. For the combined technique the mean false negative rate was 7.5±8.7% (0-33%) [33-37, 40-42, 44, 45, 48-50]. Looking at the blue dyes separately, the mean false negative rate for Patent Blue V was 9.9±8.4% (4-23%) [33, 35, 41, 45, 48, 49], methylene blue 6.4±8.2% (4-16%) [34, 37, 50] and iso-sulphan blue 13.3±2.0% (11-15%) [36, 42, 44].

With random effects it is seen that the probabilities of a false negative for iso-sulphan blue and Patent Blue V differ significantly from methylene blue. Hence, methylene blue has significantly fewer false negative nodes than either of the other blue dyes, with probabilities of 0.076±0.022 (0.026, 0.113), 0.027±0.009 (0.011, 0.046) and 0.055±0.016 (0.021, 0.084) for respectively iso-sulphan blue, methylene blue and Patent Blue V. The mean probability for a false negative node with radioisotope is 0.524±0.114 (0.179, 0.740).

The blue dyes alone have probabilities of 0.146±0.015 (0.119, 0.018), 0.0523±0.014 (0.0304, 0.0829) and 0.106±0.024 (0.067, 0.159), respectively.

Breast studies using blue dye only had a false negative rate of 11.5%±7.4%. No studies with melanoma reported false negative rates for blue dye alone. With addition of radioisotope

the false negative rate was 5.4%±4.0% for breast and 33%±0.0% for melanoma. No difference was seen between breast and melanoma studies due to a lack of information on false negative rates in melanoma studies.

Histopathology

Histopathologic characteristics are shown in table 3 and 4. A total of 4093 patients (67.3%) had normal SLNs, 1698 patients (27.9%) involved SLNs (micro- or macro-metastases) and for 291 patients (4.8%) nodal involvement was not reported. The proportion of macro- and micro-metastases was not reported in almost all articles.

Out of the 1698 patients with involved nodes, 555/2433 patients (22.8%) treated with iso-sulphan blue, 192/726 patients (26.4%) with methylene Blue and 831/2322 patients (35.8%) with Patent Blue V had involved nodes. In one comparative study [39] no separation was made for involved nodes between the two blue dye groups.

Adverse event and recurrence rates

Adverse events were documented in five studies [34, 38-40, 43]. Three studies [34, 40, 43] reported no adverse events and two studies [38, 39] reported allergic reactions in 0.2% (3/1824, Patent Blue V, peri-tumorally injection of 2-5 ml), local inflammation at the injection site in 0.3% (6/1824, five methylene blue and one Patent Blue V, sub-areolar injection of 1-2ml) and skin discoloration in 3.2% (59/1824, 22 methylene blue and 37 Patent Blue V, sub-areolar injection of 1-2ml).

Tumour recurrence was documented in three studies [32, 40, 49] of which two studies [32, 49] did not found any recurrence and one study [40] reported on recurrence in 1.4% (9/644).

Discussion

The combined technique has a high mean identification rate of 95.0±5.7%, a lymph node retrieval rate of 1.8±0.3 nodes per patient and a false negative rate of 7.5±8.7%. It should be used by centres with access to radioisotopes as standard of care. For blue dye alone, the mean identification rate was 86.2±10.0%, lymph node retrieval rate was 1.6±0.3 nodes per patient and false negative rate was 11.5±7.4%. Blue dye alone is an inferior technique to the

combined technique and the blue dye technology still has a long way to go to be perfected. This can be done by improving the type of blue dye and by further identifying the anatomy of the lymph nodes [52].

Several articles reported a learning curve associated with using blue dye for SLNB [33, 35, 37, 41, 46, 47]. The more recent published literature does not report on the learning curve or correct for it, which would explain the observed differences in identification and false negative rates. Also, not all studies performed lymph node clearance, which would enable a more accurate assessment of false negative rate.

The outcomes also differed with type of blue dye used. The identification rates were highest with methylene blue (92.7±8.4%) and lowest with Patent Blue V (83.2±10.3%); lymph node retrieval rates were lowest with Patent Blue V (1.5±0.3) and highest with iso-sulphan blue (1.8±0.4); false negative rates were lowest with methylene blue (6.4±8.2%) and highest with iso-sulphan blue (13.3±2.0%). The diversity in identification rates for the different blue dyes could also be attributed partially to differences in surgeons' learning curve, as this was not reported in the included studies.

Methylene blue had the highest identification rates and lowest false negative rates suggesting that it may be superior to the other blue dyes. Statistical analysis confirmed that methylene blue is superior to the other blue dyes with respects to false negative rates. For the identification rates, it appeared that methylene blue was superior as well, however, after adding two random effects it was shown that there was no significant difference between the blue dyes in terms of identification rates. Only three studies (670 patients) compared different blue dyes and larger studies are required in order to standardise the SLNB technique. This is particularly important from a clinical perspective (avoidance of wide variations in SLNB technique) and for future trials comparing novel tracers against the combined technique.

The ideal blue dye would be the dye with the highest identification rate but also with the lowest adverse event rate. This is predominantly important in large breast cancer centres where even a low incidence of anaphylaxis could significantly impact on practice. In remote centres or satellite day-surgery centres, a small but significant risk of anaphylaxis is an important issue. The incidence and severity of adverse events following injection were

under reported and only described in five out of 21 studies (1824 patients). The allergic reaction rate of 0.2% (3/1824) is lower than the 1% rate reported by both the ALMANAC trial and NEW START [10, 53]. Tattooing of the skin is rarely reported suggesting that this is not of particular concern to patients or clinicians. There is insufficient evidence to compare the incidence of allergic reactions between the different blue dyes.

There is wide variation in dyes and techniques used for SLNB [2]. National guidelines for SLNB are recommended in order to ensure correct documentation of technique and reporting of adverse events. Furthermore it would resolve the current wide variation in blue dyes used. Patent Blue V is currently licensed as a medical product in France. Irrespectively, it is the most common blue dye used for SLNB in some countries, for instance in the UK. Iso-sulphan blue is used in North America and less often in Europe as it is unlicensed. Methylene blue dye is CE-marked as an injectable device with one the indications for use being visualisation of sentinel lymph nodes (Colonis Pharma Ltd, UK). Despite this, it is not often used for SLNB in the UK. Furthermore in a cost effectiveness performed by Gold *et al*. [54] it was shown that methylene blue is much more cost effective compared to iso-sulphan blue and the costs are also lower compared to Patent Blue V. This is potentially important in developing were lack of access to radioisotope prevents introduction to SLNB [7].

The current combined technique has a mean SLN identification rate of 97% in breast cancer and 98.1% in malignant melanoma [10, 28, 53]. Hence, finding a non-inferior surrogate for the combined technique remains challenging. Techniques using microbubbles and magnetic nanoparticles are currently under investigation, with a view to overcome the drawbacks portended by the radioisotope use and to make the technique more widely available.

Conclusion

The SLNB identification rates are high with all the commonly used blue dyes. Furthermore, methylene blue is superior to iso-sulphan blue and Patent Blue V with respect to false negative rates. The combined technique remains the most accurate and effective surgical technique for SLNB and radioisotope should continue to be used together with blue dye. In order to standardise the SLNB technique, comparative trials are required to determine the most effective blue dye for SLNB. National guidelines for SLNB are required in order to ensure documentation of technique and reporting of adverse events.

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Future perspective

In the future, SLNB may not be offered routinely as currently over 70% of breast cancer patients are found to be node negative. Until there are significant improvements in axillary imaging, SLNB will continue to be used routinely for staging early breast cancer. It is important that the SLNB technique is standardised and this requires national or international guidelines. This should also include the minimum dataset required for appropriate operative documentation to enable subsequent assessment of outcome and for auditing purposes. The combined technique remains the most accurate and effective surgical technique for SLNB and radioisotope should continue to be used together with blue dye. Any future novel SLNB technique should be evaluated against the combined technique within a randomised controlled trial. The type of blue dye will need be standardised and this will depend on the most readily available blue dye used at the participating sites.

Executive Summary

Introduction

- The combined technique is the gold standard for sentinel lymph node biopsy (SLNB), however there is wide variation in techniques and blue dyes used.
- A systematic review was performed to assess the need for radioisotope and the optimal blue dye for SLNB.

Methods

- We identified all studies published up to June 2015, evaluating the role of blue dyes for SLNB in breast cancer and malignant melanoma.
- Studies were considered eligible if they compared a SLNB technique which included blue dye and reported on the identification and/or complication rates.

Results

- A total of 21 studies were included using Patent Blue V in ten studies, methylene blue in six studies and iso-sulphan blue in eight studies.
- The combined and blue dye alone techniques had mean identification rates of 95.0±5.7% and 86.2±10.0%. The identification rates of iso-sulphan blue, methylene blue and Patent Blue V alone were 86.7±9.3%, 92.7±8.4% and 83.2±10.3%.

- Lymph node retrieval rates of iso-sulphan blue, methylene blue and Patent Blue V alone were 1.8±0.4, 1.7±0.2 and 1.5±0.3 nodes and false negative rates were 13.3±2.0%, 6.4±8.2% and 9.9±8.4%.

Discussion

- The blue dye alone technique is inferior to the combined technique.
- Standardisation of procedures is important for future trials comparing novel tracers like microbubbles and magnetic nanoparticles, against the combined technique.
- There is insufficient evidence to compare the incidence of allergic reactions between the different blue dyes.

Conclusions

- The SLNB identification rate is high with all the commonly used blue dyes but higher with the combined technique. Methylene blue is superior to Patent Blue V and iso-sulphan blue with respect to false negative rates.
- In order to standardise the SLNB technique, comparative trials to determine the most effective blue dye and national guidelines are required.

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Reference annotations

- Ref 2*: Guidance which are of interest as is states the different types of blue dyes used for SLNB.
- Ref 24**: A comparative trial, as recommended by our study, comparing methylene blue with iso-sulphan blue for SLNB in patients with breast cancer.
- Ref 27*: A systematic review in which 69 trials were evaluated performing SLNB followed by axillary lymph node clearance for early breast cancer.
- Ref 28*: A meta-analysis of 71 studies which used SLNB for staging of malignant melanoma.
- Ref 39**: A comparative trial, as recommended by our study, comparing methylene blue with Patent Blue V for SLNB in patients with breast cancer.
- Ref 43**: A comparative trial, as recommended by our study, comparing methylene blue with iso-sulphan blue for SLNB in patients with malignant melanoma.