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Quantitative Analysis of Detected Sentinel Lymph Nodes and the Incidence of Micrometastases Using Two Different Methods of Surgical Melanoma Treatment – Pilot Study

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Introduction

Melanoma is a malignant tumour of melanocytes, cells that produce the pigment melanin. During the last few decades, the morbidity rate of cutaneous melanoma (CM) among the Caucasian population in developed countries has increased significantly (1). A standardized comparison of International Cancer Registry data (2) showed that the morbidity rate of CM in Baltic states was similar to or lower than that of other European countries. Male and female morbidity rates of cutaneous melanoma standardized according to age in Lithuania between 1978 and 2002 (per 100,000 population) has increased from 1.7 to 5.0 and from 2.3 to 7.0 cases respectively.

Growing mortality and insignificant changes in incidence of first stage melanoma show a lack of early diagnosis. The survival rate of patients with cutaneous melanoma in Lithuania is significantly lower than the average European survival rate of such patients (2).

Our literature search did not show any prospective studies comparing the results of sentinel lymph node biopsy (SLNB) using different approaches to surgical melanoma management. This included single-stage melanoma excision with sentinel lymph node biopsy and two-stage melanoma excision; (the latter consisting of primary melanoma excision followed by a subsequent re-excision of the post-operative scar and respective skin area in addition to SLNB according to the estimated cutaneous melanoma Breslow thickness).

The data on the expedience of sentinel lymph node biopsy following a wide melanoma excision is rather conflicting. According to MARIANI et al., sentinel lymph node biopsy should not be performed as the surgery traumatizes lymphatic pathways and it is likely that lymph would flow to places other than the actual sentinel lymph node (3). Other authors advise against sentinel lymph node biopsy after prior excision of melanoma > 2 cm from margins of the lesion and if the defect following CM excision is covered with revolving flaps of surrounding tissue. Sentinel lymph node biopsy is recommended if the melanoma excision site was closed by primary suture, or autodermoplastics was performed (3). However; there is a consensus on the thickness of cutaneous melanoma that requires identification of the sentinel lymph node. The American Joint Committee on Cancer (AJCC) has provided a recommendation to perform sentinel lymph node biopsy in cases of melanoma thickness 1 mm or more (4).
The issue of “false negative” SLN has also been widely discussed in the literature. “False negative” SL is defined as the actual SL with a micrometastasis which was not detected by SLNB. According to various authors 10 to 20% of patients have a false SLN excised during SLNB. A lymph node is considered a false SLN when the patient develops a clinically evident CM metastasis in the same regional lymphatic basin during the post-operative follow-up period (5).

In some clinical studies, sentinel lymph node biopsy was accompanied by a radical lymphadenectomy in order to detect “false negative” lymph nodes, i.e. missed metastases. Histological examination of excised lymph nodes showed that less than 10% of the patients with no pathology in sentinel lymph nodes had metastases detected in other regional lymph nodes (6-10).

The aim of our study was to compare the number of detected sentinel lymph nodes and the incidence of micrometastases between two groups of patients with cutaneous melanoma. The patients were divided into two groups: group V and group D. Group V patients with melanoma underwent a single-stage surgery – radical excision of the tumour with sentinel lymph node biopsy (study group “V”). Group D patients with melanoma underwent two-stage surgery; initially primary diagnostic excision of the tumour (0.5 cm from margins of the lesion) followed by a radical re-excision of the post-operative scar and sentinel lymph node biopsy (study group “D”).

Materials and methods

This prospective clinical study was conducted in The Department of Plastic and Reconstructive Surgery of Kaunas University of Medicine Hospital (KUMH) from January of 2004 to October of 2008. One hundred patients with a diagnosis of stage I-II cutaneous melanoma were recruited to the study. After dermatoscopy of the skin lesion and clear clinical diagnosis of cutaneous melanoma, patients were referred for further examination in order to detect metastases. Ultrasound scan of upper abdominal organs and regional lymph nodes was performed in addition to a chest X-ray. Melanoma depth was measured using a linear 14 MHz frequency ultrasound sensor (Toshiba Xario XG). In patients that no distant or regional lymph node metastases were detected and that had a melanoma depth exceeding 1 mm on ultrasound met the criteria to be enrolled in the study.

If a patient met inclusion criteria and agreed to participate in the study, he/she signed a consent form with a responsible researcher and the consent document was attached to his/her personal inpatient medical records. Patients were randomly allocated to one of the 2 groups: one-stage surgery (V) or two-stage surgery (D). This random allocation was carried out by a general practice nurse blindly picking one of 100 unmarked envelopes. 50 envelopes had a card marked “V” (one-stage surgery) inside and the remaining 50 envelopes had “D” (two-stage surgery). According to the letter on a card picked by the nurse a patient was allocated into one of aforementioned groups and surgery was scheduled. Group “V” patients underwent radionuclide lymphoscintigraphy in the CKUM Department of Nuclear Medicine on the day of hospitalization. 0.1 to 0.2 ml of radiopharmacological agent (RA) 99mTc-albumine nanocolloid (Nanocoll, Amersham Health) was injected into the skin and spread evenly around the primary tumour (total of 50 to 250 MBq). Dynamic lymphoscintigram using planar...
gamma camera (Siemens e.cam) was performed following the injection of RA in order to register lymph drainage from the tumour and to detect peak accumulation of RA in the sentinel lymph node. Lymphatic pathways leading from the marker injection site to one or several sentinel lymph nodes were noted. Static lymphoscintigrams were performed 20 minutes after the injection of RA. Projections of sentinel lymph nodes that contained RA were detected and marked on the skin. Surgery was started < 4 hours after the injection of the radiopharmacologic agent to prevent distant lymph nodes filling with radionuclide. The tissue stain isosulfane blue was used to detect SLN (Patentblau V, Guerbet GmbH, Sulzbach, Germany) as this flows into lymphatic capillaries and SLNs (3, 10) Isosulfane blue (0.5 to 1 ml) was injected into the skin surrounding the tumour or into margins of the tumour 15-20 min prior to surgery. Group “D” patients underwent two-stage surgery. At stage one the tumor was excised with 0.5 cm margins. The subsequent defect was closed with a primary suture. The excised tumour was sent for histology. Stage two was carried out once histology results were obtained. The period between the two stages of surgical treatment did not exceed 4 weeks. 0.1 to 0.2 ml of radiopharmacological agent (RA) 99mTc-albumine nanocolloid (Nanocoll, Amersham Health) was injected into the skin and spread evenly around the post-operative scar (total of 50 to 250 MBq). Tissue stain Isosulfane blue (0.5 to 1 ml) was injected into the skin surrounding the post-operative scar 15-20 min prior to surgery.

Statistics

Statistical analysis was performed using IBM SPSS Statistics Version 20 (Armonk, NY : IBM Corp. Software). To determine the required sample size for achieving an 80% power, the number of detected SLNs was considered as the primary end point. It was believed that a difference of 1 in the detected number of SLNs between “V” and “D” groups is of clinical importance. Assuming that the standard deviation is 1.7 (obtained from pilot study), the required sample size is 50 patients per arm. Data were presented as mean ( $\bar{x}$ ) ± standard deviation (SD). After testing for normality, parametric and non-parametric criteria, the Student’s t and Mann-Whitney U tests were used to compare two independent samples. For testing hypotheses of independence, the exact chi-square test (for small samples) and asymptotic chi-square tests were used. For comparison of probabilities, exact (for small samples) or normal approximation criteria were used. The level of statistical significance by testing statistical hypothesis was chosen to be 0.05. Kaplan-Meier method, log rank test was applied for CM disease free survival analysis.

Results

A total of 100 patients with a clear clinical diagnosis of stage I-II cutaneous melanoma were enrolled in this study. Fifty of them underwent one-stage surgery involving radical excision of the tumour and sentinel lymph node biopsy (group “V”). Another 50 patients underwent two-stage surgery consisting of primary diagnostic excision of the tumour (0.5cm from margins of the lesion) followed by a radical re-excision of the post-operative scar and sentinel lymph node biopsy (group “D”).

Study groups “V” and “D” were tested for homogeneity with regard to age, melanoma thickness, location of melanoma, type of melanoma, and ulceration. The groups were found to be homogenous.

The average number of removed sentinel lymph nodes in group “D” was 1.0 more than in group “V” (p < 0.05). The averages were 3.7 and 2.7 respectively with a SD of 1.8.

The relationship between the SL node staining and type of surgery was (p < 0.05). 49.6% stained radioactive sentinel lymphnodes at the time of surgery was found in group “V”, while 33.9% in group “D”. Our study showed no significant difference between the accumulation of SL markers in head-neck, torso, upper and lower limb regions or between study groups with regard to SL staining and RA accumulation. There was no significant difference (p < 0.05), between study groups when comparing number of SLNs with micrometastases. 8 SLNs with micrometastases were detected in group “D” and 7 in group “V” (Fig. 4). In group “V”, SLNs with micrometastases
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was detected in all anatomical regions. In group “D”, SLNs with micrometastases were detected only in those patients who had melanoma on their torso (4 cases) or legs (4 cases) (Fig. 5). 7 patients in group V had 9 SLNs with micrometastases. 8 patients in group D had 8 SLNs with micrometastases. One patient from Group V had a non-radioactive SLN with a micrometastasis (Table 1).

Our study revealed that 15/100 patients had micrometastases in excised sentinel lymph nodes. Patients with detected micrometastases underwent a radical lymphadenectomy.

Patients were followed up for 60 months, during this time 11 recurrences were detected. To detect factors influencing recurrence a disease free survival analysis was made.

We have compared survival without relapse between patients with a micrometastasis in SL (SL+ patients) and patients with no SL micrometastasis detected (SL− patients). The mean survival without relapse of SL+ patients was 39 months (95%; CI 30–49 months), whereas the SL− patient mean survival without relapse was 55 months (95%; CI 52–58 months). The difference was statistically significant (p = 0.002) (Fig. 6). Results showed that the presence of micrometastases increases the risk of relapse (hazard ratio) 6.98 times (95% PI 2.13 – 22.9; p < 0.01; the Cox proportional hazards regression model).

Comparisons between groups V and D were made looking at the rate of survival without relapse and at those who had no detected micrometastases in excised SLNs. The mean survival without relapse in the 43 group V patients was 54 months (95%; CI 49–59 months). The mean survival without relapse in 42 group D patients was 56 months (95%; CI 52–60 months). There was no significant difference between groups (p = 0.539) (Fig. 7).

Discussion

Our study showed that the accumulation of SLN markers in head-neck, torso, and upper and lower limb regions did not differ significantly between study groups with regard to SLN staining and RA accumulation. However, in cases of melanoma on lower limbs we found more non-stained yet radioactive SLNs in group “D”. They comprised 28% and 17% of total non-stained radioactive SLNs in groups “D” and “V” respectively. This finding could have been determined by a larger number of detected SLN in study group “D”.

C. Gannon who conducted a retrospective trial with patient groups similar to our study groups, found no significant difference in the number of detected SL between groups. According to his results, during a single-stage melanoma excision with SLNB, the number of detected and excised SLNs was 2.2 per patient, whereas our respective findings were 2.68 SLNs per patient. The group consisting of the two-stage surgical approach in Gannon’s study showed the mean number of detected SLNs to be 2.4, whereas we have detected and excised 3.72 SL per patient (11). Thus, our study showed a statistically significant difference between the groups according to this parameter. The discrepancy between these two studies
may have been caused by the use of different RA as a SLN marker - C. Gannon used 99mTc sulfur colloid. The differing techniques could have affected lymphatic drainage to SLNs (12). According to C. Gannon, there was no significant difference in the number of SLNs with micrometastases between groups. The author found SLNs with micrometastases in 18% of patients who had SLNB following a wide melanoma excision, and in 17% of patients who underwent a single-stage melanoma excision with SLB. A study by McCready et al has also compared two groups of melanoma patients who had SLB either during the primary excision of the tumour, or following a wide melanoma excision (13). McCready et al showed that the number of SLNs with micrometastases increased after a wide excision of melanoma. In comparison there was no significant difference between our study groups with regard to the number of SLNs with micrometastases. We have detected SL with micrometastases in 8 group “D” patients and in 7 group “V” patients (p = 0.779).

There are many publications comparing survival without relapse of CM patients who had a SL with micrometastasis detected by SLB with survival of those without micrometastases in SL. Much of the literature is consistent; showing significantly higher survival without relapse in SLN groups (14-18). Our study revealed a total of 15 patients who had micrometastases in excised sentinel lymph nodes. Patients with detected micrometastases underwent a radical lymphadenectomy. The mean survival without relapse of SL+ patients was 39 months (95% CI 30-49 months), whereas SL- patient mean survival without relapse was 55 months (95% CI 52-58 months). Our results corresponded with the findings of other authors: survival rates were higher in the SL- group and the difference was statistically significant (p = 0.002).

Further follow up of 85 SL- group patients revealed 6 cases (7%) of clinically diagnosed melanoma metastases in the lymphatic basin of SLNs. It is possible this represents the group of patients in which “false negative” SLNs were detected during SLB. The cases in which relapse manifested as local or distant metastases were not included in the “false negative” SL group.
All investigators performing SLB in CM patients encounter cases of “false negative” SL detection discussed in the literature: surgeon’s failure, pathologist’s failure, and biological failure (20-22). Surgeon’s failure is usually dependent on the learning curve, and occurs due to the lack of experience. The literature provides conflicting data on this issue, however, most researchers suggest that the probability of “false-negative” SL detection is higher following a wide CM excision (20, 22-25). Failure at the pathology level may be determined by insufficient number of SLN sections. According to the literature, higher numbers of serial sections and immunohistochemical staining increases the number of detected SL+ if H+E staining is negative. Therefore a respective decrease in the number of “false-negative” SL should be observed (21, 22, 24, 25). Biological failure may occur in two cases: firstly, when lymphatic pathways are obstructed by melanoma cells, and secondly, when inadequate primary CM excision is performed and residual melanoma cells are able to reach other lymph nodes through lymphatic pathways.

We have also compared survival without relapse between groups D and V who had no detected micrometastases in excised SL. The mean survival without relapse in 43 group V patients was 54 months (95% CI 49-59 months). The mean survival without relapse in 42 group D patients was 56 months (95% CI 52-60 months). There was no significant difference between the groups (p = 0.539) (Fig. 7).

Conclusions

Using two different early-stage cutaneous melanoma management techniques (consisting of one-stage surgery: radical excision of the tumour (0.5 cm from margins of the lesion) followed by a radical re-excision of the post-operative scar and sentinel lymph node biopsy), significantly more sentinel lymph nodes (p = 0.006) were detected using the two-stage surgery approach. However, there was no significant difference between the two approaches regarding the number of sentinel lymph nodes with micrometastases that were detected and excised.

References


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