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TITLE

Technological (R)Evolution Leads to Detection of More Sentinel Nodes in Patients with Melanoma in the Head and Neck Region

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Running Head

(R)Evolution of Sentinel Node Biopsy

Keywords

Melanoma; head and neck; sentinel lymph node biopsy; false negative

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ABSTRACT

Background

Sentinel lymph node (SN) biopsy (SLNB) has proven to be a valuable tool for staging melanoma patients. Since its introduction in the early 1990s, this procedure has undergone several technological refinements, including the introduction of single photon emission computed tomography combined with computed tomography (SPECT/CT) as well as radio- and fluorescence-guidance. The purpose of the current study was to evaluate the effect of this technological evolution on SLNB in the head and neck region. Primary endpoint was the false-negative (FN) rate. Secondary endpoints were number of harvested SNs, overall operation time, operation time per harvested SN and postoperative complications.

Patients and Methods

A retrospective database was queried for cutaneous head and neck melanoma patients who underwent SLNB at The Netherlands Cancer Institute between 1993 and 2016. The implementation of new detection techniques was divided in 4 groups: (1) 1993-2005, with preoperative lymphoscintigraphy and intraoperative use of both a gamma ray detection probe and patent blue (n=30); (2) 2006-2007, with addition of preoperative roadmaps based on SPECT/CT (n=15); (3) 2008-2009, with intraoperative use of a portable gamma camera (n=40); and (4) 2010-2016, with the addition of near-infrared fluorescence guidance (n=192).

Results

A total of 277 patients were included. At least one SN was identified in all patients. A tumor-positive SN was found in 59 patients (21.3%), 10 in group 1 (33.3%), 3 in group 2 (20.0%), 6 in group 3 (15.0%) and 40 in group 4 (20.8%). Regional recurrences of patients with tumor-negative SNs resulted in an overall FN rate of 11.9% (FN groups 1: 16.7%; 2: 0%; 3: 14.3%; 4: 11.1%). The number of harvested nodes increased with advancing technologies (p=0.003) whereas Breslow thickness and operation time per harvested SN decreased (p=0.003 and p=0.017, respectively). There was no significant difference in percentage of tumor-positive SNs, overall operation time and complication rate between the different groups.

Conclusion

The use of advanced detection technologies led to a higher number of identified SNs without increase in overall operation time, which may indicate an improved surgical efficiency. Operation time per harvested SN decreased, the average FN rate remained 11.9% and unchanged over 23 years. There was no significant change in postoperative complication rate.

INTRODUCTION

One fifth of all cutaneous melanomas occur in the head and neck region.[1] Sentinel lymph node (SN) biopsy (SLNB) for head and neck melanoma was introduced at our institute in the early 1990s for patients with clinically localized disease.(2,3) SLNB improves survival of node positive patients and the tumor status of the SN is the strongest prognostic factor.(4) The more accurate staging facilitates selection of patients for adjuvant therapy and for trials.(5)

Detection of SNs in the head and neck is often challenging because of the complex anatomy and interlacing lymph vessels can yield unexpected drainage patterns to multiple and bilateral sites.[6] Moreover, nodes in the head and neck region, especially in the parotid gland, are easily overlooked on lymphoscintigrams due to their proximity to the injection site where most of the radioactive tracer remains. These factors are responsible for a median false negative (FN) rate of 20.4% in the reviewed reports with a range of 3.3-44%.(6–9)

In recent years various complementing SLNB technologies facilitated the procedure for lymphatic mapping. First, ^{99m}Tc-nanocolloid was used for dynamic and static lymphoscintigraphy to map the lymphatic drainage and for intraoperative gamma ray detection probe tracing in combination with patent blue to visualize the afferent lymph vessels and the SNs.(10,11) Due to disadvantages like allergic reactions, coloring the skin and its fast shifting, patent blue is nowadays omitted in the head and neck.(12–15) The addition of preoperative single photon emission computed tomography combined with computed tomography (SPECT/CT) visualized SNs in their anatomic context.(16) With the introduction of intraoperative use of a portable gamma camera a better overview of the SNs in the surgical field was provided.(17) Lastly, a complex of indocyanine green (ICG) with ^{99m}Tc-nanocolloid was implemented to integrate near-infrared fluorescence imaging in the procedure.(18–20) This facilitated detection of superficial (<1cm deep) lymphatic vessels and SNs, using a dedicated fluorescence camera. So, the currently used technologies enable preoperative visualization of the lymphatic drainage pattern, imaging of the SNs within the surrounding anatomy, intraoperative tracing of radioactive SNs and visualization of the afferent lymphatic ducts and lymph nodes.

The purpose of this study was to determine the impact of the sequential technical advances, over a period of 23 years, on the outcomes of the SLNB in patients with a melanoma in the head and neck

region. Primary endpoint was the FN rate. Secondary endpoints were number of harvested SNs, duration of the procedure, operation time per harvested SN and postoperative complication rate.

PATIENTS AND METHODS

This retrospective analysis concerned 277 patients with primary cutaneous head and neck melanoma who underwent re-excision and SLNB at The Netherlands Cancer Institute between December 1993 and January 2016. SLNB was performed for ≥pT1b head and neck melanoma without clinical lymph node involvement as determined by palpation, ultrasound and ultrasound-guided fine needle aspiration cytology of suspicious lymph nodes according to the guidelines of the Dutch Head and Neck Society based on American Joint Committee on Cancer – Union for International Cancer Control 8th edition.(21) Patients with a history of previous melanoma in the head and neck region and those who had already undergone their wide excision or had radiotherapy of the melanoma site, were not eligible.

The medical charts were reviewed for tumor characteristics, overall operation time (start surgery – closure incision), operation time per harvested SN (overall operation time/number of SNs) and SLNB characteristics as well as other clinicopathologic features. The institutional review board approved this retrospective study and the requirement to obtain informed consent was waived. All procedures involving patients were in accordance with the ethical standards of the Medical Ethical Committee of The Netherlands Cancer Institute, conform to the declaration of Helsinki (1964) and later amendments.

Imaging and Sentinel Lymph Node Biopsy

A SN was defined as any lymph node receiving direct lymphatic drainage from the primary tumor.(22) Depending on the patient admission date different lymph node mapping techniques were used and divided in 4 groups. Group 1 (n=30) covered the period 1993-2005, when preoperative lymphoscintigraphy as well as an intraoperative gamma ray detection probe (Neoprobe, Johnson & Johnson Medical, Hamburg, Germany) and patent blue (Laboratoire Guerbet, Aulnay-Sous-Bois, France) were used. Group 2 covered 2006-2007 when preoperative SPECT/CT (Symbia, Siemens, Erlangen, Germany) was added to visualize SNs in their anatomic habitat. Patent blue was omitted after 2007. Group 3 (n=40) reflected 2008 and 2009, and included the addition of intraoperative use of a portable

gamma camera (Sentinella, OncoVision, Valencia, Spain). Group 4 (n=192) covered 2010-2016 and included the addition of near-infrared fluorescence guidance in lieu of patent blue using the hybrid tracer ICG-^{99m}Tc-nanocolloid and a fluorescence camera (Photo Dynamic Eye, Photo Dynamic Eye-modallity and/or Fluorescence Imaging System-00, Hamamatsu Photonics K.K., Hamamatsu City, Japan).

For lymphoscintigraphy, the radiopharmaceutical (^{99m}Tc-nanocolloid or ICG-^{99m}Tc-nanocolloid 80 Mega-Becquerels (MBq) for one-day procedure and 120 MBq for two-day procedure) was injected intradermally around the tumor or biopsy wound in four deposits of 0.1 milliliter (mL).(23) Immediately after injection, anterior and lateral dynamic planar imaging was performed for 10 minutes to identify first-echelon nodes (SNs) and distinguish these from higher-echelon nodes. This was followed by acquisition of 5-minute static images roughly 10 minutes and 2 hours after injection to identify SNs in other (aberrant) regions. SPECT/CT imaging was performed directly after the 2-hour static imaging. SN locations were marked on the skin and indicated on SPECT/CT key images (so-called surgical roadmaps) transferred to picture archiving and communication system. The imaging results were discussed with the head and neck surgeon prior to the operation.

When used in the operation (prior to 2007), 1 mL patent blue was injected intradermally around the melanoma site and the area was massaged for 5 minutes. Subsequently, the SNs were traced using a combination of the probe and the patent blue. To maintain visibility of the lymphatic ducts, patent blue injection was repeated every 90 minutes. With the introduction of a portable gamma camera pre- and intraoperative images were acquired.

With the availability/advent of the hybrid tracer, a dedicated near-infrared fluorescence camera was also incorporated. When a presumed SN was roughly located using the gamma ray detection probe and portable gamma camera, the lights in the operating room were dimmed and its precise location was determined using fluorescence imaging.

To verify completion of SLNB, the wound was inspected, palpated and intraoperative imaging (fluorescence and/or portable gamma camera) was repeated. When a residual signal was observed at the location of the original SN, this node was considered a missed SN or part of a cluster of multiple adjacent SNs and also removed.

Histopathologic Examination

Following the European Organization for Research and Treatment of Cancer recommendations, multiple levels of the SN were analyzed using haematoxylin and eosin staining (H&E) and immunohistochemistry for the melanocytic differentiation antigens S-100 and Glycoprotein 100/Human Melanoma Black 45 (1993-2003) or Melanocyte-Antigen/Melanoma Antigen Recognized by T Cell 1 (2004 onwards). Since February 2014, our protocol changed from examining 3 to 6 levels of 50–150 micrometer with H&E and immunohistochemistry on each level.(24).

Follow-up

SN-negative patients were followed every 3 months in year 1, every 6 months in year 2-5 and annually thereafter. SN-positive patients were followed every 3 months in year 1-2, and every 6 months in year 3-10.

Statistical Analysis

The procedure was considered to be FN if a recurrence developed in the nodal region from which a tumor-free SN had been removed without any signs of local, other regional or distant tumor activity. The FN rate was calculated by dividing the number of patients who present with a nodal recurrence following a tumor-negative SLNB by the sum of those with a true-positive SN (TP) and those with a nodal recurrence (FN/(TP+FN)), which is 1-sensitivity.(25–27)

Descriptive statistics are presented with means/medians and confidence intervals (CI)/interquartile ranges (IQR) or numbers in case of nominal data. In case of continuous or ordinal variables, one-way ANOVA and/or Chi-square tests were performed to assess a difference between the different imaging techniques, respectively. Significance was defined as $p<0.05$. All statistical analyses were performed using SPSS version 22.0 or STATA version 13.

RESULTS

Clinicopathologic Features and Follow-up

Clinicopathologic features for the entire cohort are described in table 1. Age differed between the four groups (table 2). The Breslow thickness decreased over the years (p=0.003). Follow-up details are presented in table 2.

(Post)Operative Findings

Between 1993-2016, 14 head and neck surgeons performed SLNB our institute. In all 277 patients, all preoperatively visualized SNs were identified during surgery. A mean of 3.8 (CI 95%, 3.5-4.1) SNs per patient was excised in a median operation time of 115 minutes (IQR, 87-153) (table 2). The overall operation time remained stable over the years (p=0.74). The number of harvested SNs increased over time (p=0.003), whereas the number of tumor-positive SNs remained equal. The operation time per SN decreased with the advancing technology (p=0.017).

Twelve patients (4.3%) developed postoperative complications that were related to SLNB. The complication rate was similar in the groups. Hemorrhage required treatment in 7 patients, 1 in group 1 (3.3%), 1 in group 2 (6.7%), 1 in group 3 (2.5%) and 4 in group 4 (2.1%). Wound infection developed in 1 patient in group 3 (2.5%) and 2 in group 4 (1.0%). One patient in group 1 (3.3%) developed transient facial nerve palsy and a case of transient spinal accessory nerve dysfunction was seen in group 3.

Sentinel Lymph Node Biopsy Outcomes

A tumor-positive SN was found in 59 patients (21.3%) (table 3). In group 1: 33.3% of the patients had a tumor-positive SN, in group 2: 20.0%, in group 3: 15.0% and in group 4: 20.8%. There was no significant difference between the groups. Eight patients with tumor-negative SNs and no disease elsewhere recurred in their nodal region resulting in an overall sensitivity of 88.1% and a FN percentage of 11.9. Two patients in group 1 had a FN procedure (16.7%), none in group 2, 1 in group 3 (14.3%) and 5 in group 4 (11.1%). FN nodes were located infra-auricularly, retro-auricularly, in the parotid gland, in level II and V. With the exception of the level II recurrence, all were located in the proximity to the primary

melanoma where most of the injected radioactivity remained. Follow-up details and recurrences are visualized in table 4.

DISCUSSION

This study assessed the sequential impact of SPECT/CT, intraoperative use of a portable gamma camera and fluorescence guidance using a hybrid tracer on SLNB in patients with melanoma in the head and neck region. With the introduction of these sophisticated techniques more SNs were harvested per patient while the duration of the operations remained the same. So, SNs were identified quicker, improving the surgical workflow. In this respect, the increasing experience of the individual surgeons in SN identification should also be considered. The greater number of SNs could also imply an increase in postoperative morbidity, but this was not the case. The observed downward trend of the FN rate over time from 16.7 to 11.1 could suggest that some – tumor positive – SNs were missed in the early days.

Several large series of patients with head and neck melanomas report the removal of an average of 2.0 to 2.5 SNs per patient, which is less than the 3.8 in our entire population.(8,28,29) Although the current data suggest that this higher yield is due to the portable gamma camera and the fluorescence imaging, depiction of extra sentinel nodes by SPECT/CT in the preoperative work-up cannot be neglected.(30) The high tissue penetration of radioactive gamma rays in combination with the high spatial resolution of the fluorescence signal increased the identification of SNs within clusters of lymph nodes.(23) If the SN could not be identified separately, the entire cluster was harvested and all nodes were classified as SNs. Since not all of these were necessarily on a direct drainage pathway from the tumor, the lack of intraoperative visualization of afferent lymph vessel(s) may thus inadvertently have increased the number of “SNs”.

Our tumor-positive SLNB rate of 21.3% is higher than for melanomas elsewhere in the body.(31,32) In a systematic review of 12 studies on head and neck melanoma, De Rosa et al. demonstrated an average tumor-positive rate of 15.1%.(9) The high number of thick melanomas in our first cohort may well explain our relatively high rate of tumor-positive SNs.

The current overall FN rate of 11.9% concurs with other studies, including a wide spectrum of melanoma sites (mean 14.0%, range 2.8-32.1%).(25,27,32–34) Although one would intuitively expect

innovative detection methods to improve FN rates, we could not establish significant difference between the groups. Results to improve melanoma staging by ICG vary in literature. In a large prospective cohort of melanoma patients, ICG and lymphoscintigraphy resulted in higher SN positive rates than the predicted true positive SLNB rate based on the literature and their cohort.(35) However, another recent prospective study of 121 melanoma patients demonstrated that the combination of lymphoscintigraphy, probe and ICG fluorescence improved the SN detection rate only marginally.(36)

In addition to the introduction of new detection techniques and advancing surgical skills, the more elaborate histopathological examination plays an important role in the sensitivity of SLNB. After increasing the number of levels of examination of SNs from 3 to 6 in 2014, the number of FN procedures dropped from 8 in 193 patients to none in the subsequent 84.

The variety in definitions of a SN and of a FN SLNB hampers meaningful comparison of results of different studies. Some investigators consider the procedure to be FN in case of a nodal recurrence anywhere after a tumor-negative SLNB, while in most studies only in-field nodal recurrences count.(25,37) Instead of using the formula FN/(TP+FN)(27), some other investigators calculate the rate of FN over the entire group of patients or over the group of tumor-negative SLNBs.(1,29,38) The differences in follow-up duration are a further limiting factor, as the number of FN cases goes up as more recurrences develop with longer follow-up.(9)

There is a great geographical variability in the type of radiotracer used. Human serum albumin-based radiocolloids (particle range 15-100 nm) are generally used in Europe, while sulfur colloids (particle range 20-1000 nm) are commonly used in the USA and antimony sulfide colloids (particle range 10-15 nm) in Australia.(39,40) The more recently approved ^{99m}Tc-Tilmanocept is now also being studied in the head and neck region.(41) No randomized studies have been performed to establish superiority of one radiotracer over another.

The retrospective design, single institution and the relatively small sample size in the time span of the first two modalities (table 3) can also be considered limitations of this study. Although all participating surgeons involved were dedicated head and neck specialists, their substantial number and varying experience might possibly be a limiting factor for the secondary endpoints of the study. Furthermore, the

consecutive addition of new technologies without associated randomized studies makes it difficult to discern the independent contribution of each of these.

CONCLUSION

Using advanced image guidance technologies we found more SNs without increasing overall operation time or postoperative complication rate, which may indicate an improvement of surgical efficiency. Operation time per harvested SN decreased, the average FN rate remained 11.9% and unchanged over 23 years.

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STATEMENT OF IMPACT

Question

Does the technological evolution on SLNB in the head and neck region have impact on the outcome of the false negative rate?

Pertinent Findings

This is a retrospective study that results in a higher number of identified SNs with an unchanged false negative rate.

Implications for Patient Care

To optimize the results of the sentinel lymph node biopsy procedure and thereby make the patient's prognosis more accurate.

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Table 1. Baseline characteristics.

Characteristic	N	%
Gender		
Female	100	36.1
Male	177	63.9
Age at SLNB		
Median (IQR)	59 (46-68)	
Location of primary tumor		
Scalp	77	27.8
Face	103	37.2
Ear	55	19.9
Nose	14	5.1
Neck	28	10.1
Breslow (mm)		
Median (IQR)	2.2 (1.5-3.6)	
T*		**(SLNB +)**
T1b	21	7.7 (4.8%)
T2	94	34.3 (13.8%)
T3	93	34.1 (30.1%)
T4	65	23.8 (26.2%)
Ulceration		
Absent	195	70.9
Present	69	25.1
Unknown	11	4.0

*IQR = interquartile range. Percentages may not equal 100 because of rounding. *According American Joint Committee on Cancer – Union for International Cancer Control 8th edition*

Table 2. Patient and tumor characteristics

	Overall 1993-2016 (n=277)	Group 1 Lymphoscintigraphy 1993-2005 (n=30)	Group2 SPECT/CT 2006-2007 (n=15)	Group3 Portable gamma camera 2008-2009 (n=40)	Group4 Hybrid tracer 2010-2016 (n=192)	*p*-value
Age						
Median (IQR)	59 (46-68)	49 (39-62)	53 (45-66)	54 (45-68)	60 (50-70)	**0.007***
Breslow Thickness						
Median (IQR)	2.2 (1.5-3.6)	3.0 (1.8-4.9)	2.9 (1.9-6.0)	2.2 (1.5-3.4)	2.0 (1.4-3.5)	**0.003***
Number of excised SNs						
Mean (CI)	3.8 (3.5-4.1)	2.8 (2.0-3.5)	2.5 (1.9-3.2)	3.3 (2.7-3.8)	4.2 (3.8-4.6)	**0.003***
Number of tumor-positive SNs						
Mean (CI)	1.5 (1.3-1.7)	1.8 (0.9-2.7)	1.3 (0-2.0)	1.2 (0.7-1.6)	1.5 (1.2-1.7)	0.90*
Operation time (minutes)						
Median (IQR)	115 (87-153)	118 (96-149)	149 (90-164)	98 (77-142)	115 (89-154)	0.74*
Time per SN (minutes)						
Median (IQR)	38 (25-54)	51 (33-71)	54 (39-66)	40 (30-51)	34 (22-51)	**0.017***
Complications						
n (%)	12 (4.3)	2 (7.1)	1 (7.1)	3 (8.1)	6 (3.1)	0.49**
Number of surgeons						
n	14	6	5	7	10	***NA***
Follow-up in months						
Median (IQR)	41 (25-65)	85 (31-138)	106 (65-125)	69 (53-88)	35 (22-53)	***NA***

*CI = confidence interval 95%; NA = not applicable. Percentages may not equal 100 because of rounding. *One-way ANOVA, **Chi-square (exact) test.*

Table 3. SLNB outcomes

	Overall 1993-2016 (n=277)	Group1 Lymphoscintigraphy 1993-2005 (n=30)	Group2 SPECT/CT 2006-2007 (n=15)	Group3 Portable gamma camera 2008-2009 (n=40)	Group4 Hybrid tracer 2010-2016 (n=192)
SLNB – n (%)	210 (75.8%)	18 (60%)	12 (80%)	33 (82.5%)	147 (76.6%)
SLNB + n (%)	59 (21.3%)	10 (33.3%)	3 (20%)	6 (15%)	40 (20.8%)
False-negative (rate)* n (%)	8 (11.9%)	2 (16.7%)	0 (0%)	1 (14.3%)	5 (11.1%)
Sensitivity %	88.1	83.3	100	85.7	88.9
Specificity %	100	100	100	100	100

**False-negative rate is calculated as: (False-negatives / (True-positives + False-negatives)*100%)*

Table 4. Follow-up (in months) and recurrences

	Overall (n=277)	SLNB – (n=218)	SLNB + (n=59)
Follow-up			
Median (IQR)	41 (25-65)	44 (24-67)	37 (23-60)
Follow-up recurrence			
Median (IQR)	33 (18-60)	35 (19-61)	26 (10-52)
Recurrence			
n (%)	81 (29)	50 (23)	31 (53)
Local	21	11	10
Regional	17	12*	5
Distant	43	27	16

*SLNB - = tumor-negative sentinel lymph node biopsy; SLNB + = tumor-positive sentinel lymph node biopsy; *consisted of 8 false negative nodes, 3 locolregional recurrences and 1 contralateral node recurrence with simultaneous distant metastasis*

GRAPHICAL ABSTRACT