Published in final edited form as:

Eur J Cancer. 2015 September ; 51(13): 1780–1793. doi:10.1016/j.ejca.2015.05.023.

EFFECT OF TIME TO SENTINEL-NODE BIOPSY ON THE PROGNOSIS OF CUTANEOUS MELANOMA

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Conflict of interest: None declared.

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Abstract

Instroduction—In patients with primary cutaneous melanoma, there is generally a delay between excisional biopsy of the primary tumor and sentinel-node biopsy. The objective of this study is to analyze the prognostic implications of this delay.

Patients and method—This was an observational, retrospective, cohort study in four tertiary referral hospitals. A total of 1963 patients were included. The factor of interest was the interval between the date of the excisional biopsy of the primary melanoma and the date of the sentinel-node biopsy (delay time) in the prognosis. The primary outcome was melanoma-specific survival and disease-free survival.

Results—A delay time of 40 days or less (HR, 1.7; CI, 1.2 to 2.5) increased Breslow thickness (Breslow ≥2 mm, HR >3.7; CI 1.4 to 10.7), ulceration (HR 1.6; CI, 1.1 to 2.3), sentinel-node metastasis (HR, 2.9; CI, 1.9 to 4.2), and primary melanoma localized in the head or neck were independently associated with worse melanoma-specific survival (all $P<0.03$). The stratified analysis showed that the effect of delay time was at the expense of the patients with a negative sentinel-node biopsy and without regression.

Conclusion—Early sentinel-node biopsy is associated with worse survival in patients with cutaneous melanoma.

Keywords

Melanoma; prognosis; sentinel lymph node; waiting list; skin surgery

Introduction

Sentinel lymph node is a standard staging procedure in melanoma management. Although its relevance in overall survival has not been clearly demonstrated so far, its value to stratify melanoma patients into prognostic groups is unquestionable[1].

There is, usually a delay between excision of the primary tumor and performance of sentinel-node biopsy, attributable to multiple factors, including surgical scheduling, preoperative assessment and planning, and sometimes, limited health care resources. The influence of this delay in prognosis remains unclear to date. Three decades ago, Sim et al[2] evaluated the therapeutic value of elective lymph node dissection and compared immediate lymphadenectomy versus delayed lymphadenectomy between 2 and 4 months bearing in mind the possible role of the regional lymph defensive system of the host against melanoma. They found no significant differences in survival but the arm of patients treated by immediate lymphadenectomy (n=54) showed a slight tendency to metastasize before with a worse prognosis (n=55). More recently, Parrett et al. evaluate the effect of time to sentinel-node biopsy on sentinel-node involvement, recurrence, and mortality, and found no significant differences in survival on comparing a delay time of less than 40 days with one of 40 days or more[3]. They did, however, detect a trend towards higher melanoma-specific mortality in patients who underwent early sentinel-node biopsy (less than 40 days) and attributed this to a higher frequency of ulceration and thicker tumors in this group. They also admitted that their results were underpowered due to the relatively small number of patients evaluated (n=492).

This delay time has not been sufficiently studied regarding the prognosis of these patients.

The objective of this study was to further evaluate in a large series of patients the effect on survival of the delay between excision of a primary melanoma and performance of sentinel-node biopsy.

Patients and Methods

We performed a multi-institutional retrospective observational study in which we selected all cases of primary cutaneous melanoma registered in databases at four hospitals: Hospital Universitario “Virgen de la Victoria” (HUVV) in Malaga, Spain; Instituto Valenciano de Oncología (IVO) in Valencia, Spain; Hospital Clínic Universitari de Barcelona (HCUB) in Barcelona, Spain; and Gustave-Roussy (GR) in Villejuif-Paris, France. It is noteworthy that all databases included patients’ data in a prospective way. Relevant ethical standards regarding the use of databases were applied in all cases.

The study included patients with a single primary melanoma (clinical stage I or II) who had undergone sentinel-node biopsy within 120 days of excision of the primary tumor and who were still alive at the end of this period (n=1977). Patients who developed a recurrence during this time (n=14) were excluded. The final number of patients evaluated was 1963.

The inclusion dates and number of patients evaluated at each of the hospitals were as follows: October 1, 2001 to December 31, 2012 at the HUVV (n=189); January 1, 2000 to December 31, 2012 at the IVO (n=415); February 1, 1997 to December 31, 2012 at the HCUB (n=847); and January 1, 2003 to December 31, 2012 at GR (n=512). The dates corresponded to the periods during which the patient data had been prospectively recorded. The study was approved by local ethic committee.

The main variable was the interval between the date of the excisional biopsy of the primary melanoma and the date of the sentinel-node biopsy. This interval, defined as *delay time*, was used as continuous variable and also categorized using the minimum p-value approach,[4] which consisted of performing multiple log-rank tests to compare survival curves and determine the optimal cutoff point for separating the patients into two groups. We analyzed intervals of 10 days, from day 10 up to day 60. The optimal cutoff was established at 40 days (40 days or less vs. more than 40 days) based on the minimum p-value obtained. This value was contrasted with a recursive partitioning method for categorize variable (Classification and regression tree)[5] and the result was 40.5 days which was rounded to 40 days.

The following clinical covariates were also included: age (both, continuous and dichotomized ≤65 vs. >65 yrs.), sex, and anatomical site (head and neck, trunk, extremities, and hands/feet).

We also analyzed the following histologic features: Breslow thickness (continuous and categorized in four groups: ≤1.00, 1.01–2.00, 2.01–4.00, or >4.00 mm) [6], ulceration (present vs. absent), regression (present vs. absent), and sentinel-node status (positive vs. negative).

Pathologic examination of the sentinel lymph nodes at each hospital was performed using standard procedures that have previously been described[7, 8] The HCUB has been applying the Minitub protocol (EORTC 1208: Minitub registration study) since 2011.

Associations between delay time and other variables were investigated using chi-square tests.

The main outcome was disease-free survival and melanoma-specific survival. Survival was defined as time from baseline, that is, the time from the date of the excision of the primary melanoma plus 120 days, to the date of the first recurrence or death (events for disease-free and melanoma-specific survival, respectively) or to the date of the last follow-up, whichever occurred first. Disease-free and melanoma-specific survival curves for delay time were generated using the Kaplan-Meier method. The log-rank test was used to perform univariate analyses. Multivariate analyses were performed with Cox proportional hazard models. The assumption of proportionality was evaluated graphically using "*log-log*" plots in the two-

sample comparison case. An analysis of missing values was also performed. These values were imputed using a complete-case (multiple imputation) model[9] for which we ran five iterations and combined estimates and standard errors using Rubin's rules. Prior to developing the model, we tested if the data were randomly missing using the missing values add-on module in the SPSS statistical package. A P value of less than 0.05 was considered to indicate statistical significant. SPSS software version 20.0 (SPSS) was used for all statistical analyses. Kaplan-Meier curves were drawn using SAS software version 9.3, Cary, North Carolina, USA.

Vital status of patients lost to follow-up was systematically reviewed through the respective National Mortality Registry.

Results

We evaluated 1963 patients with a single primary melanoma who had undergone sentinel-node biopsy. There were 967 women (49.3%) and 996 men (50.7%), with a median age of 53 years (interquartile range, 41 to 65) (Table 1).

Patients with a delay time of 40 days or less had a higher frequency of ulceration (38.6% vs. 32.1%, P=0.014) and melanoma located on the hands and feet and a lower frequency of melanoma on the extremities (P<0.001). No significant association was found for age, sex, stratified o continuous Breslow thickness, regression, or a higher frequency of sentinel-node involvement.

After a median follow-up of 46 months (interquartile range, 20–77), 209 (10.6%) patients had died and 368 (18,7%) had developed a recurrence (uncensored patients for melanoma-specific survival and disease-free survival, respectively).

We identified 594 patients (30.2%) lost to follow-up until 31 December 2012. Vital status was systematically reviewed in all cases and it was ascertained that 5 patients (1%) had died. A sensitivity analysis was performed on the reviewed data and the results were similar (data not shown).

A delay time of more than 40 days was associated with better 5-year disease-free survival (80.1% vs. 73.8%, P<0.0839) (Figure 1A) and better 5-year melanoma-specific survival (89.5% vs. 82%; P=0.0002) (Figure 1B).

On analyzing cases of regional lymph node involvement during follow-up, we also found no differences between the rate of false-negative sentinel-node in the two groups (21,3% vs. 18.1%). False-negative sentinel-node was calculated as the amount of false negative results divided by the amount of false-negative + true positives as suggested van Akkooi et al.[10]

There was no evidence of difference in the type of recurrence in both groups (Table 1).

The main prognostic factor for disease-free and melanoma-specific survival in the overall group was sentinel-node positivity. Five-year disease-free survival was 85.5% for patients with a negative biopsy and 56% for those with a positive biopsy. The corresponding rates for 5-year melanoma-specific survival were 92.4% and 71.4% respectively. The stratified

analysis by sentinel-node status showed better disease-free and melanoma-specific survival for patients with a delay time of 40 days or more in both the node-positive and node-negative groups (Figures 1C–1F). Although there was a statistical significance only in the negative sentinel lymph node group. In this group, a delay time of 40 days or less was associated with melanomas located in hands and feet. The rest of variables were quite homogenous (Table 4).

A delay time of 40 days or less or as continuous variable retained its statistical significance after adjusting for all co-variates in the multivariate analysis for melanoma-specific survival in the whole population of patients (Table 3.). In these models, sentinel-node involvement, Breslow thickness, sex, ulceration, and anatomical site were all also significant prognostic factors for both disease-free and melanoma-specific survival. Delay time did not retain its significance as an independent prognostic factor for disease-free survival. Multivariate analysis stratifying by sentinel-node metastasis status showed that delay time was only significant for patients with a negative sentinel-node biopsy (Table 5).

We also performed stratified analysis based on the presence or absence of regression, patients showed a lower survival rate in the group of less than 40 days only in the case of the absence of regression. In the presence of histologic regression, a worsening of survival did not exist for patients with a shorter delay to 40 days (Figure 2). In a multivariate analysis the delay time retained its statistical significance only for cases without regression (Table 6).

Discussion

In this study of 1963 patients from four leading hospitals in Spain and France, we have shown for the first time that the interval between excision of a primary cutaneous melanoma and performance of sentinel-node biopsy has prognostic significance, with worse melanoma-specific survival observed in patients who undergo early biopsy, specifically, in those with a negative sentinel-node biopsy and absence of regression in the primary tumor.

Ulceration and Breslow thickness were associated with a worse prognosis in our patients, particularly in the absence of nodal involvement, as it has previously been shown[11–14]. Location on head and neck and, to a lesser extent, on acral sites was associated with worse MSS and DFS, findings that are consistent with previous reports[11, 15].

To our knowledge, only two studies to date, published by Parrett et al.,[3] and at the same time, in this volume, the study of Oude Ophuis et al.[16], have studied the impact of the timing of sentinel-node biopsy on survival in melanoma patients. In Parrett's study, surprisingly, they observed an increased frequency of recurrence and melanoma-specific mortality in patients with a delay time of less than 40 days. The authors attributed the higher mortality and recurrence rates observed in the early group to the fact that sentinel-node biopsy tends to performed sooner in patients with thicker or ulcerated melanomas.

In a similar way, Oude Ophius et al., have analyzed the same topic on a database of the EORTC Melanoma Group exclusively in patients with a positive SLN. Observing that, the timing of sentinel-node biopsy for any interval is not an independent prognostic factor in this group of patients[16].

In our series, thicker and/or ulcerated melanomas were also overrepresented in the ‘early group’. In addition, we noticed a shorter delay for patients with melanomas located on the hands and feet, possibly because sentinel-node biopsy is sometimes performed at the same time as excision of the primary tumor in large or acral melanomas which are often surgically more complex.[17] Our study is in agreement with the results of Oude Ophius et al., since the time to sentinel-node biopsy did not prove to be an independent prognostic factor in the group of patients with a positive SLN. However, time to sentinel-node biopsy retained its significance as an independent prognostic factor for MSS after adjusting for all these variables in the multivariate analysis for patients with a negative SLN.

There are no studies to our knowledge, apart from that by Parrett et al.[3], that offers a possible explanation for the biological plausibility of our findings. Sentinel-node biopsy is predicated on the assumption that melanoma spreads from the primary tumor to the sentinel lymph node before reaching the other nodes in the regional basin.[18]

The sentinel node is the first organ in the lymphatic system that acts as a barrier to tumor spread; accordingly it is also the first structure encountered by tumor antigens traveling through the lymph system from the primary lesion.[19]

The immunogenic capacity of melanoma is well established and forms the basis of various immune-based therapies targeting different immune pathways.[20]

Induction of a specific antitumor T-cell response depends on the priming of specific naïve T cells by dendritic cells in the draining lymph nodes[21, 22]. When a specific antigen is presented by dendritic cells, the naïve T cells are activated.[23] Priming of helper and cytotoxic anti-tumor T cells seems to take place in the SN and potentially causes an antitumor T-cell response in melanoma.

The immunosuppressive effect of melanoma, however, has been well documented, in particular in draining lymph nodes where several mechanisms impairing the activation of regional immunity have been described.[24] This immunosuppression occurs even in the absence of sentinel-node involvement[25], suggesting that it may be partly mediated by the release of different cytokines from the primary tumor.[26–29]

The above-described immunosuppressive state could be reversible following excision of the primary melanoma.[28] In this sense, it has been observed a correlation in the maturation of dendritic cell with respect to a prolonged delay time between the excision of primary tumor and the sentinel lymph node biopsy[30].

Considering what is known about the immunobiology of melanoma and based on the findings of our study, it could be hypothesized that the immunosuppressive sentinel-node microenvironment would disappear following excision of the primary tumor, allowing the induction of an efficient antitumor-specific immune response over the following weeks. In this scenario, early removal of the sentinel node would prevent this response and be detrimental to patients. Thus, it is possible that our apparently paradoxical results could be explained by the fact that, at the early stages (melanoma stages I and II), a short time interval between primary excision and sentinel-node biopsy could be deleterious for

mounting an efficient antitumor immune response. Accordingly, in the setting of the presence of regression in primary melanoma, which is supposed to be a sign of immune response against tumor, it was not possible to observe an effect in delay time.

Our study has certain limitations. Given that our conclusions are based on the retrospective analysis of prospectively collected data, the possibility of bias must be considered. A high proportion of patients were lost-to follow-up. To minimize the effect of this weakness, we systematically reviewed the status of these patients using National Mortality Registries. We also did not include mitotic rate in the analyses, since there was not a systematic and centralized review of the melanoma histologies, the criteria used varied both between centers. It is possible that tumors with higher proliferative activity and faster clinical growth may have been candidates for earlier sentinel-node biopsy. However, to minimize this risk, we performed the necessary adjustments in the multivariate model.

Finally, it is noteworthy that the risk of selection or treatment bias is also limited by the fact that we studied a large group of patients and analyzed prospectively collected data that had not been collected for the purpose of the present study.

In conclusion, our results raise important questions and the implication that early sentinel-node biopsy reduces melanoma-specific survival in patients, needs to be further and prospectively explored. But, what is clear is that a delay in the procedure did not worse the prognosis in any case. Future studies will also need to determine the underlying etiologic and pathogenic mechanisms in order to guide optimal management strategies for our patients.

Acknowledgments

Founding:

This work was supported by partially (Grants 03/0019, 05/0302, 06/0265, 09/1393 and 12/00840) from Fondo de Investigaciones Sanitarias, Spain; by the CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain; by the AGAUR 2009 SGR 1337 of the Catalan Government, Spain; by the European Commission under the 6th Framework Programme, Contract no: LSHC-CT-2006-018702 (GenoMEL); and by the National Cancer Institute (NCI) of the US National Institute of Health (NIH) (CA83115).

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis and interpretation of data; or in the preparation, review, or approval of the manuscript.

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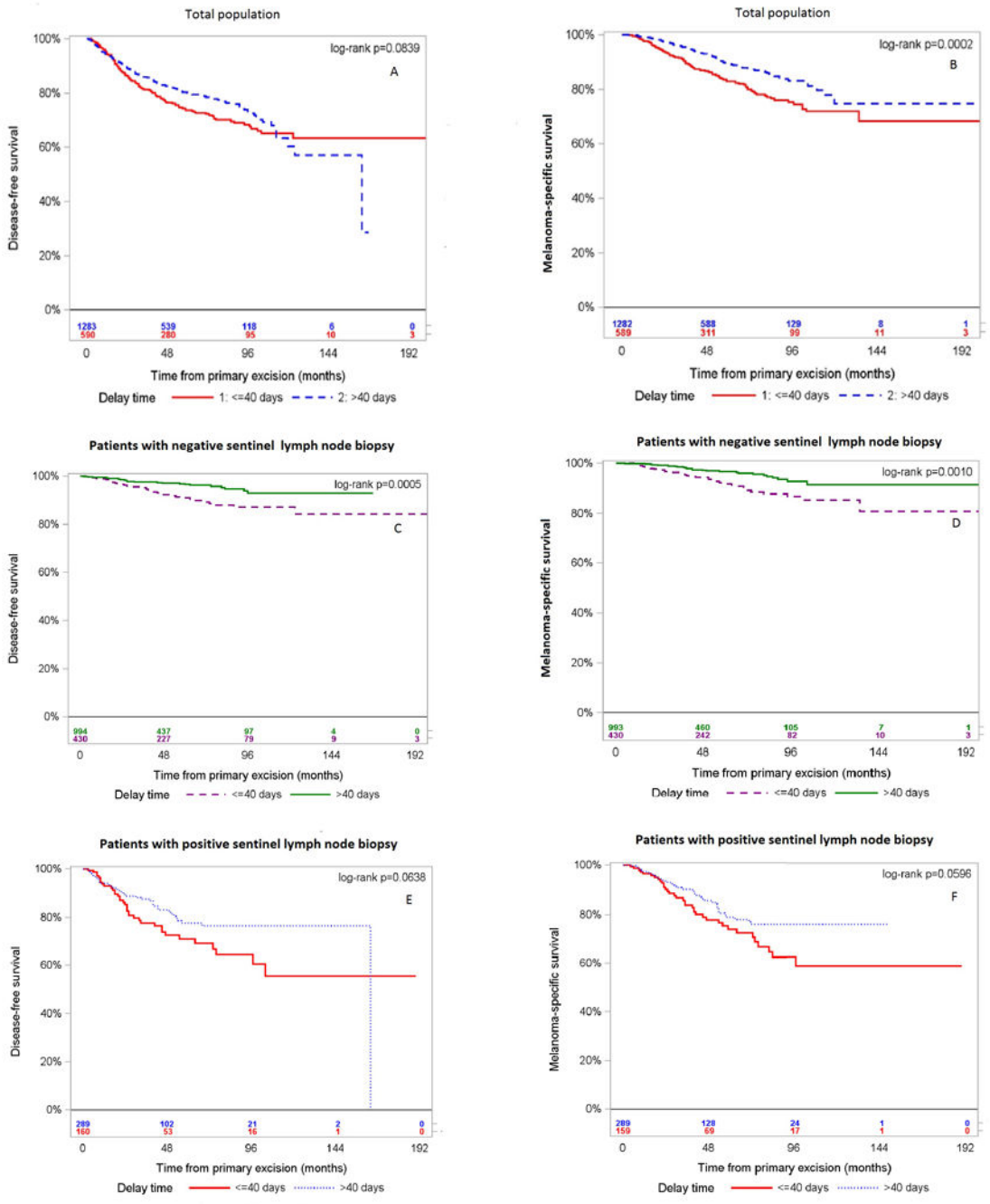

Figure 1.

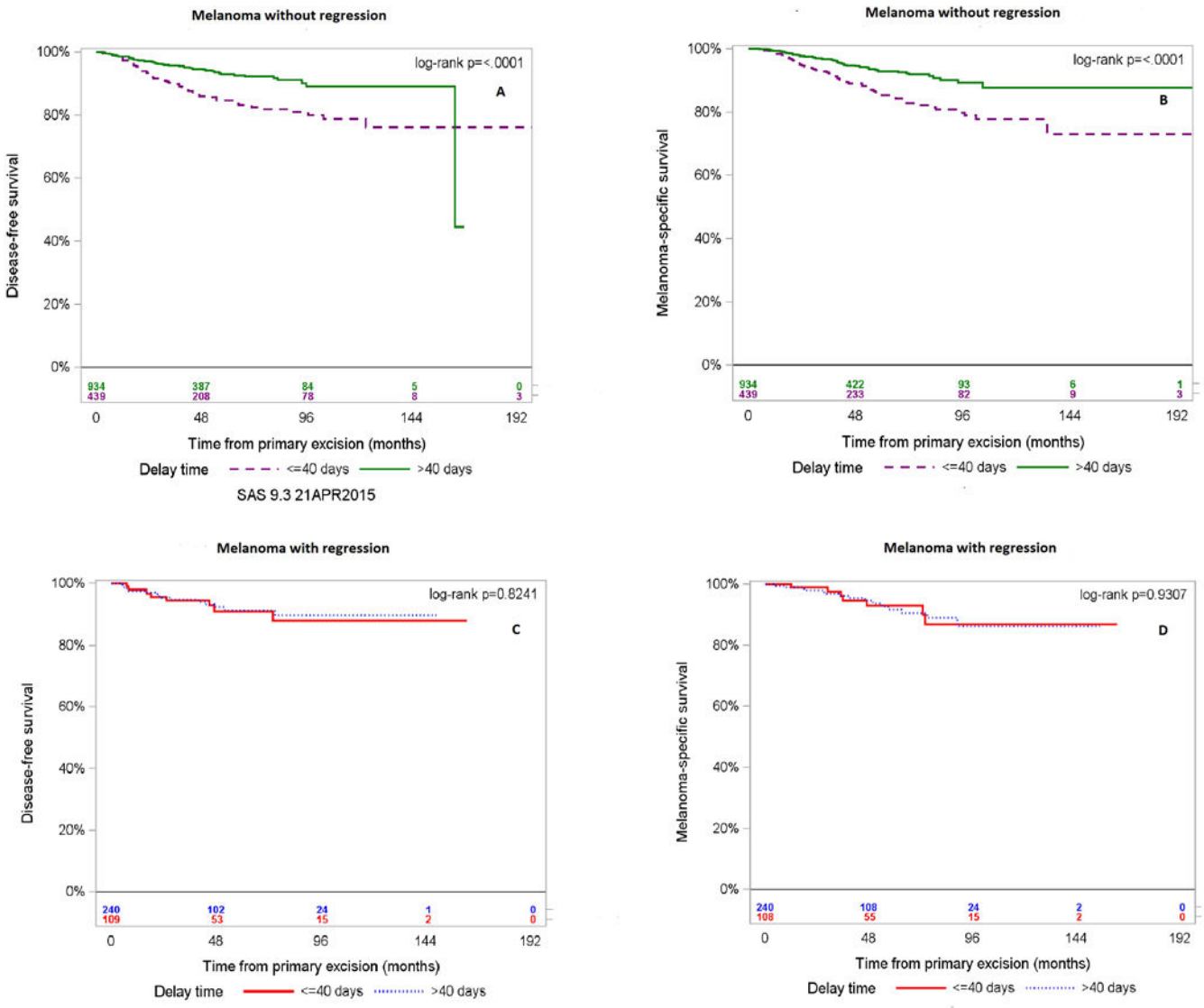

Figure 2.

Table 1

Clinical and Pathological Characteristics of the Study Population (n=1963)*.

	Delay Time			
Variable	**≤40 days**	**>40 days**	**Total Patients**	**P Value**
Age (yr)	N=625	N=1320	N=1945	
≤65	446 (71.4)	975 (73.9)	1421 (72.1)	0.245
>65	179(28.6)	345 (26.1)	524 (26.9)	
Mean (year; SD)	54.2 (16.2)	54.6 (15.7)		0.6
Sex	N=631	N=1324	N=1955	
Male	317 (50.2)	646 (48.8)	963(49.3)	0.55
Female	314 (49.8)	678 (51.2)	992 (50.7)	
Breslow thickness (mm)	N=630	N=1315	N=1945	
<1	102 (16.2)	249 (18.9)	351 (18)	0.132
1.01–2	254 (40.3)	535 (40.7)	789(40.6)	
2.01–4	183 (29)	323 (24.6)	506 (26)	
>4	91 (14.4)	208 (15.8)	299 (15.4)	
Mean (mm; SD)	2.6 (2.4)	2.6 (2.8)		0.7
Ulceration	N=461	N=1096	N=1557	
Present	178 (38.6)	352 (32.1)	530 (34)	0.014
Absent	273 (61.4)	784 (67.9)	1158 (66)	
Regression	N=593	N=1219	N=1812	0.64
Present	117 (19.7)	252 (20.7)	369 (20.4)	
Absent	476 (80.3)	967 (79.3)	1443 (79.6)	
Sentinel-node status	N=633	N=1329	N=1962	
Positive	163 (25.8)	301 (22.6)	464 (23.6)	0.131
Negative	470 (74.2)	1028 (77.4)	1498 (76.4)	
Anatomical site	N=634	N=1329	N=1963	
Head and neck	63 (9.9)	188 (14.1)	251 (12.8)	<0.001
Trunk	221 (34.9)	559 (42.1)	780 (39.7)	
Extremities	240 (37.9)	474 (35.7)	714 (36.4)	
Hands/feet	110 (17.4)	108 (8.1)	218 (11.1)	
Time of follow-up (months)	51 (1–192)	45 (1–170)		
Recurrence	N=586	N=1274	N=1860	
Yes	135 (23)	204 (16)	339 (18.2)	<0.001
No	451 (77)	1070 (84)	1521 (81.8)	
Death	N=586	N=1275	N=1861	
Yes	93 (16.9)	95 (7.8)	188 (10.5)	<0.001
No	493 (84.1)	1180 (92.5)	1673 (89.9)	
Type of recurrence	N=82	N=134		

	Delay Time			
Variable	**≤40 days**	**>40 days**	**Total Patients**	**P Value**
Local	4 (4.9)	9 (6.7)	13 (6)	0.23
Satellitosis/In-transit	12 (14.6)	34 (25.4)	21 (19.6)	
Regional lymph node	25 (30.5)	32 (23.9)	57 (26.4)	
Systemic	41 (50)	59 (44)	100 (46.3)	

*Data shown as number (%) of patients unless otherwise indicated. SD: Standard deviation.

Table 2

Univariate Analysis of Prognostic Factors for Disease-Free and Melanoma-Specific Survival in Patients Who Underwent Sentinel-Node Biospy (n=1963).

	Disease-Free Survival		Melanoma-Specific Survival	
Independent variable	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**
Age (yr) ≤65 >65	<0.001	1 (Reference) 1.5 (1.2–1.8)	0.02	1 (Reference) 1.4 (1.1–1.9)
Sex Female Male	<0.001	1 (Reference) 1.7 (1.4–2.1)	<0.001	1.8(1.4–2.4) 1 (Reference)
Time to sentinel-node biopsy ≤40 days >40 days	<0.004	1.4 (1.1–1.7) 1 (Reference)	<0.001	1.9 (1.4–2.7) 1 (Reference)
Breslow thickness (mm) ≤1.00 1.01–2.00 2.01–4.00 >4.00	0.003 <0.001 <0.001	1 (Reference) 2 (1.3–2.3) 5.1 (3.2–8.2) 10.2 (6.4–16.4)	0.004 <0.001 <0.001	1 (Reference) 2.6(1.4–4.9) 5.6(3–10.5) 9.4(5–17.8)
Ulceration Present Absent	<0.001	2.2 (2.2–3.4) 1 (Reference)	<0.001	2.8 (2.1–3.8) 1 (Reference)
Regression Present Absent	0.001	0.6 (0.4–0.8) 1 (Reference)	…	…
Localization Head and Neck Trunk Extremities Hands/feet	<0.001 <0.001 <0.001 0.65	1 (Reference) 0.5 (0.3–0.7) 0.5 (0.3–0.8) 0.9 (0.6–1.4)	… <0.001 0.001 0.43	1 (Reference) 0.4 (0.2–0.7) 0.5 (0.3–0.8) 0.8 (0.4–1.4)
Sentinel lymph node status Positive Negative	<0.001	3.6 (3–4.4) 1 (Reference)	<0.001	4.1 (3.1–5.4) 1 (Reference)
Hospital HVV IVO HCUB GRC	… … 0.023 …	… … 0.7 (0.6–0.9) …	…	… … … …

HVV: Hospital Virgen de la Victoria; IVO: Instituto Valenciano de Oncología; HCUB: Hospital ClínicUniversitari de Barcelona; GRC: Gustave-Roussy Center.

Table 3

Multivariate Analysis of Prognostic Factors for Disease-Free and Melanoma-Specific Survival in Patients Who Underwent Sentinel-Node Biospy (n=1963).

Disease-free Survival		
MODEL 1		
Independent variable	P value	Odds ratio (95% CI)
Breslow thickness (mm)		
≤1.00		1 (Reference)
1.01–2.00	0.03	2.9(1.1–7.5)
2.01–4.00	<0.001	6.9(2.7–17.8)
>4.00	<0.001	8 (3–21.4)
Ulceration		
Present	0.01	1.7 (1.2–2.5)
Absent		1 (Reference)
Localization		
Head and Neck		1 (Reference)
Trunk	0.011	0.5(0.3–0.8)
Extremities	<0.001	0.3(0.2–0.6)
Hands/feet	0.35	0.7 (0.4–1.3)
Sentinel lymph node status		
Positive	<0.001	2.5 (1.8–3.3)
Negative		1 (Reference)
MODEL 2		
Sex		
Female		1 (Reference)
Male	0.003	1.4 (1.1–1.8)
Age	0.001	1.01(1.05–1.02)
Breslow Thickness (mm)	<0.001	5.2 (3.6–7.5)
Ulceration		
Present	0.01	1.4 (1.1–1.8)
Absent		1(Reference)
Sentinel lymph node status		
Positive	<0.001	2.6 (2.1–3.2)
Negative		1 (Reference)
Localization		
Head and Neck		1 (Reference)

Trunk	0.001	0.6 (0.4–0.8)
Extremities	<0.001	0.5 (0.4–0.6)
Hands/feet	0.06	0.7 (0.5– 1.01)
Melanoma-Specific Survival		
MODEL 1		
Time to sentinel-node biopsy	0.007	1.7 (1.2–2.5)
≤40 days		1 (Reference)
>40 days		
Breslow thickness (mm)		
≤1.00		1 (Reference)
1.01–2.00	NS	…
2.01–4.00	0.007	3.7 (1.4–10.7)
>4.00	0.003	4.5(1.7–12.1)
Ulceration		
Present	0.029	1.6 (1.1–2.3)
Absent		1 (Reference)
Localization		
Head and Neck		1 (Reference)
Trunk	<0.001	0.4 (0.2–0.6)
Extremities	<0.001	0.4 (0.2–0.6)
Hands/feet	0.018	0.5 (0.2–0.9)
Sentinel lymph node status		
Positive	<0.001	2.9 (1.9–4.2)
Negative		1 (Reference)
MODEL 2		
Sex		
Female		1 (Reference)
Male	0.02	1.4 (1.1–2)
Age (per year)	0.03	1.01 (1.001–1.02)
Brewlow thickness	<0.001	3.4 (2.1–5.8)
Localization		
Head and Neck		1 (Reference)
Trunk	0.002	0.5 (0.3-.8)
Extremities	0.006	0.6 (0.4–0.8)
Hands/feet	…	…
Sentinel lymph node status		
Positive		

Negative	<0.001	2.9 (2.1–3.9)
Time to sentinel-lymph biopsy	0.01	0.6 (0.4–0.9)

Table 4

Clinical and pathological characteristics of patients with negative sentinel lymph node biopsy (n=1497).

	Delay Time			
Variable	**≤40 days**	**>40 days**	**Total Patients**	**P Value**
Age (years)	N=464	N=1019	N=1483	
≤65	325 (70)	753 (73.9)	1078 (72.7)	0.12
>65	139 (30)	266 (26.1)	405 (27.3)	
Mean (years; SD)	54.3 (16.9)	54.2 (15.2)		0.9
Sex	N=468	N=1022	N=1490	
Male	225 (48.1)	513 (50.2)	738 (49.5)	0.44
Female	243 (51.9)	509 (49.8)	752 (50.5)	
Breslow thickness (mm)	N=468	N=1015	N=1483	
<1	98 (20.9)	233 (23)	331 (22.3)	0.15
1.01–2	220 (47)	444 (43.7)	664 (44.8)	
2.01–4	109 (23.3)	215 (21.2)	324 (21.8)	
>4	41 (8.8)	123 (12.1)	164 (11.1)	
Mean (mm; SD)	2.1 (1.6)	2.3 (2.8)		0.1
Ulceration	N=335	N=835	N=1170	
Present	102 (30.4)	229 (27.4)	331 (28.3)	0.3
Absent	233 (69.6)	606 (76.6)	839 (71.7)	
Regression	N=444	N=944	N=1388	0.94
Present	99 (22.3)	212 (22.5)	311 (22.2)	
Absent	345 (77.7)	732 (77.5)	1077 (77.6)	
Anatomical site	N=471	N=1026	N=1497	
Head and neck	45 (9.6)	171 (16.7)	216 (14.4)	<0.001
Trunk	161 (34.2)	395 (38.5)	556 (37.1)	
Extremities	189 (40.1)	383 (37.3)	572 (38.2)	
Hands/feet	76 (16.1)	77 (7.5)	153 (10.2)	
Time of follow-up (months)	53 (1–192)	45 (1–188)		
Recurrence	N=433	N=985	N=1418	
Yes	66 (15.2)	113 (11.5)	179 (12.6)	0.049
No	367 (84.8)	872(88.5)	985 (69.5)	
Death	N=433	N=986	N=1419	
Yes	46 (10.6)	45 (4.6)	188 (10.5)	<0.001
No	387 (89.4)	941 (95.4)	1328 (93.6)	
Type of recurrence	N=38	N=69		
Local	3 (7.9)	7 (10.1)	10 (9.3)	0.7
Satellitosis/In-transit	6 (15.8)	15 (21.7)	21 (19.6)	
Regional lymph node	11 (28.9)	14 (20.3)	25 (23.4)	
Systemic	18 (47.4)	33 (47.8)	51 (47.7)	

*Data shown as number (%) of patients unless otherwise indicated. SD: Standard deviation.

Table 5

Multivariate Analysis of Prognostic Factors for Disease-Free and Melanoma-Specific Survival According to Sentinel Lymph Node Status (n=1963).

	Disease-Free Survival				**Melanoma-Specific Survival**			
MODEL 1	**Negative Sentinel- Node Biopsy**		**Positive Sentinel- Node Biopsy**		**Negative Sentinel- Node Biopsy**		**Positive Sentinel- Node Biopsy**	
Independent Variable	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)
Age (yr)								
≤65	…	…	…	…	…	…	…	…
>65								
Sex								
Female	0.018	1 (Reference)	…	…	…	…	…	…
Male		1.6 (1.1–2.2)						
Time to sentinel-node biopsy	…	…	…	…	<0.001	2.6 (1.5–4.6)	…	…
≤40 days						1 (Reference)		
>40 days								
Breslow thickness (mm)								
≤1.00		1 (Reference)		1 (Reference)		1 (Reference)	…	…
1.01–2.00	0.038	2.4 (1.1–4.4)	…	…	…	…	…	…
2.01–4.00	0.001	3.6 (1.8–7.6)	…	…	0.051	2.9 (0.9–8.7)	…	…
>4.00	<0.001	5.8 (2.7–12.4)	0.04	4.4 (1.1–18.4)	0.006	4.9 (1.6– 15.6)	…	…
Ulceration								
Present	0.001	1.8 (1.2–2.5)	…	…	<0.001	2.1 (1.3–3.6)	…	…
Absent		1 (Reference)				1 (Reference)		…
Regression								
Present	…	…	…	…	…	…	…	…
Absent								
Localization								

Head and Neck		1 (Reference)			…	1 (Reference)	…	…
Trunk	0.001	0.4 (0.3–0.7)	…	…	<0.001	0.2 (0.1–0.5)	…	…
Extremities	0.012	0.6 (0.4–0.8)	….	…	0.006	0.5 (0.2–0.8)	…	…
Hands/feet	…	…	….	…	0.017	0.8 (0.2–0.8)	…	…
MODEL 2	**Negative Sentinel- Node Biopsy**		**Positive Sentinel- Node Biopsy**		**Negative Sentinel- Node Biopsy**		**Positive Sentinel- Node Biopsy**	
Independent Variable	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)
Age (yr)	0.003	1.01 (1.01–1.02)	…	…	0.001	1.02 (1.01–1.04)	…	…
Sex								
Female	0.008	1 (Reference)	…	…	…	…	…	…
Male		1.6 (1.1–2.2)						
Time to sentinel-node biopsy	…	…	…	…	0.003	0.4 (0.2–0.7)	…	…
Breslow thickness (mm)	<0.001	6.1 (3.6–10.3)	<0.001	3.4 (2–5.6)	0.001	3.6 (1.6–7.9)	0.004	2.6 (1.4–4.9)
Ulceration								
Present	0.01	1.5 (1.1–2.5)	0.04	1.4 (1.1–1.9)	<0.001	2.1 (1.3–3.6)	…	…
Absent		1 (Reference)		1 (Reference)		1 (Reference)		…
Regression								
Present	…	…	…	…	…	…	…	…
Absent								
Localization								
Head and Neck		1 (Reference)		1 (Reference)	…	1 (Reference)	…	1 (Reference)
Trunk	0.001	0.4 (0.3–0.7)	0.02	0.5 (0.3–0.9)	0.001	0.4 (0.2–0.6)	0.02	0.4 (0.3–0.6)
Extremities	0.012	0.6 (0.4–0.8)	0.002	0.4 (0.3–0.7)	0.02	0.5 (0.2–0.9)	0.04	0.5 (0.2–0.9)
Hands/feet	…	…	….	…	…	…	…	…

Table 6

Multivariate Analysis of Prognostic Factors for Disease-Free and Melanoma-Specific Survival According to the present or absent of histological regression in primary tumor (n=1963).

	Disease-Free Survival				**Melanoma-Specific Survival**			
MODEL 1	**Present of regression**		**Absent of regression**		**Present of regression**		**Absent of regression**	
Independent Variable	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**
Age (yr)								
≤65	…	…	…	…	…	…	…	…
>65								
Sex								
Female	…	…	…	…	…	…	…	…
Male								
Time to sentinel-node biopsy	…	…	…	…	<0.001	2.6 (1.5–4.6)	<0.001	1.4 (1.2–1.7)
≤40 days						1 (Reference)		1 (Reference)
>40 days								
Breslow thickness (mm)								
≤1.00		1 (Reference)		1 (Reference)		1 (Reference)	…	…
1.01–2.00	…	….	…	…	…	…	…	…
2.01–4.00	0.004	4.1 (1.6–10.7)	…	3.4 (1.6–7.6)	0.051	2.9 (0.9–8.7)	…	…
>4.00	0.004	5.7 (1.7–17.1)	0.04	5.3 (2.4–11.7)	0.006	4.9 (1.6– 15.6)	0.05	2.4 (1–6.1)
Ulceration								
Present	…	…	<0.001	1.7 (1.3–2.3)	<0.001	2.1 (1.3–3.6)	0.001	2 (1.3–3)
Absent				1 (Reference)		1 (Reference)		1 (Reference)
Sentinel lymph node status								
Positive	<0.001	4.2 (2.2–8.1)	<0.001	2.3 (1.7–3.1)	…	…	<0.001	2.4 (1.6–3.6)
Negative		1 (Reference)		1 (Reference)				1 (Reference)

Localization								
Head and Neck		1 (Reference)		1 (Reference)	…	1 (Reference)	…	1 (Reference)
Trunk	<0.001	0.2 (0.1–0.5)	…	0.4 (0.3–0.7)	<0.001	0.2 (0.1–0.5)	<0.001	0.3 (0.2–0.6)
Extremities	<0.001	0.2 (0.1–0.4)	<0.001	0.6 (0.4–0.8)	0.006	0.5 (0.2–0.8)	0.045	0.6 (0.3–0.9)
Hands/feet	…	…	0.004	…	0.017	0.8 (0.2–0.8)	0.035	0.5 (0.3–0.9
MODEL 2	**Present of regression**		**Absent of regression**		**Present of regression**		**Absent of regression**	
Independent Variable	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**	**P Value**	**Odds Ratio (95% CI)**
Age (yr)	…	…	0.001	1.01 (1–1.02)	…	…	0.01	1.01 (1.01–1.02)
Sex	…	…						
Female			0.03	1 (Reference)	…	…	…	…
Male				1.3 (1.1–1.5)				
Time to sentinel-node biopsy	…	…	…	…	…	…	0.03	0.6(0.3–0.9)
Breslow thickness (mm)	<0.001	8.1 (3.1–20.5)	<0.001	4.3 (3.4–6.4)	0.01	6.5 (1.6–26)	<0.001	2.9 (1.6–5.1)
Ulceration	…	…						
Present			<0.001	1.6 (1.2–2.1)	…	…	0.008	1.6 (1.1–2)
Absent								1 (Reference)
Sentinel lymph node status								
Positive	<0.001	4.1 (2.1–8.2)	<0.001	2.3 (1.8–3.1)	0.001	4.2 (1.8–10.1)	<0.001	2.6 (1.8–3.6)
Negative		1 (Reference)		1 (Reference)		1 (Reference)		1 (Reference)
Localization								
Head and Neck		1 (Reference)		1 (Reference)	…	1 (Reference)	…	1 (Reference)
Trunk	0.002	0.3 (0.1–0.5)	<0.001	0.3 (0.1–0.5)	0.026	0.3 (0.1–0.8)	0.002	0.4 (0.2–0.7)
Extremities	<0.001	0.2 (0.1–0.4)	<0.001	0.3 (0.1–0.4)	…	…	…	…
Hands/feet	…	…	…	…	…	…	…	….