

# Ultrasound of the Sentinel Node in Melanoma Patients: Echo Free Island (EFI) is a Discriminatory Morphologic Feature for Node Positivity

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*Melanoma Res* 2016, 26:267-271 doi: 10.1097/CMR.0000000000000223

## Abstract

**Objective** Unlike breast and thyroid cancer, the use of Ultrasound (US) guided Fine Needle Aspiration Cytology (FNAC) for preoperative staging is limited in melanoma. New US morphology criteria have shown that US-FNAC can correctly identify 50% of all involved sentinel nodes (SN) in melanoma patients prior to surgical excision. Aim of this study was to examine a new criterion, the Echo-Free Island (EFI).

**Methods** 1,000 consecutively staged melanoma patients (Breslow thickness > 1mm or < 1mm, but ulcerated, Clark IV/V or regressed) scheduled for SN staging underwent preoperative US. US morphology items were assessed: Peripheral Perfusion (PP), Loss of Central Echoes (LCE), Balloon Shape (BS) and EFI. FNAC was performed in case of suspicious and malignant US patterns. All patients proceeded to undergo an SN biopsy or direct CLND (in case of positive FNAC).

**Results** 7% was male. Mean / median Breslow thickness was 2.58 / 1.57 mm. Mean / median follow-up was 56 / 53 months. SN was positive in 21%. EFI information was available in 95.3%. It was seen in 40 patients (4%). EFI sensitivity was 10.8%, specificity 97.6%, PPV 50% and NPV 80.2%. EFI was significantly correlated to PP (67.5%). There was no correlation to BS or LCE. 5-year MSS of patients with EFI was significantly worse: 80% versus 92% when absent.

**Conclusions** The Echo-Free Island (EFI) can be useful in the early detection of SN melanoma metastasis. It is an early sign of involvement and thus associated with a decreased survival.

## Introduction

Unlike staging for breast or thyroid cancer, preoperative ultrasound (US) of the regional sentinel nodes (SNs) is not routinely performed for melanoma<sup>1-4</sup>. Previously, our experience with preoperative US guided fine needle aspiration cytology (FNAC) for the early detection of SN involvement in melanoma was described<sup>5-7</sup>. Amongst others, the pattern of an echo free island (EFI) was presented. Other morphologic factors, such as peripheral perfusion (PP), balloon shaped (BS) lymph node and loss of central echoes (LCE) were key characteristics in these first analyses. EFI was a relatively infrequent finding. With increased experience, the discriminative role of this US morphologic characteristic has now been investigated.

In a 2011 meta-analysis Catalano et al.<sup>8</sup> found that 31 out of 201 articles matched their inclusion criteria to provide a description of the use of US scanning for the detection of melanoma lymph node metastases. This compilation of articles showed that, in contrast to older systems, modern high-resolution scanners now allow for recognition of very subtle abnormalities within the lymph node. Predictive criteria should therefore be modified according to the improved devices and resolution.

Especially the color Doppler imaging should be seen differently nowadays. Even if set adequately for detecting slow flow, the scanners used in the past would frequently not have been able to allow for the recognition of relevant signals in malignant lymph nodes, simply because of the low intrinsic sensitivity of those machines<sup>8</sup>. Instead, modern equipment would be very sensitive to slow flow and optimal depiction of the abnormal angiographic architecture of superficial lymph node metastases<sup>8</sup>. The previous limitations of Doppler systems would help explain why many authors did not consider color Doppler findings in their assessment of melanoma lymph node metastases in the past<sup>8</sup>.

Unfortunately though, all meta-analyses comprise a certain time-period, and Catalano et al. used the years 1989 – 2009<sup>8</sup>. Our pivotal papers from 2009-2010 and obviously the most recent from 2014, reporting the largest prospective collected database on melanoma lymph node US patterns, were not used in the meta-analysis<sup>5-7</sup>. In this large database up to 65% of all metastases could be potentially identified preoperatively<sup>5</sup>. The most sensitive morphologic criterion was PP, which could potentially identify 77% of metastases preoperatively, but had a low specificity of 52%<sup>6</sup>. BS and LCE had a lower sensitivity, but a high positive predictive value (PPV) of 96% and 65%, respectively<sup>6</sup>. Moreover, a clear correlation between these US patterns and SN tumor burden could be established. Smaller lesions showed PP, whereas only in advanced lesions BS and LCE was seen<sup>5</sup>.

Aim of the current study was to report on the infrequently observed morphologic criterion, the echo free island (EFI), which often indicates presence of micrometastatic involvement of the lymph node in melanoma patients.

## Patients and Methods

### *Patients*

This prospective database included all patients presenting with a newly diagnosed, histopathologically proven, primary malignant melanoma (at least 1.00 mm Breslow thickness, or if less, at least Clark level IV/V, ulcerated and/or with regression) and who were planned for an SN procedure at the department of dermatology, Charité, University Medicine Berlin, Germany. The institutional ethical review board (ERB) approved the study and informed consent was obtained from all patients enrolled. For the current analyses of the US patterns, inclusion was halted after the first 1,000 consecutive patients with sufficient follow-up (July 2001 – November 2010). Results in the first 400 patients and the follow-up in the first 1,000 patients have been previously reported<sup>5-7</sup>.

### *Methods*

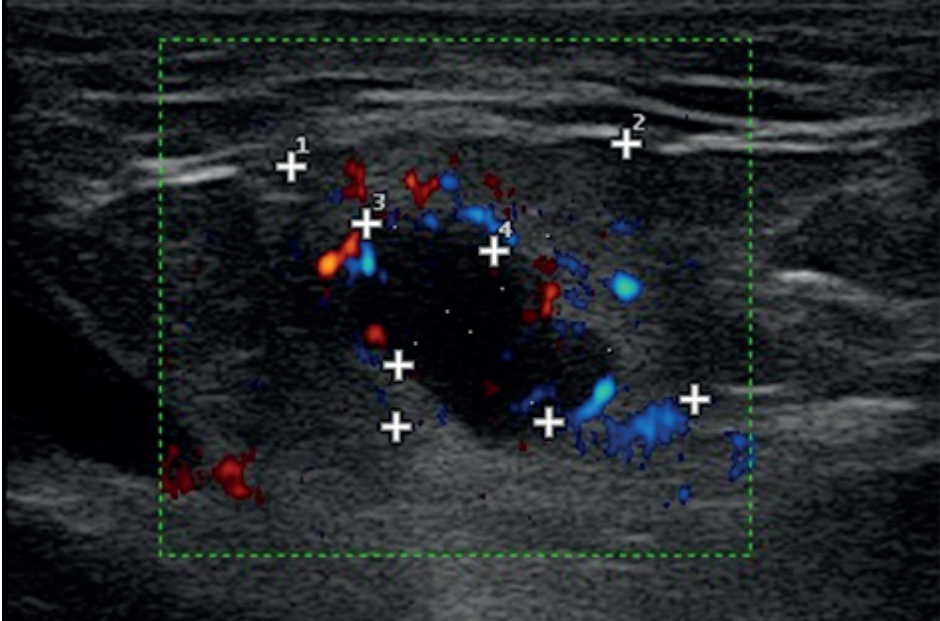
The US patterns were examined, determined and listed in a prospective database. All patients were scheduled for an SN procedure in either a 1 or 2 day protocol. In all cases, patients first underwent a lymphoscintigraphy, which allowed for a targeted US examination of the SNs and adjacent lymph nodes. In the timeslot between lymphoscintigraphy and surgery, patients were examined by US in B-Mode and Power Doppler. US was aimed at clearly depicting the location of the suspected SN and at clearly stating whether it seemed involved or not. If US depicted a suspicious or malignant SN, FNAC was performed for verification of the lesion (3-4 repeat FNACs were performed within one single procedure). If a clearly malignant US pattern could not be verified by FNAC, patients proceeded to undergo an SN nevertheless, since the decision to alter a planned SN directly into a CLND was always based on positive cytology.

Micro-anatomic location of the SN metastases was evaluated according to the criteria by Dewar et al.<sup>9</sup>. SN tumor burden was assessed according to the Rotterdam Criteria for SN tumor burden<sup>10-12</sup>.

### *Ultrasound Technique and Image Analysis*

All US examinations were performed using the high-end device MyLab 70 (ESAOTE, Genova, Italy) equipped with 3 transducers (1-18 MHz) (B-mode, 30 pictures per second, color Doppler, Power Mode). The lymph node was measured, the pattern was described and it was classified as benign [b], suspicious [s] or malignant [m].

In general, an US was considered suspicious, when PP was present or if the central echo was wandering towards the rim. US was considered malignant if there was a total LCE or if the lymph node was enlarged and BS. If an echo-poor disruption of the lymph node architecture was seen, the lymph node was described as EFI (**Figure 1**). Details of these Berlin US morphology criteria have been reported previously elsewhere<sup>6</sup>.



**Figure 1.** Illustration of the Echo-Free Island (EFI) Phenomenon on Ultrasound Examination. Line 1 and 2 indicate the maximum diameters of the lymph node, line 3 and 4 indicate the maximum diameters of the EFI within the lymph node.

### *Fine Needle Aspiration Cytology (FNAC) in detail*

FNAC was performed with a hand-held Binder-valve as described in detail elsewhere<sup>5</sup>. The fine needle used for superficial lymph nodes has a diameter of approximately 0.4 mm (26G), especially for small targets. A smear was considered technically sufficient, if it contained approximately 100 cells.

Cytology results were reported to the surgeons and it was left to their discretion how to proceed with surgery, either SN or LND. If the US did not show any suspicious (EFI, PP) or malignant (BS, LCE) patterns or if the cytology was negative, the patient proceeded to undergo the scheduled SN.

### *Histopathological evaluation of excised SN*

In brief, the EORTC Melanoma Group protocol according to Cook et al. was followed<sup>13</sup>. Lymph nodes were fixed for 24 hours in buffered formalin. After fixation they were cut in half through the hilum and its longest dimension, and embedded in paraffin. In rare cases, exceptionally large lymph nodes were sectioned parallel to the first cut in order to fit into the blocks. Five serial step sections of 4  $\mu$ m each were cut from each face of the lymph node and staining with H&E, S100 and HMB-45 was performed. Micro-anatomic location of the metastases and SN tumor burden were assessed according to the Dewar and Rotterdam Criteria, respectively<sup>9-12</sup>.

## Statistics

To assess the predictive value of individual and combination of US patterns for involved SNs; sensitivity, specificity, PPV (Positive Predictive Value) and NPV (Negative Predictive Value) were calculated. Associations were tested with Pearson's chi square test. Disease-Free Survival (DFS) and Melanoma Specific Survival (MSS) were calculated for SN date until recurrence (DFS) or death due to melanoma (MSS). Patients were censored at the date of last known follow-up if no events had taken place. Univariate analyses of survival were performed using the Kaplan-Meier method and log-rank test. Multivariate analyses to determine the prognostic value of covariates regarding survival were performed using the Cox's proportional hazard model. Statistical analyses were all performed with Stata®, version 10.0 (Stata Corporation, College Station, TX, USA). P values of less than 0.05 were considered as significant.

## Results

Table 1 summarizes the most important patient, primary tumor, SN and US characteristics. Mean and median follow-up of all 1,000 patients was 56 and 53 months, respectively. The EFI pattern could be assessed in 953 patients (95.3%), the pattern was present in 40 patients (4%).

EFI showed a sensitivity of 10.8% for metastasis in the final histology of the SN. Specificity was high (97.6%), PPV was 50%, NPV was 80.2% (**Table 2**). Moreover, a clear correlation was seen with PP. In 27/40 cases with EFI, PP was also seen (67.5%) (**Table 2**).

However, in case of PP, the EFI was only seen in a minority of cases 27/273 (9.9%). There was no correlation between EFI and BS ( $P=0.852$ ) (Table 2). In most cases, when an EFI was seen, the central echo would still be normally present 24/40 (60%) (**Table 2**).

Univariate analysis showed that patients with an EFI had a significantly worse 5-year MSS compared to patients without the EFI US pattern (80% vs. 92%) ( $P<0.001$ ) (**Figure 2**). The univariate hazard ratio (HR) for MSS was 3.32 (95% CI 1.66 – 6.68) ( $P=0.001$ ).

Multivariate analysis for MSS demonstrated that EFI was non-significant when analyzed together with other known independent prognostic factors (ulceration, Breslow thickness and PP, data not presented).

When the multivariate analysis for MSS was performed separately for US patterns only, the presence of any of these patterns was associated with a detrimental survival. EFI had a HR of 2.06 (95% CI 1.01 – 4.20) ( $P=0.048$ ). BS had a HR of 2.62 (95% CI 1.01 – 6.79) ( $P=0.048$ ). LCE has a HR of 3.05 (95% CI 1.26 – 7.40) ( $P=0.013$ ). PP was associated with a HR of 2.57 (95% CI 1.49 – 4.41) ( $P<0.001$ ).

**Table 1.** Baseline Patient, Primary Tumor, Sentinel Node and Ultrasound Characteristics of All 1,000 Patients

Characteristic	N	%	Characteristic	N	%
<i>Gender</i>			<i>SN Tumor Burden (n = 208)</i>		
Male	567	57%	≤ 0.1 mm	30	14%
Female	433	43%	0.1 – 1.0 mm	62	30%
<i>Histological subtype</i>			> 1.0 mm	62	30%
SSM	595	60%	LND / Unknown	54	26%
NM	242	24%	PP		
LMM	37	4%	Absent	663	66%
ALM	44	4%	Present	273	27%
Unknown / other	82	8%	Unknown	64	7%
<i>Breslow Thickness</i>			<i>LCE</i>		
Mean / Median (Range)	2.58 / 1.57 (0.2 – 44) mm		Central Echo Present (Normal)	791	79%
T1 (≤ 1.00 mm)	288	29%	Wandering to Rim	97	10%
T2 (1.01 – 2.00 mm)	308	31%	Lost	66	6%
T3 (2.01 – 4.00 mm)	231	23%	Unknown	46	5%
T4 (> 4.00 mm)	173	17%	BS		
<i>Ulceration</i>			Absent	881	88%
Absent	758	76%	Present	53	5%
Present	242	24%	Unknown	66	7%
<i>SNs Removed</i>			<i>EFI</i>		
Mean / Median	1.72 / 1 (1 – 13)		Absent	913	91%
SN result			Present	40	4%
Negative	792	79%	Unknown	47	5%
Positive	208	21%	<i>US-FNAC Result</i>		
Direct LND (after + FNAC)	43	4%	Not Performed & Negative	892 / 342	89% / 34%
	43 / 208	21%	Positive	98 / 342	10% / 26%

SN, sentinel node; SSM, superficial spreading melanoma; LND, lymph node dissection; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acrolentiginous melanoma; PP, peripheral perfusion; LCE, loss of central echo; BS, balloon shape; EFI, echo-free island; US-FNAC, ultrasound – fine needle aspiration cytology

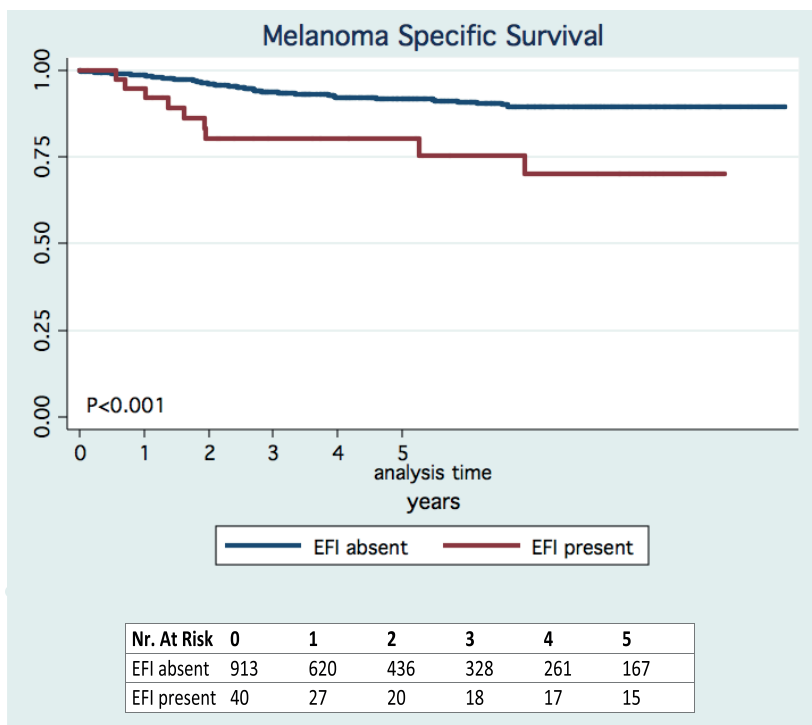
## Discussion

The final results of the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1) have been published<sup>14</sup>. Although the MSLT-1 trial did not demonstrate a significant benefit for wide local excision (WLE) + SN staging (in case of a positive result followed by CLND) versus WLE + sequential nodal observation, the authors concluded that biopsy-management prolongs DFS for all patients and prolongs DMFS and MSS for node positive

**Table 2.** Sensitivity, Specificity, PPV and NPV of the Echo-Free Island (EFI) and Correlation to Peripheral Perfusion (PP), Loss of Central Echoes (LCE) and Balloon Shape (BS)

	SN negative	SN positive	P-value	
EFI absent	732	181	P<0.001	
EFI present	18	22		
<b>Correlations</b>	<b>PP absent</b>	<b>PP present</b>		
EFI absent	650	246	<0.001	
EFI present	13	27		
	<b>BS absent</b>	<b>BS present</b>		
EFI absent	843	51	0.851	
EFI present	38	2		
	<b>Central Echo</b>	<b>Wandering to Rim</b>	<b>LCE</b>	<b>P-Value</b>
EFI absent	766	86	61	<0.001
EFI present	24	11	5	
	<b>Central Echo / Wandering to Rim</b>		<b>LCE</b>	
EFI absent	852		61	0.156
EFI present	35		5	

SN, sentinel node; EFI, echo-free island; PP, peripheral perfusion; BS, balloon shape; LCE, loss of central echo



**Figure 2.** Kaplan-Meier 5-year Melanoma Specific Survival (MSS) for Patients With or Without Echo-Free Island (EFI)



patients with intermediate thickness melanoma<sup>14</sup>. These last conclusions are very much topic of an ongoing debate<sup>15-17</sup>.

Perhaps more important is the fact that the SN biopsy provides accurate and important staging information<sup>14,15</sup>. This SN result provides information on the chance of additional non-SN involvement of the CLND and with respect to survival. SN tumor burden further stratifies these risk assessments<sup>10, 12</sup>. Nowadays, patients with significant SN tumor burden (> 1 mm in maximum diameter), who have a high risk for relapse, might be eligible for adjuvant therapy (studies). Recently, the first positive results with respect to an improved RFS have been reported for patients undergoing adjuvant Ipilimumab<sup>18, 19</sup>.

Even if SN staging itself does not provide a survival benefit to the individual patient, effective adjuvant therapy might. Moreover, patients should be adequately staged to be eligible for participation into new adjuvant therapy trials. It would be unethical to withhold them this possibility.

When comparing the situation in melanoma to that of breast or thyroid cancer, the role of US staging has been minimal. US-guided-FNAC is even less invasive than surgical SN staging. Previous results have shown that US-guided-FNAC can correctly identify 50% up to 80% in selected subgroups, of all SN metastases, prior to the surgical excision by SN biopsy<sup>5,6</sup>. Important aspects to achieve these improved results are the use of newly defined US patterns and a low threshold for FNAC<sup>7</sup>.

The current paper is an addendum to the previously reported Berlin morphology patterns. Another potential useful item is presented, the EFI. Although the EFI is a rare phenomenon, the EFI is significantly associated with PP in 67.5% of cases, and at the same time the Central Echo is still present in the majority of cases (60%), indicating that it is an early sign of disruption by a developing metastasis. This is illustrated by the survival curves, which show that EFI is associated with a significantly decreased MSS.

As these US morphologic signs can be used to detect early (sub) micrometastases in SNs, standard US of the lymph node basins prior to surgery may once more be considered. Ultimately, further improvement of the technique of US scanning and targeted FNAC of the SN may even replace the surgical SN procedure as the routine staging of new stage I/II melanoma patients. Currently ongoing research is being performed to investigate minimally invasive alternatives to the surgical SN procedure (Dutch trial: Gamma Probe and Ultrasound Guided Fine Needle Aspiration Cytology of the Sentinel Node - GULF trial, NL52091.078.15).

In conclusion, the Echo-Free Island (EFI) as an US morphologic sign can be useful in the early detection of SN metastases in melanoma patients. It is an early sign of involvement and thus associated with a decreased survival.

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