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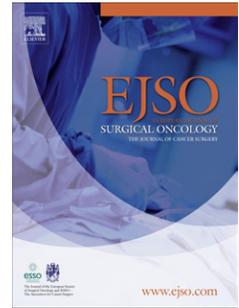
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The value of pre-operative S-100B and SUV in clinically Stage III melanoma patients undergoing therapeutic lymph node dissection

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Synopsis: Preoperative serum S-100B and Fluorodeoxyglucose (FDG) Standardized Uptake Value (SUV) in clinically stage III melanoma are not correlated. S-100B is a strong predictor for Disease Free Survival (DFS) in stage III melanoma.

ACCEPTED MANUSCRIPT

Abstract

Introduction: High preoperative serum S-100B values and Standardized Uptake Values (SUV) of Fluorodeoxyglucose (FDG) in PET for clinically stage III melanoma patients could be indicators of recurrence after surgical treatment. Aim was to assess the correlation and the prognostic value of these markers.

Methods: All melanoma patients with palpable nodal metastases, without distant metastases, were included from February 2004 to December 2007. Preoperative SUV and S-100B was determined. The correlation between SUV and S-100B and their relations with DFS and DSS were calculated by Cox Proportional Hazard Analysis.

Results: 62 Patients, median age 56.9 years, were included in the study. An elevated S-100B was found in 31 patients (50%) and elevated SUV in 24 patients (38.7%). No relation was found between S-100B and SUV. DFS was reduced (31.1%) for patients with an elevated S-100B (HR 3.1; $p=0.02$) in comparison to a normal S-100B (44.6%). The DFS was 42.0% for patients with a SUV below the cut-off point and 29.0% for patients with an elevated SUV (HR 1.1; $p=0.8$). DSS was 60.7% in a normal S-100B and 44.7% for patients with an elevated S-100B (HR 2.2; $p=0.07$). DSS was 59.1% for patients with a normal SUV and 43.5% for patients with elevated SUV (HR 1.1; $p=0.8$).

Conclusion: S-100B and SUV in stage III melanoma are not correlated and each have different associations with various histopathological factors. S-100B, in contrast with SUV, is associated with nodal tumor load, and when elevated, predicts a shorter DFS.

Introduction

Melanoma causes more than 75% of all deaths related to skin cancer and its incidence has increased dramatically worldwide, especially in Caucasian populations.¹ During the period 1998-2007 the Dutch incidence increased from 13.2 to 20.0 newly diagnosed melanoma patients per 100 000 inhabitants.² The past 12 years, melanoma incidence rates have increased rapidly and are expected to keep on rising in the future. The absolute total number of new cases in the Netherlands is estimated to be well over 4800 in 2015, compared to approximately 2400 cases in 2000.³

Despite being diagnosed in an earlier phase of disease, which may be a result of increased awareness and better surveillance, melanoma patients presenting with palpable nodal metastases today still have a poor 5-year survival of 59% and 40% for stage IIIB and IIIC respectively.⁴ Staging studies with whole-body FDG-PET and/or spiral CT in patients with stage IIIB melanoma have shown that about 27% of patients are upstaged and treatment was changed for one of five patients.⁵ Recently Balch et al analyzed 2,313 patients with AJCC stage III disease and for the complete cohort 5-year overall survival was 63%. However, when focusing on more specific patient groups, a tremendous heterogeneity in 5-year survival rates was observed (23%-87% 5-year survival).⁶

More than 60 years ago, Hill published about increased Lactate Dehydrogenase (LDH) as a prognostic serum marker in melanoma patients.⁷ The last decades various biomarkers besides LDH have been studied, such as Melanoma Inhibitory Activity (MIA), S-100B and Standardized Uptake Value (SUV) in FDG-PET.^{8,9} Weighing the evidence, LDH has high specificity for melanoma and literature demonstrates this marker to be elevated in advanced disease, predominantly in case of dissemination to the liver. However, today the most extensively studied melanoma biomarker is S-100B. This 21 kDa protein was first isolated from the central nervous system in vertebrates.¹⁰ A preoperatively elevated serum S-100B in

FDG-PET and CT evaluated patients with palpable nodal metastases is associated with a significantly worse survival.¹⁰⁻¹³ Another prognostic marker was studied by assessment of the degree of Fluorodeoxyglucose (FDG) accumulation during Positron Emission Tomography in a melanoma metastasis; the Standardized Uptake Value (SUV).¹⁴ Bastiaannet et al proved that the uptake of FDG in the lymph node metastases in melanoma patients with clinical stage III melanoma is of prognostic value.¹⁵

High values of S-100B and FDG Standardized Uptake Value (FDG-SUV) measured preoperatively in stage III melanoma patients could both be highly specific indicators of early recurrence after surgical treatment. Possibly, S-100B values and FDG-SUV form a measurable reflection of the presence of a subclinical process of dissemination. Therefore, the aim of this study was to assess the correlation between both markers and to study their association with Disease Free Survival (DFS) and Disease Specific Survival (DSS).

Material and methods

Patients

Melanoma patients, previously staged as AJCC I or II, now presenting with palpable and pathologically proven lymph node metastases (AJCC stage IIIB or IIIC) were prospectively included in this study from February 2004 to December 2007. Patients were staged with whole-body FDG-PET and spiral CT. In case of negative test results for distant metastases, patients were eligible for therapeutic lymph node dissection (TLND) and were included in this study. Sentinel lymph node positive patients were excluded from this study. Patients received adjuvant radiotherapy (20 x 2.4 Gy) in case of a nodal metastasis of ≥ 3 cm in diameter, ≥ 3 tumor positive lymph nodes or the presence of extranodal growth.^{16,17}

Patient characteristics and follow up

Age, sex, treatment strategy of primary melanoma, date of primary melanoma diagnosis, characteristics of the primary melanoma (Breslow thickness, localization, ulceration status, Clark level) and characteristics of the lymph node metastases (localization, number of lymph nodes removed, number of tumor positive nodes, presence of extranodal growth, lymph node size and AJCC stage III B/C) were recorded, as well as the date of recurrence or death. Any form of melanoma recurrence after TLND was scored as recurrent disease.

Follow-up strategy was the same for all patients. Three monthly history and physical examination in the first year after TLND, 4 monthly in year 2, 6 monthly in years 3 to 5 and annual visits and chest radiographs for the subsequent five years.

PET protocol

FDG-PET and CT were performed preoperatively in a random order. FDG was produced on site.¹⁸ Before FDG injection, patients were instructed to fast for at least 6 hours and drink 1 liter of water. After intravenous injection of FDG (Range 220 to 690 MBq), whole body PET-imaging was performed; two/three dimensional mode, emission scans 5 minutes per bed position, starting 90 minutes after injection of FDG. Patients were scanned from scalp to feet, using a Siemens ECAT EXACT HR + scanner (Siemens/CTI, Knoxville, TN). FDG-PET readings were performed by attending staff nuclear medicine physicians.

CT protocol

In the CT protocol a 64-slice spiral CT (Siemens Somatom Sensation) was performed of neck, chest and abdomen. Before starting the procedure oral (800 ml) and intravenous contrast agents were administered to the patient using standard imaging protocols. CT images were interpreted by attending staff radiologists.

Standardized Uptake Value (SUV)

The SUV depends on the amount of injected radioactivity, the patient's weight and the calibration factor of the camera. The value is calculated according to the following formula: $SUV\ mean = \frac{\text{radioactivity concentration in tissue (Bq/Kg)}}{(\text{injected dose [Bq]} / \text{patient weight [Kg]})}$. Three dimensional regions of interest were placed semi-automatically over the tumor on multiple slices using a software program and a threshold of 70 % of the maximum pixel value within the tumor. In case of multiple metastases in the lymph node basin, the lesion with the most intense uptake was analyzed. For SUV the median of 8.4 was used as cut-off value in absence of a standardized cut-off value. Currently no cut-off point is described in literature although initiatives have been undertaken to create a new protocol using standardized uptake values.¹⁹

S-100B

Serum S-100B levels were calculated on the basis of a calibration curve and checked against internal standards with a known concentration of S-100B. In this assay, a cut-off point of 0.15 $\mu\text{g/l}$ was set. The reference values for the S-100B assay (Liason Sangtec 100) were established by analysis of S-100B values of 120 healthy men and women according to the CLSI C28A2 guideline. Concentrations below the cut-off point were considered to be normal, whereas concentration above this point were considered to be elevated. S-100B levels were measured one day before operation.

Statistics

The association between elevated S-100B and elevated SUV was tested with a linear regression model and a scatter plot. Factors correlated with DFS and DSS were analyzed with

univariate (Log rank test) and multivariable (Cox Proportional Hazard) tests. Factors associated with elevated S-100B concentrations and elevated SUV were tested using univariate logistic regression. Finally, a stratified survival analysis for DFS and DSS was performed to assess the strata in which the marker S-100B has the highest prognostic value. Data were analyzed with STATA version 10.0. A difference was considered significant when $p \leq 0.05$.

Results

Patient characteristics

Overall, 62 patients (30 males and 32 females), with a median age of 56.9 (range 24.7-93.2) years, were included in the study. The majority of lymph node metastases were found in the groin (48.3%), followed by the axilla (35%) and the neck (16.7%). Size of the lymph node metastases was less than 3.0 cm in 45.2% and 3.0 cm or bigger in 54.8% of patients. An extranodal growth pattern was found in 37.1% of cases. Patient and tumor characteristics are summarized in Table 1.

S-100B and SUV

Thirty-one patients (50%) had a normal preoperative S-100B level ($< 0.15 \mu\text{g/l}$) and thirty-one patients (50%) had an elevated S-100B level ($\geq 0.15 \mu\text{g/l}$). Thirty-eight patients (61.3%) had a low preoperative SUV (< 8.4) and twenty-four patients (38.7%) had a high preoperative SUV (≥ 8.4). No association was found between preoperative S-100B and SUV ($p=0.7$) in a linear regression model.

Disease free survival (DFS)

For DFS, recurrent disease was interpreted as any form of melanoma recurrence. However, all patients who returned with recurrent disease after TLND had distant metastases and none of the patients presented with local or regional recurrences.

As shown in Table 2 and Figure 1 DFS was 31.1% for a elevated S-100B and 44.6% for S-100B below cut-off point (univariate: HR=1.5; p= 0.2). The DFS was 29% for a elevated SUV and 42% for a SUV below cut-off point (univariate: HR=1.7; p= 0.1).

In multivariate analysis only variables with $p \leq 0.2$ were entered in the multivariable model. SUV and S-100B were entered separately. In multivariate analyses the DFS was significantly correlated with S-100B (HR 3.1; $p=0.02$). There was no correlation of DFS found with SUV (HR 1.1; $p=0.8$). Primary melanoma ulceration ($p=0.008$ and $p=0.01$) and ≥ 2 lymph nodes positive ($p=0.02$ and $p=0.03$) were both associated with significant reduced DFS.

Disease Specific Survival (DSS)

As shown in Table 3 and Figure 1 DSS was 44.7% for an elevated S-100B and 60.7% for a S-100B below the cut-off point (univariate: HR=2.0; $p=0.06$). The DSS was 43.5 % for a elevated SUV and 59.1% for a SUV below cut-off point (univariate: HR=1.7; $p=0.1$).

In multivariate analysis an elevated S-100B showed a trend for DSS (HR= 2.4; $p=0.06$), but an elevated SUV was not correlated with DSS at all (HR=1.0; $P=0.9$). Primary melanoma ulceration was also correlated with DSS ($p=0.05$ and $p=0.06$).

Marker association with histopathological factors

Table 4a reflects the association between histopathological factors and an elevated S-100B or SUV. For FDG-SUV primary melanoma ulceration ($p=0.04$), size of the lymph node

metastases ($p=0.01$) and extranodal growth ($p=0.03$) were associated. For S-100B, a large tumor size in the lymph node metastasis ($p=0.04$) was associated with elevated S-100B levels.

Stratified analysis of the prognostic values S-100B

Table 4b reflects the prognostic value of S-100B for DFS in both males ($p=0.03$) and females ($p=0.01$), for patients aged 46-64 yrs ($p=0.03$), patients with groin metastases ($p=0.02$) and with ≥ 2 positive lymph nodes ($p=0.02$) or a tumor size <3.0 cm ($p=0.04$) and patients with extranodal growth ($p=0.01$).

For DSS, S-100B was of prognostic value especially in elderly patients (≥ 65 years) ($p=0.05$), in patients with groin metastases ($p=0.01$) and with size of the lymph node metastases <3.0 cm ($p=0.02$). A trend towards significance was seen in patients with presence of ≥ 2 positive lymph nodes ($p=0.05$).

Discussion

General

No relation was found between the prognostic markers S-100B and SUV in melanoma patients with palpable lymph nodes. A preoperative elevated S-100B predicts a reduced DFS in stage III melanoma and is a prognostic marker in case of high lymph node tumour burden. An elevated SUV, according to the present data, has no correlation with survival. Therefore, evaluating these data, S-100B could be used as a prognostic marker in the stratification for new adjuvant trials, in order to better stage and select clinically AJCC stage III melanoma patients for systemic treatment.

Prognostic markers

This is the first study, to our knowledge, evaluating both prognostic markers S-100B and SUV in melanoma patients before undergoing a therapeutic lymph node dissection. After a lymph node dissection, most melanoma patients die within three years as a consequence of distant metastases. Prediction of survival in AJCC stage III has traditionally been based on the number and size of lymph nodes, the presence of extranodal growth and ulceration of the primary tumor.²⁰ In the search for effective systemic therapy in stage III melanoma, the development of prognostic markers and staging will be of increasing interest in order to improve patient-tailored adjuvant therapy.

Staging

Accurate staging before treatment is essential for all melanoma patients. However, for the evaluation of early regional lymphatic dissemination in AJCC stage I and II melanoma, determining S-100B or performing a PET/CT scan is unsuitable.²¹ Fortunately, nowadays the technique of lymphatic mapping and sentinel lymph node biopsy (SLNB) can be used as a standard method of selecting patients with micro metastases. The technique has undisputedly proven to be a reliable staging method and the fourth interim analysis of the MSLT I study reveals that SLNB indeed leads to improved melanoma specific survival.^{22,23} In staging studies analyzing patients with palpable lymph nodes (AJCC stage III), whole-body FDG-PET and/or spiral CT have proved their value with 27% upstaging to stage IV disease. A sensitivity of 79-92% and a specificity of 86-90% to detect distant metastases was found.^{5,24} The combined FDG PET/CT leads to a change in the planned dissection in 37% of the patients.²⁵ Acknowledging the heterogeneity of stage III melanoma, the suspicion rises that numerous patients have a form of subclinical dissemination, which can even remain undetected by standard FDG-PET and CT imaging.⁶

SUV and S-100B not correlated

SUV can be easily calculated after a FDG-PET in the diagnostic work-up and might recognize, when elevated, high risk stage IIIB patients. By assessing the degree of FDG accumulation SUV was recorded earlier as a prognostic marker by other researchers.¹⁵ If treatment response can be predicted by measurement of increased glucose metabolism, this would be of particular value in choosing an adequate patient-tailored adjuvant therapy. Earlier, several other studies have recommended the use of S-100B. Successful treatment with lymph node dissection, chemotherapy or immunotherapy was associated with decreased S-100B concentrations whereas increased concentrations were an expression of disease progression.²⁶ Also, elevated S-100B was thought to be an expression of early dissemination and an independent prognostic marker of risk for mortality.¹¹⁻¹³ Our results show that the tumour markers SUV and S-100B are not related. Both tumour markers are correlated with tumor size in the lymph node and SUV is also correlated with extranodal growth and primary ulceration (Table 4a).

Consideration

Interpreting these results leads to the conclusion that serum S-100B elevation seems correlated with lymphatic dissemination and nodal tumor burden. Elevation of S-100B might detect a process of haematogenic and lymphogenic dissemination in melanoma patients. Because S-100B is located in the cytoplasm of melanoma cells, elevated serum S-100B probably occurs as a result of loss of melanoma cell integrity.¹³ Earlier evidence concerning FDG uptake suggests that the intensity of uptake does not correlate with lymph node tumor burden, but more with biological aggressiveness and is associated with cell viability and particularly cell proliferative activity.²⁸

In the current study SUV did not prove its role as prognostic marker despite earlier findings by Bastiaannet et al.¹⁵ However, the cohorts differed significantly in localization of the primary melanoma, number of positive nodes and extra nodal growth. Furthermore, earlier Bastiaannet et al used a cut-off point of 6.14 and in the current study 8.4. The cut-off point for SUV is data driven and therefore depends on the patient group. In literature no consensus for a cut-off point for SUV has been formed yet.¹⁹ In general, SUV values are often subject to different sources of variability, such as the timing of PET acquisition after tracer injection, patient size, region of interest definition, partial volume and image reconstruction.^{29,30}

Conclusion

Preoperative elevation of S-100B is a prognostic marker for AJCC stage III melanoma patients. S-100B, although not significantly correlated with DSS ($p=0.06$), is a prognostic marker for DFS. The results of the current study, although with a relatively small group, again confirm the value of preoperative S-100B in stage III melanoma.¹¹ Furthermore, Standardised Uptake Value (SUV) is not associated with S-100B and the marker does not reconfirm its status as independent prognostic factor.

For the future we predominantly recommend the use of S-100B in combination with the established prognostic parameters for better stratification in new adjuvant therapeutic trials. Pre-operative S-100B levels provide information for stage III melanoma patients who want to be optimally informed about their disease prognosis. Ongoing research for prognostic biomarkers in larger prospective, randomized, high-quality methodological studies is essential in establishing treatment better tailored to the characteristics of individual tumor biology in stage III melanoma.

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Table 1 : Characteristics of 62 AJCC stage III

melanoma patients unthergoing lymph node dissection

		N	%
Sex	Male	30	48.4
	Female	32	51.6
Age (yrs)	≤45	16	25.8
	46-64	28	45.2
	≥65	18	29.0
Breslow thickness	≤2.0	30	48.4
	>2.0	32	51.6
Clark Level	I-III	17	27.4
	IV-V	36	58.1
	Unknown	9	14.5
Ulceration	No	51	82.3
	Yes	11	17.7
Region	Neck	10	16.7
	Axilla	21	35.0
	Groin	29	48.3
Number removed	<15	27	43.6
	≥15	35	56.4
Positive nodes	<2	26	41.9
	≥2	36	58.1
Lymph node size	<3.0	28	45.2
	≥3.0	34	54.8
Extranodal growth	No	39	62.9
	Yes	23	37.1

Table 2: Univariate and multivariate analysis on Disease-Free Survival (DFS) for SUV and S100-B with any recurrence as event.

Characteristic	3-years DFS	Univariate	Univariate p-	Multivariable	Multivariable	Multivariable	Multivariable	
		HR	value	HR	p-value	HR	p-value	
Breslow	≤2.0	44.9	<i>Ref</i>	<i>0.09</i>	<i>Ref</i>	<i>0.4</i>	<i>Ref</i>	0.7
Thickness	>2.0	30.0	1.7 (0.9-3.3)		1.4 (0.6-3.0)		1.2 (0.5-2.5)	
Melanoma	No	43.2	<i>Ref</i>	<i>0.01</i>	<i>Ref</i>	<i>0.008</i>	<i>Ref</i>	<i>0.01</i>
Ulceration	Yes	11.4	2.5 (1.2-5.3)		2.8 (1.3-6.2)		2.7 (1.2-5.9)	
Lymph node positive	<2	52.7	<i>Ref</i>	<i>0.02</i>	<i>Ref</i>	<i>0.02</i>	<i>Ref</i>	<i>0.03</i>
	≥2	26.8	2.3 (1.2-4.6)		2.7 (1.2-5.8)		2.5 (1.1-5.4)	
Extranodal growth	No	46.5	<i>Ref</i>	<i>0.01</i>	<i>Ref</i>	<i>0.07</i>	<i>Ref</i>	0.2
	Yes	22.4	2.3 (1.2-4.5)		2.1 (0.9-4.6)		1.7 (0.8-3.9)	
Region	Neck	72.9	<i>Ref</i>	0.1	<i>Ref</i>	0.1		
	Axilla	24.5	4.9 (1.1-1.7)		3.2 (0.7-15.3)			
	Groin	25.9	4.8 (1.1-0.7)		4.6 (1.1-20.0)			
S-100B	Low	44.6	<i>Ref</i>	0.2	<i>Ref</i>	<i>0.02</i>		
	High	31.1	1.5 (0.8-2.9)		3.1 (1.2-7.8)			
SUV	Low	42.0	<i>Ref</i>	0.1			<i>Ref</i>	0.8
	High	29.0	1.7 (0.9-3.2)				1.1 (0.5-2.4)	

All variables with $p \leq 0.2$ were entered in the multivariable model; the following variables were not entered:

sex ($p=0.4$), age ($p=0.9$), clark level ($p=0.7$) localization of the primary tumor ($p=0.9$), number of lymph nodes removed ($p=0.3$) and tumor size of the lymph nodes metastasis ($p=0.6$). **SUV and S100 were entered separately in the model.

Table 3: Univariate and multivariate analysis on Disease-Specific Survival (DSS) for SUV and S-100B with death due to melanoma as event.

Characteristic		3-years DSS	Univariate HR	Univariate p-value	Multivariable HR	Multivariable p-value	Multivariable HR	Multivariable p-value
Sex	Male	39.7	Ref	0.2	Ref	0.1	Ref	0.1
	Female	64.2	0.6 (0.3-1.3)		0.5 (0.2-1.1)		0.5 (0.2-1.2)	
Breslow thickness	< 2.0	63.0	Ref	0.2	Ref	0.1	Ref	0.2
	≥ 2.0	42.8	1.6 (0.8-3.3)		2.1 (0.9-5.0)		1.8 (0.8-4.4)	
Melanoma Ulceration	No	58.9	Ref	0.04	Ref	0.05	Ref	0.06
	Yes	27.3	2.2 (1.0-4.7)		2.3 (1.0-5.1)		2.3 (0.9-5.4)	
Lymph node positive	<2	51.9	Ref	0.6	Ref	0.2	Ref	0.3
	≥2	44.5	1.2 (0.6-2.5)		1.7 (0.7-3.9)		1.5 (0.7-3.5)	
Region	Neck	66.7	Ref	0.2	Ref	0.8	Ref	0.4
	Axilla	29.9	2.4 (0.8-7.3)		1.4 (0.4-4.9)		2.0 (0.6-6.9)	
	Groin	51.9	1.3 (0.4-3.9)		1.2 (0.4-3.8)		1.0 (0.3-3.3)	
S-100B	Low	60.7	Ref	0.06	Ref	0.06		
	High	44.7	2.0 (1.0-4.0)		2.4 (0.9-5.9)			
SUV	Low	59.1	Ref	0.1			Ref	0.9
	High	43.5	1.7 (0.8-3.4)				1.0 (0.4-2.6)	

All variables with $p \leq 0.2$ were entered in the multivariable model; the following variables were not entered: age ($p=0.8$), clark level ($p=0.3$) localization of the primary tumor ($p=0.3$), number of lymph nodes removed ($p=0.3$), number of nodes positive ($p=0.5$), tumor size of the lymph nodes metastasis ($p=0.7$) and extranodal growth ($p=0.3$). **SUV and S100 were entered separately in the model

Table 4a: Correlations of different patient and tumor characteristics for prognostic markers S-100B and SUV

		Associated with high S-100B	Associated with high SUV
Sex	Male	0.9	0.8
	Female		
Ulceration primary melanoma	No	0.9	0.04
	Yes		
Tumorsize lymph node metastasis	<3.0	0.04	0.01
	≥3.0		
Extranodal growth	No	0.8	0.03
	Yes		

Table 4b: Prognostic value of S-100B stratified for different groups

		DFS* HR (95%CI); p-value	DSS** HR(95%CI); p-value
Sex	Male	4.6 (1.1-19.1); p=0.03	2.4 (0.7-8.4); p=0.2
	Female	6.0 (1.5-24.8); p=0.01	2.6 (0.7-9.5); p=0.1
Age (years)	≤45	0.8 (0.1-7.2); p=0.8	3.0 (0.1-75); p=0.5
	46-64	9.1 (1.6-52.1); p=0.03	1.0 (0.2-3.9); p=0.9
	≥65	2.9 (0.2-42.1); p=0.2	22.9 (1.0-520); p=0.05
Localization lymph node metastases	Neck	p=0.9***	p=0.8***
	Axilla	0.4 (0.1-2.7); p=0.3	0.7 (0.1-5.4); p=0.7
	Groin	3.4 (1.2-10.0); p=0.02	5.2 (1.5-18.9); p=0.01
Lymph nodes positive	<2	4.2 (0.9-20.3) ; p=0.07	3.4 (0.7-15.7) ; p=0.1
	≥2	3.6 (1.3-10.0) ; p=0.02	2.7 (1.0-7.6) ; p=0.05
Tumorsize lymph node metastasis	<3.0	4.7 (1.1-21.0); p=0.04	7.4 (1.4-38.3); p=0.02
	≥3.0	2.6 (0.8-8.0); p=0.1	1.7 (0.5-5.2); p=0.4
Extranodal growth	No	2.3 (0.8-6.3); p=0.1	2.6 (0.9-7.8); p=0.1
	Yes	6.0 (1.5-24.3); p=0.01	2.9 (0.8-10.0); p=0.1

*DFS adjusted for ulceration, Breslow, number of positive nodes, localization and extranodal growth

DSS adjusted for sex, ulceration, Breslow, number of positive nodes and localization. *no estimation of the HR due to small numbers.

Fig 1 : Disease-Free Survival (DFS) and Disease Specific Survival (DSS) for the prognostic markers S-100B and SUV

