ONCOLOGY

Prognostic factors for in-transit metastasis in patients with malignant melanoma

Vlad Alexandru Gâta[1,2], Andrei Roman[3], Maximilian Muntean[1,2], Dragoș Ștefan Morariu[2], Cătălin Ioan Vlad[1,2], Eduard Alexandru Bonci[1,2], Alexandru Irimie[2,4], Patriciu Achimaș-Cadariu[1,2]

1) Department of Oncology, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Surgical Oncology, "Prof. Dr. Ion Chiricuta" Institute of Oncology, Cluj-Napoca, Romania

3) Department of Radiology, "Prof. Dr. Ion Chiricuta" Institute of Oncology, Cluj-Napoca, Romania

4) The Academy of Medical Sciences, Cluj-Napoca Branch, Romania

Abstract

Background and aims. Malignant melanoma represents an aggressive and unpredictable malignancy, with high locoregional recurrence rates, regardless of tumor stage and therapeutic management. This study aims to identify the main histopathological prognostic factors involved in the development of in-transit metastasis in patients with malignant melanoma.

Methods. The study includes only patients that were diagnosed with malignant melanoma and with histologically confirmed in-transit metastasis who were treated in a comprehensive cancer center between 2010-2021. Histopathological parameters were investigated, univariate and multivariate analysis was performed.

Results. A total of 26 patients were included in the analysis. On univariate and multivariate analysis, only primary cutaneous melanomas located on the thorax correlated with the risk of developing in-transit metastasis, whereas clinicopathological factors such as an increased Breslow thickness and Clark level, the presence of ulceration, positive lymph nodes, a non-brisk TIL density, a high mitotic rate, a nodular subtype, and age >50 years may represent risk factors, even though we could not find any correlations.

Conclusions. Primary cutaneous melanomas that arise on the thorax present a high risk for the occurrence of locoregional disease, whereas other clinicopathological characteristics could not be used to predict local recurrence. However, prospective and more extensive cohort studies are needed in order to validate these important prognostic factors.

Keywords: in-transit metastasis, malignant melanoma, prognosis, risk factors, tumor-infiltrating lymphocytes

Introduction

Malignant melanoma, the cancer of melanocytes, represents a highly aggressive tumor with an incidence that continued to rise in the past 30 years [1]. Even though it is the least common histological type, it is responsible for the most deaths involving skin cancer, accounting for up to 60% of skin cancer-related deaths, primarily due to rapid proliferation and metastasis, mainly by lymphatic spread [2]. Moreover, unfortunately, due to higher rates of chronic sun exposure, either by direct sunlight or using tanning salons, malignant melanoma occupies the first place in the United States regarding cancer incidence among young people aged 20-35 years [3,4]. In this regard, in countries with the highest incidence, such as Australia and the USA, numerous public health campaigns were undertaken in order to establish a more rapid diagnosis, as prevention and rapid diagnosis of high-risk patients remain the most effective management of this disease [5]. However, in the last decade, the results of the numerous randomized clinical trials helped in developing new therapeutic agents, such as mitogen-activated kinase

DOI: 10.15386/mpr-2173

Manuscript received: 19.04.2021
Received in revised form: 29.06.2021
Accepted: 30.07.2021

Address for correspondence:
gatavlad@yahoo.com

and BRAF inhibitors for BRAF positive patients and monoclonal antibodies (anti CTLA4, anti PDL1), used as immunotherapy in non-resectable or metastatic melanoma, managing to decrease the rates of mortality and improve overall survival of these patients [6-11].

The most important prognostic factors include the Breslow index, Clark scale, the presence of ulceration, microsatellitosis, regression, and lymph node metastasis [5]. Moreover, malignant melanoma is very aggressive, managing to easily stimulate an immune reaction in the host organism and being considered as the tumor with the highest mutational load, harboring three main oncogenes: BRAF, N-RAS, and c-Kit [12,13]. Therefore, the role of the immune system has been investigated in recent years, as tumor proliferation and progression are considered to be linked to a series of complex interactions between the tumor and the host's immune response [14]. In this regard, the value of tumor-infiltrating lymphocytes has gained much interest, and the result of numerous studies confirmed that a peritumoral brisk infiltrate correlates with a lower rate of lymph node metastasis and improved overall survival [15-18].

Malignant melanoma can metastasize by hematogeneous or by lymphatic pathway, the mortality being related to the high potential of metastasis and the visceral spread. Moreover, a unique feature of melanoma is represented by the capacity of locoregional metastasis, with the occurrence of in-transit or satellite metastasis. In-transit metastasis is considered as the recurrence of the disease, located between the primary tumor and the regional lymph nodes, in the dermis or the subcutaneous tissue, isolated or extensive, occurring with an incidence of 5-10%, and having an unfavorable prognosis, with low survival rates [19]. The physiopathology of this type of recurrence is not entirely understood, but numerous studies concentrated on identifying the main prognostic factors involved in the development of the recurrence [19-23].

In this study, we analyzed 26 patients with pT3 and pT4 malignant melanoma, with histologically confirmed in-transit metastasis, who were treated exclusively in a tertiary cancer center. The main objective was to evaluate the main histopathological parameters as prognostic factors for developing in-transit metastasis.

Methods

Patient selection and data collection

We analyzed patients with histologically confirmed primary invasive malignant melanoma and histologically confirmed in-transit metastasis that were treated exclusively in our center, a tertiary cancer center in Romania. We consulted the databases, which comprised a number of 232 patients, over a period of 10 years, January 2010-December 2020, and selected a number of 26 patients. The occurrence of in-transit metastases was synchronous with the diagnosis in one case, whereas in the other patients the recurrence appeared after a disease-free interval. Clinical and histopathological parameters were identified by analyzing the patients' clinical records and pathology reports. Malignant melanoma was staged according to the latest American Joint Committee on Cancer (AJCC-8th edition-2018) staging system, and the treatment and follow-up of the patients were done according to European Melanoma Guidelines (ESMO). The patients were considered eligible for the study based on the following inclusion criteria: (i) histologically confirmed malignant melanoma; (ii) histologically confirmed in-transit metastasis; (iii) patients that have undergone surgery exclusively at our Cancer Center; (iv) patients with no other cancers; (v) patients with adequate follow-up. Lymph node status was evaluated by surgery, either by direct lymph node dissection (LND) or by sentinel lymph node biopsy (SLNB), and completed with lymph node dissection if positive within two months maximum from the initial diagnosis. All study participants provided informed consent on admission, and all clinical information for each patient was coded; finally, the study was approved by the institutional ethical committee.

Pathology

Patient age, sex, and site of the primary tumor were recorded from the clinical observation papers, and the following features of the primary tumor were extracted from the histopathology reports: histological subtype, tumor thickness (Breslow index), Clark level of invasion, mitotic rate (per mm^2), presence or absence of ulceration, TIL density grade, microsatellitosis, presence or absence of regression, lymph node status, number of positive lymph nodes, BRAF mutational status. BRAF mutation was performed by analyzing the primary cutaneous tissue sample. All pathology reports for the patients included in the study were reviewed by another pathologist, and there was 100% concordance with the databases.

Statistical analysis

Qualitative and quantitative data were described, and an evaluation of the association between qualitative variables was made by contingency tables, with absolute frequencies and percentages on lines, respectively by mosaic/bar graphs, and the existence of the association was tested by the $\chi 2$ test or the exact Fisher test. In the situation where the relationship between dichotomous qualitative variables was evaluated, indicators were used that quantify the importance of the association: odds ratio, attributable risk, with 95% associated confidence intervals. The evaluation of the multivariate association was evaluated by logistic regressions. For multivariate models, the presence of multicollinearity was evaluated. The fit of the models was evaluated with the Hosmer-Lemeshow test. The model's predictive capacity was evaluated by the overall percentage of correct classification and the area under the receiver operating characteristic. The results are expressed with a 95% confidence interval. The Mann-Whitney U test was used to assess whether there were differences between two independent groups of quantitative data.

Comparisons between groups targeting survival data were made using the log-rank test. The hazard rate was calculated for various explanatory variables to assess their association with survival, using Cox regression, providing the hazard ratio with the associated 95% confidence interval. The presumption of proportional hazard was verified graphically with Schoenfeld residuals and using a statistical test. For all tests, the value of 0.05 was used as a significance threshold, and the bilateral p-value was taken into account in the tests that provided it. The statistical environment for statistical calculations and graphs R version 4.0.2 was used for statistical processing.

Results

A number of 26 patients were included in the current study. The median age of the patients was 60.5 years (46 - 64.75). Men were more frequently affected by the in-transit metastasis than women, whereas the primary tumor had a higher tendency to arise on the thorax and the legs. Nodular histological subtype was the most common among the cohort, whereas ulceration was present in more than 80% of the cases. Also, 50% of the patients were treated by sentinel lymph node biopsy and 50% by complete lymph node dissection. All clinicopathological features are presented in table I.

Table I. Clinicopathological characteristics of the patients included in the study.

Characteristic	Number (%) (n=26)
Age (years), median (IQR)	**60.5 (46 - 64.75)**
Age >= 50 years (Yes vs. No)	19/26 (73.08)
Gender (Female vs. Male)	9/26 (34.62)
Histological type	acral: 3/26 (11.54) nodular: 16/26 (61.54) superficial spreading melanoma: 7/26 (26.92)
Breslow, median (IQR)	5 (4 - 6)
Breslow >= 4 (Yes vs. No)	20/26 (76.92)
Clark level	3: 6/26 (23.08) 4: 11/26 (42.31) 5: 9/26 (34.62)
Ulceration (Yes vs. No)	21/26 (80.77)
Mitotic rate, median (IQR)	8.5 (6 - 15.75)
Mitotic rate >= 5 (Yes vs. No)	21/26 (80.77)
Perineural invasion (Yes vs. No)	1/26 (3.85)
Angiolymphatic invasion (Yes vs. No)	5/26 (19.23)
Regression (Yes vs. No)	1/26 (3.85)
TIL (brisk vs. non-brisk)	3/26 (11.54)
Microsatellitosis (Yes vs. No)	8/26 (30.77)
pT	3a: 1/26 (3.85) 3b: 8/26 (30.77) 4a: 5/26 (19.23) 4b: 12/26 (46.15)
pN	0: 3/26 (11.54) 1: 7/26 (26.92) 2: 7/26 (26.92) 3: 9/26 (34.62)
Lymph nodes number, median (IQR)	13 (7.75 - 20.75)
Positive lymph nodes number, median (IQR)	2 (1 - 4)
Positive lymph nodes (Yes vs. No)	23/26 (88.46)
BRAF	Negative: 8/26 (30.77) Positive: 13/26 (50)
BRAF mutation type	V600E: 12/26 (46.15) V600K: 1/26 (3.85)
Primary tumor site	Abdomen: 4/26 (15.38) Lower limb: 9/26 (34.62) Thorax: 10/26 (38.46) Upper limb: 3/26 (11.54)
Intervention type (CLND vs. SLNB)	13/26 (50)

Table II. Univariate analysis according to disease-free survival.

	HR unadjusted	(95% CI)	p
Histological type (nodular vs. acral)	1.7	(0.48 - 5.96)	0.409
Histological type (superficial spreading melanoma vs. acral)	1.87	(0.47 - 7.4)	0.37
BRESLOW >= 4 (Yes vs. No)	1.06	(0.41 - 2.7)	0.906
Ulceration (Yes vs. No)	0.84	(0.31 - 2.26)	0.725
Mitotic rate >= 5 (Yes vs. No)	1.17	(0.43 - 3.18)	0.758
TIL (non-brisk vs. brisk)	2.52	(0.58 - 10.93)	0.218
Positive lymph nodes (Yes vs. No)	1.29	(0.38 - 4.41)	0.686
BRAF (Positive vs. Negative)	1.1	(0.44 - 2.76)	0.844
BRAF mutation type (V600K vs. V600E)	0.71	(0.09 - 5.71)	0.746
Localization (Lower limb vs. Abdomen)	1.26	(0.37 - 4.22)	0.713
Localization (Thorax vs. Abdomen)	4.15	(1.14 - 15.12)	0.031
Localization (Upper limb vs. Abdomen)	2.86	(0.57 - 14.42)	0.203
Intervention type (SLNB vs. CLND)	0.6	(0.27 - 1.35)	0.216

Table III. Multivariate analysis according to disease-free survival.

	HR adjusted	(95% CI)	P-value
Ulceration (Yes vs. No)	1.06	(0.36 - 3.13)	0.92
Mitotic rate >= 5 (Yes vs. No)	0.77	(0.25 - 2.36)	0.644
TIL (non-brisk vs. brisk)	3.63	(0.71 - 18.59)	0.123
Localization (Lower limb vs. Abdomen)	1.74	(0.46 - 6.63)	0.416
Localization (Thorax vs. Abdomen)	5.41	(1.32 - 22.16)	0.019
Localization (Upper limb vs. Abdomen)	5.29	(0.89 - 31.3)	0.066

On univariate analysis, only the primary tumor site located on the thorax influenced the recurrence of the disease, with an HR of 4.15 (95% CI:1.14-15.12, p=0.031), whereas other clinicopathological characteristics did not have a statistically significant impact. Also, on multivariate analysis, patients with primary tumor site on the thorax and the upper limb were more likely to develop an early recurrence, as thorax localization with an HR of 5.41 (95% CI:1.32-22.16) and upper limb localization with an HR of 5.29 (95% CI:0.89-31.3) were risk factors for developing in-transit metastasis.

Discussion

This study shows that a primary invasive cutaneous melanoma located on the thorax is strongly correlated with the occurrence of in-transit metastasis. Other clinicopathological characteristics showed no statistically significant difference, probably due to the small number of patients included in the study, as the incidence of locoregional recurrence is low, accounting for less than 10% of the cases.

The Breslow index remains the most important prognostic factor, and depending on it, the management of the disease is guided, according to NCCN and ASCO recommendations [24,25]. A Breslow index >4 mm was correlated with a high risk of developing in-transit metastasis in our study. However, other clinicopathological parameters are also considered as independent prognostic factors in malignant melanoma. Age, in a study including 2268 patients, represents an independent factor for prognosis, as older age is correlated with a lower risk of lymph node metastasis, but with a higher rate of locoregional recurrence, in comparison with young patients that present a higher risk of lymph node metastasis, but with a favorable prognosis [26]. In another study on 849 patients, authors observed that patients over 65 years old presented with a higher tumoral stage, therefore age influenced the prognosis directly, especially for patients with numerous comorbidities that might have had relative or absolute contraindications for performing a radical treatment, both by surgery or by chemotherapy [27]. Also, gender represents another clinical feature that was incriminated as an important prognostic factor. Over time, numerous studies were conducted; Mervic et al., in a study including 7738 patients, observed that men had a higher probability of developing lymph nodes and distant metastasis, with lower overall survival than females. Moreover, women had a lower rate of distant metastasis but a higher rate of in-transit metastasis, concluding that hormonal or immunological factors may be responsible

for these differences [28]. In our study, a large proportion of the patients were over 50 years old (73.08%), which concurs with the existing data in the literature, whereas the male gender was more affected by the recurrence compared to females.

The site of the primary tumor may also play an essential role in developing locoregional recurrence. Therefore, the lymphatic drainage may be responsible for differences in the behavior and prognosis of melanoma, as the number of lymph nodes to be passed until entering the bloodstream could be a weapon of the organism against tumor cells [28]. In our study, the most frequent primary site was the thorax, thus a shorter lymphatic pathway; therefore, we found a strong correlation, statistically significant, between the primary tumor site on the thorax and the recurrence, which may validate the hypothesis mentioned above.

There are four different histological subtypes of cutaneous malignant melanoma, the superficial spreading subtype being the most common. On the other hand, the nodular subtype is the most aggressive, being correlated with a higher rate of lymph node metastasis, a higher risk of recurrence, and a lower overall and disease-free survival [29]. After data analysis, we observed that 61.54% of the patients presented with a nodular histological subtype and in-transit metastasis; therefore, even if we did not find a statistically significant correlation, the nodular subtype may be an independent risk factor for developing locoregional metastasis, both in-transit and lymph nodes.

Ulceration represents another crucial prognostic factor, being included in the TNM classification of malignant melanoma since 2009, its presence upstaging the tumor [14]. Several studies validated Balch's hypothesis, formulated in 1980, namely that the dimension of the ulceration is correlated with the overall survival [30]. More specifically, Haut et al. stratified the extent of the ulceration (extended if >70% or moderate / minimum if <70%) and demonstrated that the presence of extended ulceration had a negative impact, influencing the prognosis and being correlated with lower overall survival [31]. Moreover, in 2 clinical trials regarding adjuvant therapy for melanoma, ulceration was investigated as a prognostic factor. The result showed that adjuvant therapy correlated with improved recurrence-free survival and overall survival in patients with ulceration compared with patients without ulceration. Ulceration was present in 21 patients included in our study but without being directly correlated with the occurrence of in-transit metastasis.

Tumor-infiltrating lymphocytes have gained much interest in the past ten years, as melanoma is considered an immunogenic tumor, quickly stimulating immune responses and easily escaping immune surveillance through different mechanisms. Numerous studies confirmed the independent prognostic value of TILs in malignant melanoma, and classification was elaborated: brisk, non-brisk, or absent [32]. Therefore, studies have demonstrated that a non-brisk or absent peritumoral lymphocytic infiltrate correlates with a high rate of lymph node metastasis and low overall survival, as due to the heterogeneity of lymphocytes, immune surveillance is realized when present [16,17,33]. Moreover, the recent findings highlight the close relationship between the evolution of the tumor, the immune response of the host, and the tumor microenvironment, facilitating the use of TILs as potential therapeutic agents [15]. Thus, as expected, a non-brisk infiltrate was present in 88.46% of the cases, however, without being correlated with the occurrence of locoregional metastasis. BRAF oncogene, a serine-threonine kinase, the most common genetic alteration in melanoma, was also identified in 50% of the patients and it may contribute to developing in-transit metastasis; we could find no studies in the literature on this subject, but in the future, this hypothesis may be investigated on a larger cohort of patients.

Surgical technique was also incriminated as being a risk factor for developing locoregional recurrence. The principles of a complete surgical resection are well defined, as resection margins should be appropriate in order to avoid and also decrease the risk of local recurrence. The conclusions of several randomized clinical trials helped define a consensus regarding the appropriate surgical margins according to the stage of the tumor [24,34-36]. Moreover, a hypothesis was raised whether to perform a sentinel lymph node biopsy (SLNB) or complete lymph node dissection (CLND) to avoid a higher risk of developing in-transit metastasis. Thus, it is believed that in-transit metastasis results as an embolus from the tumor cells that becomes entrapped in the dermal lymphatic vessels, leading to local recurrence within a variable length of time [37]. Cerovac et al. included 972 patients in a study and observed that only 7.9% developed locoregional recurrence (3.7% in-transit metastasis); the authors concluded that there was no difference between the two types of lymph node dissection, as the recurrence correlated only with aggressive tumor biology, depending on the Breslow thickness and lymph node metastasis [38]. Another study conducted by Pawlik included 1395 patients with malignant melanoma who underwent sentinel lymph node biopsy; 17.3% of the patients developed disease recurrence, and a small proportion (6.6%) developed in-transit metastasis. The authors concluded that the overall incidence of in-transit metastases in patients undergoing SLNB was low and independent predictors of in-transit metastasis included age >50 years, a lower extremity location of the primary tumor, Breslow thickness, ulceration, and positive sentinel lymph nodes metastasis [19]. Also, Almiron et al. carried out a prospective study, including 404 patients with cutaneous melanoma. Twenty-eight patients (6.9%) developed in-transit metastasis, but without being linked to the approach of the surgical treatment (no statistically significant differences for patients that underwent SLNB),

but being correlated with risk factors such as age>50 years, increased Breslow thickness, and Clark level, the presence of ulceration and positive SLNB [20]. In addition, Beasley reported that early-stage melanomas (<1 mm) develop low rates of in-transit metastasis, with an incidence of only 0.4% (22/5288) of the patients [21]. Our study confirms the aforementioned data, as we did not find any statistically significant differences between SLNB and CLND regarding the occurrence of in-transit metastasis.

Certain limitations of the current study must be specified. This is a retrospective study, and we included in the analysis only patients treated in our institution, as the study is based on a single center and therefore including a small number of patients. Indeed, a more extensive study on a larger cohort of patients may be more relevant in order to validate the main prognostic factors statistically. Also, in order to eliminate any other potential errors, we revised every histopathology report, and all the patients were treated after the same protocol, in concordance with NCCN recommendations.

Conclusions

In conclusion, clinicopathological factors such as an increased Breslow thickness and Clark level, the presence of ulceration, positive lymph nodes, a non-brisk TIL density, a high mitotic rate, a nodular subtype, and age>50 years may represent risk factors for developing in-transit metastasis, even though we could not find any correlations. However, prospective and more extensive cohort studies are needed in order to validate these important prognostic factors. Therefore, these predictive factors may help clinicians identify high-risk patients and guide a more aggressive adjuvant management.

References

1. National Cancer Institute TS, Epidemiology and End Results (SEER) Programme. Cancer Stat Facts: Melanoma of the Skin 2020. Available from https://seer.cancer.gov/statfacts/html/melan.html.
2. Ballantine KR, Watson H, Macfarlane S, Winstanley M, Corbett RP, Spearing R, et al. Small Numbers, Big Challenges: Adolescent and Young Adult Cancer Incidence and Survival in New Zealand. J Adolesc Young Adult Oncol. 2017;6:277-285.
3. Watson M, Geller AC, Tucker MA, Guy GP Jr, Weinstock MA. Melanoma burden and recent trends among non-Hispanic whites aged 15-49years, United States. Prev Med. 2016;91:294-298.
4. Guy GP Jr, Zhang Y, Ekwueme DU, Rim SH, Watson M. The potential impact of reducing indoor tanning on melanoma prevention and treatment costs in the United States: An economic analysis. J Am Acad Dermatol. 2017;76:226-233.
5. Gata VA, Lisencu CI, Vlad CI, Piciu D, Irimie A, Achimas-Cadariu P. Tumor infiltrating lymphocytes as a prognostic factor in malignant melanoma. Review of the literature. J BUON. 2017;22:592-598.
6. Luther C, Swami U, Zhang J, Milhem M, Zakharia Y. Advanced stage melanoma therapies: Detailing the present and exploring the future. Crit Rev Oncol Hematol. 2019;133:99-111.
7. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med. 2015;372:30-39.
8. Schachter J, Ribas A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomized, open-label phase 3 study (KEYNOTE-006). Lancet. 2017;390:1853-1862.
9. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomized, phase 3 trial. Lancet Oncol. 2018;19:1480-1492.
10. Long GV, Atkinson V, Cebon JS, Jameson MB, Fitzharris BM, McNeil CM, et al. Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial. Lancet Oncol. 2017;18:1202-1210.
11. Jinga CD, Ciuleanu T, Negru S, Aldea C, Gales L, Bacanu F et al. Effectiveness and safety profile of ipilimumab therapy in previously treated patients with unresectable or metastatic melanoma - the Romanian Patient Access Program. J BUON. 2017;22:1287-1295.
12. Incorvaia L, Badalamenti G, Rinaldi G, Iovanna JL, Olive D, Swayden M, et al. Can the plasma PD-1 levels predict the presence and efficiency of tumor-infiltrating lymphocytes in patients with metastatic melanoma? Ther Adv Med Oncol. 2019;11:1758835919848872.
13. Sini MC, Doneddu V, Paliogiannis P, Casula M, Colombino M, Manca A, et al. Genetic alterations in main candidate genes during melanoma progression. Oncotarget. 2018;9:8531-8541.
14. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27:6199-6206.
15. Gata VA, Milan Kubelac P, Buiga R, Vlad IC, Valean D, Muntean MV et al. The value of tumor infiltrating lymphocytes as prognostic factor for lymph node status and survival amongst patients with cutaneous malignant melanoma. J BUON. 2020;25:2700-2707.
16. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J Clin Oncol. 2012;30:2678-2683.
17. Thomas NE, Busam KJ, From L, Kricker A, Armstrong BK, Anton-Culver H, et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment, and melanoma study. J Clin Oncol. 2013;31:4252-4259.

18. Lee N, Zakka LR, Mihm MC Jr, Schatton T. Tumour-infiltrating lymphocytes in melanoma prognosis and cancer immunotherapy. Pathology. 2016;48:177-187.
19. Pawlik TM, Ross MI, Johnson MM, Schacherer CW, McClain DM, Mansfield PF, et al. Predictors and natural history of in-transit melanoma after sentinel lymphadenectomy. Ann Surg Oncol. 2005;12:587-596.
20. Clemente-Ruiz de Almiron A, Serrano-Ortega S. [Risk factors for in-transit metastasis in patients with cutaneous melanoma]. Actas Dermosifiliogr. 2012;103:207-213.
21. Beasley G, Tyler D. In-transit melanoma metastases: incidence, prognosis, and the role of lymphadenectomy. Ann Surg Oncol. 2015;22:358-360.
22. Perone JA, Farrow N, Tyler DS, Beasley GM. Contemporary Approaches to In-Transit Melanoma. J Oncol Pract. 2018;14:292-300.
23. Gipponi M, Solari N, Giovinazzo D, Queirolo P, Bertoglio S, Villa G, et al. The role of sentinel lymph node biopsy in patients with local recurrence or in-transit metastasis of melanoma. Anticancer Res. 2014;34:3197-3203.
24. Coit DG, Thompson JA, Albertini MR, Barker C, Carson WE, Contreras C, et al. Cutaneous Melanoma, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2019;17:367-402.
25. Wong SL, Faries MB, Kennedy EB, Agarwala SS, Akhurst TJ, Ariyan C, et al. Sentinel Lymph Node Biopsy and Management of Regional Lymph Nodes in Melanoma: American Society of Clinical Oncology and Society of Surgical Oncology Clinical Practice Guideline Update. Ann Surg Oncol. 2018;25:356-377.
26. Kretschmer L, Starz H, Thoms KM, Satzger I, Völker B, Jung K, et al. Age as a key factor influencing metastasizing patterns and disease-specific survival after sentinel lymph node biopsy for cutaneous melanoma. Int J Cancer. 2011;129:1435-1442.
27. Rutkowski P, Nowecki ZI, Zdzienicki M, Michej W, Symonides M, Rosinska M, et al. Cutaneous melanoma with nodal metastases in elderly people. Int J Dermatol. 2010;49:907-913.
28. Mervic L. Time course and pattern of metastasis of cutaneous melanoma differ between men and women. PLoS One. 2012;7:e32955.
29. Egger ME, Dunki-Jacobs EM, Callender GG, Quillo AR, Scoggins CR, Martin RC 2nd, et al. Outcomes and prognostic factors in nodular melanomas. Surgery. 2012;152:652-659; discussion 659-660.
30. Balch CM, Wilkerson JA, Murad TM, Soong SJ, Ingalls AL, Maddox WA. The prognostic significance of ulceration of cutaneous melanoma. Cancer. 1980;45:3012-3017.
31. In 't Hout FE, Haydu LE, Murali R, Bonenkamp JJ, Thompson JF, Scolyer RA. Prognostic importance of the extent of ulceration in patients with clinically localized cutaneous melanoma. Ann Surg. 2012;255:1165-1170.
32. Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969;29:705-727.
33. Burton AL, Roach BA, Mays MP, Chen AF, Ginter BA, Vierling AM, et al. Prognostic significance of tumor infiltrating lymphocytes in melanoma. Am Surg. 2011;77:188-192.
34. Wright FC, Souter LH, Kellett S, Easson A, Murray C, Toye J, et al. Primary excision margins, sentinel lymph node biopsy, and completion lymph node dissection in cutaneous melanoma: a clinical practice guideline. Curr Oncol. 2019;26:e541-e550.
35. Utjés D, Malmstedt J, Teras J, Drzewiecki K, Gullestad HP, Ingvar C, et al. 2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomized trial. Lancet. 2019;394:471-477.
36. Hayes AJ, Maynard L, Coombes G, Newton-Bishop J, Timmons M, Cook M, et al. Wide versus narrow excision margins for high-risk, primary cutaneous melanomas: long-term follow-up of survival in a randomized trial. Lancet Oncol. 2016;17:184-192.
37. Kretschmer L, Beckmann I, Thoms KM, Mitteldorf C, Bertsch HP, Neumann C. Factors predicting the risk of in-transit recurrence after sentinel lymphonodectomy in patients with cutaneous malignant melanoma. Ann Surg Oncol. 2006;13:1105-1112.
38. Cerovac S, Mashhadi SA, Williams AM, Allan RA, Stanley PR, Powell BW. Is there increased risk of local and in-transit recurrence following sentinel lymph node biopsy? J Plast Reconstr Aesthet Surg. 2006;59:487-493.