

Title: The benefit of sentinel lymph node biopsy and adjuvant therapy in thick (>4 mm) melanoma: multi-center, retrospective study of 291 Japanese patients.

Running head: SLNB and adjuvant therapy in melanoma

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Abstract

Objective: The benefit of sentinel lymph node (SLN) biopsy and adjuvant therapy for patients with thick (>4mm) melanoma has not been well studied in Asian population. We examined the benefit of SLN biopsy and adjuvant therapy on prognosis in Japanese thick melanoma patients.

Materials and methods: A review of the melanoma database collected from 26 institutions in Japan identified 291 patients with thick melanoma between 2005 and 2010. Univariate and multivariate analyses were performed to evaluate factors predictive of overall (OS) and disease-free survival (DFS).

Results: Of the 242 thick melanoma patients who received SLN biopsy, the results for 96 (40%) were positive. On multivariate analysis, increased Breslow thickness (relative risk, 1.11; 95% confidence interval, 1.05-1.17; $P=0.0002$) and SLN metastasis (2.14; 1.04-4.43; $P=0.040$) were associated with poor OS. Increased Breslow thickness (1.11; 1.04-1.18; $P=0.0018$), ulceration (3.11; 1.25-7.72; $P=0.014$), satellitosis (3.89; 1.62-9.31; $P=0.0023$)

and SLN metastasis (2.24; 1.16-4.36; $P = 0.017$) were associated with DFS. Adjuvant chemotherapy had no impact on either OS or DFS. Adjuvant use of monthly dermal injection of interferon- β (IFN β) was associated with improvement in both OS (0.34; 0.17-0.67; $P = 0.0022$) and DFS (0.42; 0.20-0.86; $P = 0.018$).

Conclusions: SLN biopsy provided useful prognostic information and adjuvant use of IFN β improved both OS and DFS in Japanese patients with thick melanoma. These results were consistent with those of previous studies conducted in Caucasians. Therefore, we suggest that SLN biopsy and adjuvant IFN should be considered for patients with thick melanoma regardless of the Breslow thickness or ethnicity.

Keywords: melanoma, sentinel node biopsy, adjuvant therapy

Introduction

Fifteen to twenty percent of patients with clinically localized melanoma of intermediate thickness (1 - 4 mm Breslow thickness) have occult lymph node metastasis. Before Morton et al [1] proposed the sentinel lymph node (SLN) concept, immediate elective lymphadenectomy was advocated to improve tumor staging and possibly survival [2,3] despite the risk of surgery-related complications. For patients with intermediate-thickness melanoma, SLN biopsy has become the standard method for determining the pathologic status of the regional nodal basin[4].

On the other hand, controversy remains as to whether SLN biopsy has any merit for patients with thin melanoma (< 1 mm Breslow thickness) or thick melanoma (>4 mm Breslow thickness). Because the risk of nodal metastasis for patients with thin melanoma is as low as 5%[4], patients without risk factors such as ulceration or mitotic activity are not likely benefit from SLN biopsy. Therefore, discussion has centered on identifying a subset of these patients who may still benefit from SLN biopsy [5] [6].

The risk of thick melanoma nodal metastasis is reported to be almost 40% [7-9]. Moreover, this patient population has a high risk for systemic occult disease [10]. Given these facts, the benefit of SLN biopsy in patients with thick melanoma is still controversial[8]. On the other hand, some reports studying the role of SLN biopsy in thick melanoma showed that the SLN status provided important prognostic information[11, 12]. According to those studies, patients with no evidence of tumor in the SLN had significantly better disease-free survival (DFS) as well as overall survival (OS) than did the SLN-positive patients .

Previous large-population studies have all been conducted in Western countries. The clinical evidence for Asian patients, especially from large-scale studies, is limited; and little is known about melanoma in Asian population. This is because melanoma is relatively rare in non-Caucasian populations: according to the Surveillance, Epidemiology, and End Results (SEER) data from the United States, the incidence of melanoma in Asians is about 1 in 100,000 persons as compared to that in Caucasians of 20 in 100,000 persons (SEER data, 2004-2008; <http://seer.cancer.gov/statfacts/html/melan.html#incidence-mortality>).

Moreover, the clinical characteristics such as the pathology, anatomical origin, and patient's prognosis differ significantly among different ethnic groups[13]. Therefore, whether previous study results can be applied to Asian populations needs to be confirmed.

The aim of this study was to evaluate the effect of SLN biopsy and adjuvant therapy on Japanese patients with thick melanoma.

Materials and methods

The Japanese Melanoma Study is a prospective cohort study involving 26 centers in Japan that started in 2005. All patients diagnosed as having melanoma are registered with this database; so far, 2126 patients have been registered. We extracted data from this database that met the following requirements: pathologically confirmed primary cutaneous melanoma, Breslow thickness >4 mm, clinically lymph node-negative, without elective lymph node dissection, without distant metastasis, and records with survival time. Because DAVFeron (dacarbazine, nimustine hydrochloride, vincristine,

interferon- β , IFN β) therapy is the only adjuvant chemotherapy method recommended in the General Rules for Clinical and Pathological Studies on Malignant Neoplasms of the Skin[14], we omitted data from patients who received other adjuvant chemotherapy so as to standardize the therapeutic methods. Finally, 291 consecutive cases were eligible for this analysis.

SLN biopsy with peritumoral intradermal injection of technetium 99m sulfur colloid or phytate colloid and intradermal patent blue dye was performed. The SLNs were serial-sectioned with hematoxylin-eosin staining and immunohistochemistry for S-100 and HMB-45 as recommended[14] in most cases. Survival information was obtained from the registered database.

This study was approved by the institutional review board of the University of Tsukuba.

Statistical methods

The associations between groups were tested using the chi-square test and the t test for categorical and continuous characteristics, respectively. Overall survival (OS) and disease-free survival (DFS) were calculated for each patient beginning from the date of the primary surgery. For OS, patients confirmed to be alive were censored using their last contact date. For DFS,

patients confirmed to be alive without locoregional relapse or distant metastasis were censored using their last contact date. The estimated OS and DFS were calculated using the Kaplan-Meier method. The Cox proportional hazard models was used to estimate the bivariate association between each end point and the available patient/ tumor characteristics and the type of adjuvant therapy. The best multivariate model was defined as the model that resulted from application of a stepwise regression (forward selection) algorithm that automatically eliminated the variables with *P* values over 0.05, until only significant characteristics remained. Significance for all statistical tests was defined as a *P* value over 0.05. All analyses were performed with commercially available software (Stat Flex version 6.0; Artech, Osaka, Japan).

Results

Patient characteristics

The patient characteristics of this study are listed in Table 1. The mean age of the patients was 69 years. The median Breslow thickness was 5.9 mm,

with a range of 4 to 25 mm. The primary tumor distribution occurred most commonly in the foot and was relatively well distributed in the head and neck, trunk, and hand. Ulceration was present in 63% of the patients, regression in 4%, and satellitosis in 8%. SLN biopsy was performed in 241 patients (83%). Adjuvant chemotherapy and IFN- β protocol-2 were performed in approximately half of the population (48% and 44%, respectively) and IFN- β protocol-1 was performed in just over one quarter of the population (28%).

SLN status

Of the 291 patients, 50 (17%) did not undergo SLN biopsy. The reason why these patients did not do so is unclear, but given the higher average age in this group than in the group that did undergo the biopsy (76.8 versus 63.8 years; *t* test, $P < 0.00001$), the patients' elderly age and comorbidities might have been determining factors. For patients who did undergo SLN biopsy, the median number of harvested SLNs per case was 2 (range, 1-10). In 12 patients, a SLN biopsy was attempted but failed.

The SLN was positive in 96 patients (40%). Factors associated with a positive SLN are listed in Table 2. Age was not associated with SLN

positivity. Female sex was relatively higher in the SLN-positive group than in the SLN-negative group ($P=0.055$). The proportion of the acral lentiginous melanoma (ALM) histologic subtype was lower in the SLN-positive group than in the SLN-negative group ($P=0.011$). Breslow thickness is known to be strongly predictive of SLN positivity, and the incidence of SLN positivity rises significantly with increasing thickness ($P=0.03$). Other factors such as ulceration, regression and satellitosis were not correlated with SLN positivity.

Of the 96 patients found to be SLN-positive, 79 underwent complete lymph node dissection (CLND). Of these patients, 23 (29%) had non-SLN metastasis. Although we do not have clear reasons for why the SLN-positive patients did not receive CLND, the higher age of these patients (mean, 73.0 years) as compared with the patients who did receive CLND (mean, 59.4 years) might be an essential factor. The SLN status showed no significant correlation with treatment choice.

Survival

The mean follow-up for the entire cohort was 24.5 months. During follow-up, 83 patients (29%) developed recurrent melanoma and 46 (16%)

died of the disseminated disease. SLN biopsy conferred no survival benefit (Figure 1).

The association between prognostic factors and both OS and DFS were studied in patients with thick melanomas who underwent successful SLN biopsy. SLN-positive patients had a shorter OS than did SLN-negative patients (Figure 2). The 3-year OS for SLN-positive patients was 65% as compared with 87% for SLN-negative patients (log-rank method, $P<0.005$). Univariate analysis showed the following 5 factors as significantly predictive of OS (Table 3): male sex (relative risk, 2.10; 95% confidence interval [CI], 1.04- 4.14; increasing Breslow thickness (1.14; 1.08- 1.20; $P<0.000001$), SLN-positivity (2.34; 1.30- 5.34; $P=0.0071$), satellitosis (3.17; 1.31- 7.63; $P=0.010$), and adjuvant IFN- β (0.31; 0.16- 0.62; $P=0.0009$). On multivariate analysis, the following 3 factors maintained significance (Table 3): Breslow thickness (1.11; 1.05- 1.17; $P=0.0002$), tumor-positive SLN (2.14; 1.04- 4.43; $P=0.040$) and adjuvant IFN- β (0.34; 0.1- 0.67; $P=0.0022$).

Next we examined the effect of these factors on DFS. SLN-positive patients had shorter DFS than did SLN-negative patients (Figure 3). The 3-year DFS for SLN-positive patients was 52% as compared with 76% for SLN-negative

patients (log-rank method, $P < 0.005$). As shown in Table 4, univariate analysis showed that DFS was significantly associated with the following 4 factors: increasing Breslow thickness (relative risk, 1.14; 95% CI, 1.04- 1.21; $P < 0.00001$), tumor-positive SLN (2.58; 1.31- 5.10; $P = 0.0063$), presence of satellitosis (2.90; 1.32- 6.37; $P = 0.0080$) and adjuvant IFN- β (0.40; 0.20- 0.79; $P = 0.0081$). Multivariate analysis showed that presence of ulceration (3.11; 1.25- 7.72; $P = 0.014$) was also significantly associated with DFS in addition to the following 4 factors (Table 4): Breslow thickness (1.09; 1.02- 1.16; $P = 0.014$), SLN-positivity (2.59; 1.28- 5.23; $P = 0.0077$), satellitosis (3.89; 1.62- 9.31; $P = 0.0023$) and adjuvant IFN- β (0.42; 0.20- 0.86; $P = 0.018$)

Discussion

In the current analysis of 291 Japanese patients diagnosed with thick melanoma, we discovered that the histologic subtype of ALM was quite common, up to 50%. This observation is consistent with previous data obtained from other Asian populations[15, 16]. Some reports suggested that patients with the histologic subtype of ALM have shorter survival than do

patients with other subtypes[15, 17]. Therefore, it was necessary to confirm whether the evidence obtained from studies using Caucasian populations can be directly applied to patients of Asian populations.

The median tumor thickness in our study population was 5.9 mm, indicating tumor considered as locally advanced and with high risk of systemic disease[18]. Although the benefit of SLN biopsy for thick melanoma is still controversial[19], recent studies on Caucasian patients with thick melanoma showed that the SLN status was an independent prognostic factor for both OS and DFS[11, 12]. In the present study, 241 patients underwent SLN biopsy, but the remaining 50 patients did not. We could not find any survival difference between the patients who underwent SLN biopsy and those who underwent nodal observation (Figure 1).

We found that nearly 42% (96 out of 229) of the patients in this population had SLN metastasis, which is a rate similar to those of previous reports [7] [21] [11] [12]. As shown in Table 2, SLN-positive patients had a lower rate of the ALM subtype and increased Breslow thickness. Other factors including ulceration were not statistically correlated with the SLN biopsy results. In this analysis, SLN-negative patients had a significantly better OS as well as

DFS (log-rank method, $P < 0.005$). The multivariate analysis showed that increased Breslow thickness and SLN status affected OS. Previous reports concluded that the OS of thick melanoma was affected by the following factors: increased Breslow thickness[11, 12], ulceration[7, 12], and SLN status[7, 11, 12]. We should note that SLN status was concluded to be an independent prognostic factor of OS in all of the studies including ours, that focused on thick melanomas[7, 11, 12]. Our results confirmed that the SLN status is also an independent prognostic factor for thick melanoma in Japanese population.

In the present study, multivariate analysis showed that increased Breslow thickness, ulceration, satellitosis, and SLN status affect DFS. Others have also reported the impact of increased Breslow thickness[12, 22], ulceration[7, 9, 11], and SLN status[7, 9, 11, 12, 22] on DFS. SLN status was identified as an independent prognostic factor of DFS, as well as for OS. Our study also showed satellitosis to be an independent prognostic factor for DFS; however, recent studies[7, 9, 12, 22] failed to show significant association. Gajdos et al[11] showed the significance of satellitosis by univariate analysis, but not by multivariate analysis. Our results support the inclusion of satellitosis in

the current American Joint Committee of Cancer (AJCC) staging system.

To improve the outcome data and to reduce the risk of recurrence for thick melanoma, several efforts have been attempted, including adjuvant chemotherapy using dacarbazine (DTIC) or nonspecific immune adjuvants. However, such attempts failed to improve survival[23]. To date, the only Federal Drug Administration approved adjuvant therapy for thick melanoma and/or regional lymph node metastasis is high-dose IFN-a. On the other hand, since Yamamoto et al [24] showed that a combination chemotherapy regimen using DTIC, nimustine hydrochloride, vincristine, and IFN-b (DAVFeron) improved survival in high-risk melanoma, this chemotherapy regimen has become widely accepted for the management of AJCC stage 2 and stage 3 melanomas in Japan. Adjuvant IFN-b is also accepted. However, no randomized controlled study had been conducted to prove the impact of DAVFeron and adjuvant IFN-b on DFS or OS. Moreover, several cases of DAV therapy-related myelodysplastic syndrome have been reported [25]. Although ours is a prospective cohort study, we concluded that chemotherapy using the DAV regimen had no effect on DFS and OS. On the other hand, IFN-b protocol-2 (3 MU dermal injection of IFN-b once a month

continued for at least 1 year) had a significant survival benefit on both DFS and OS. This result is consistent with the study by Garbe et al[26] which showed that subcutaneous injection of 3 MU IFN- α significantly improved both the DFS and the OS of AJCC stage 3 melanoma.

In conclusion, SLN biopsy provides important prognostic information in thick melanoma, and low-dose IFN β improves both DFS and OS. These observations are consistent with the previous studies on Caucasians, with no ethnic differences observed. We strongly suggest the routine use of SLN biopsy and low-dose IFN therapy in patients with thick melanoma because both procedures can be performed safely. Further studies evaluating their influence are needed.

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Figure 1 Kaplan-Meier overall survival analysis for thick primary melanoma patients who received sentinel lymph node biopsy or nodal observation

Figure 2 Kaplan-Meier overall survival analysis for sentinel lymph node-positive and –negative patients with thick primary melanoma

Figure 3 Kaplan-Meier disease-free survival analysis for sentinel lymph node-positive and -negative patients with thick primary melanoma