Analysis of Dendritic Cells in Sentinel Lymph Nodes of Patients With Endometrial and Patients With Cervical Cancers

P. Pelin Kara, MD, Ali Ayhan, MD,† Biray Caner, MD,* Murat Gultekin, MD,† Omer Ugur, MD,* M. Fani Bozkurt, MD,* Alp Usubutun, MD,‡ and Aysegul Uner, MD‡*

Objective: The aim of this study was to identify the immune response in sentinel lymph nodes (SLNs) of patients with endometrial and patients with cervical cancers by analyzing the number of S-100–, CD1a-, CD83-positive (+) dendritic cells that are the major antigen-presenting cells.

Methods: A total of 56 patients with early-stage cancer (n = 32, with cervical; n = 24, with endometrial cancer) underwent SLN biopsy. Sentinel lymph nodes and non-SLNs were stained with antibodies against S-100, CD1a, and CD83 as markers for dendritic cells to find out whether SLNs were immunomodulated compared with non-SLNs.

Results: The mean values of S-100(+) and CD1a(+) dendritic cells in both the tumor-free and the metastatic SLNs were significantly higher than those of both the tumor-free and the metastatic non-SLNs. When metastatic SLNs were compared with nonmetastatic SLNs, CD83(+) dendritic cells were found significantly more abundant in nonmetastatic SLNs.

Conclusions: Significantly higher numbers of S-100(+) and CD1a(+) dendritic cells in the SLNs compared with those in the non-SLNs may indicate that SLNs are the first sites of immunostimulation. Immunosupression may be the underlying factor for the metastatic involvement of SLNs, which might be secondary to the significantly decreased number of mature dendritic cells in metastatic SLNs compared with tumor-free SLNs.

Key Words: Endometrial cancer, Sentinel lymph node, Dendritic cell, Cervical cancer, Immunostimulation, Immunosupression, S-100, CD1a, CD 83

Received April 1, 2009, and in revised form June 16, 2009.
Accepted for publication June 21, 2009.

(*Int J Gynecol Cancer* 2009;19: 1239–1243)

Sentinel lymph node (SLN) is known as the first node/nodes affected by the primary tumor, and if SLN is negative for metastases, the metastatic involvement of the remaining lymph nodes (non-SLN) are extremely unlikely; therefore, further lymph node resection can be avoided. Sentinel lymph node mapping has become a standard procedure of eliminating unnecessary lymph node resection in the treatment of patients with malignant melanoma and breast cancer. Few studies have examined the feasibility of an SLN procedure in gynecologic tumors.[1–11]

Departments of *Nuclear Medicine, †Gynecology, and ‡Pathology, Hacettepe University Faculty of Medicine, Sihhiye, Ankara, Turkey.
Address correspondence and reprint requests to Pelin P. Kara, MD, Department of Nuclear Medicine, Hacettepe University Faculty of Medicine, 06100 Sihhiye, Ankara, Turkey. E-mail: ppelinozcan@gmail.com.

ISSN: 1048-891X
DOI: 10.1111/IGC.0b013e3181b3e616

Because the SLN is the first node affected by a primary tumor, it is also expected as the first structure encountering the tumor antigens released from the primary tumor. Therefore, it is logical to assume that the immune response of an SLN to a tumor differs from that of non-SLNs. Highly specialized antigen-presenting cells have the key role in immune response against tumor antigens. Dendritic cells (DCs) are known as the most potent antigen-presenting cells that are capable of inducing cytotoxic T lymphocytes from native T cells and therefore used as markers for immune response. Dendritic cells express not only a high level of major histocompatibility complex (MHC) molecules but also a high level of adhesion and costimulatory molecules, which are critical for activating native T cells. Thus, DCs play a central role in the regulation and maintenance of a cellular immune response against cancer. S-100 is a DC marker. Maturation markers of DCs are CD1a and CD83, where CD1a is the immature DC marker and CD83 is the mature DC marker. Sentinel lymph nodes are thought to be more closely associated with antitumor immunity than non-SLNs. Previous studies

showed that SLNs from patients with breast cancer were profoundly modified for immune response relative to non-SLNs. There was a major reduction in the area of the paracortex and the densities of paracortical DCs, as well as in the frequency and complexity of the DC dendritis.[12] The authors proposed that regional lymph node immune suppression was probably due to the influence of the primary tumor and that the selective occurrence of the early metastases in the SLNs was related to their immune modulation.[12] According to our knowledge, there has been no such study so far exploring the previously mentioned hypothesis in patients with gynecologic malignancies.

In this study, we investigated the role of immunomodulation by DC analysis of SLNs in comparison to non-SLNs in patients with early-stage endometrial and patients with early-stage cervical cancers. Our aim was to assess the immunoactivation of DCs as the most potent antigen-presenting cells in SLNs.

MATERIALS AND METHODS

A total of 56 consecutive patients with early-stage cancer (n = 32, with cervical; n = 24, with endometrial cancer) were asked to participate in this study. Patients with prior chemotherapy, radiotherapy, or retroperitoneal surgery were not included in the study. All of the patients with cervical cancer (International Federation of Gynecology and Obstetrics [FIGO] stages IA2-IIA) and endometrial cancer (FIGO stages I and II) underwent SLN biopsy. Radical hysterectomy and systematic retroperitoneal lymph node dissection including para-aortic lymph nodes were completed in all patients. The study was approved by the local ethics committee, and informed consent was obtained from all patients. The detailed method for detection of SLN has been described by our group in a previous report.[13] Briefly, preoperative lymphoscintigraphy (LS) was performed after an injection of a total of 74 MBq (4 doses of 18.5 MBq each) Tc 99m nanocolloid (Nanocis or Nanocoll from CIS Bio International, France, and Amersham Health, Italy, respectively) into the 4 quadrants of the cervix. The scintigraphy protocol consisted of dynamic imaging phase (40 frames of 20 s; matrix, 64 × 64) and static image acquisitions (500,000 counts; matrix, 256 × 256) for 4 hours in anterior projection. The first-appearing persistent focal accumulation was considered to be an SLN. All nodal areas were investigated with gamma probe (Neoprobe 2000; Neoprobe Corporation, Dublin, Ohio) for the hottest lymph nodes after removal of all lymph nodes at surgery. A lymph node was considered to be an SLN if it showed ex vivo radioactive counts more than 10-fold above the background radioactivity. After the removal of all lymph nodes, the pelvic and para-aortic regions were checked for persistent radioactivity.

Pathologic Evaluation

Routine Hematoxylin-Eosin Staining

Frozen sectioning was not performed. All excised SLNs labeled separately were sent to the pathology department to be examined in detail. All SLNs and non-SLNs were fixed in 10% formaldehyde solution and embedded in parafin followed by routine hematoxylin-eosin (H&E) staining. Routine H&E staining with standard techniques was performed for all lymph nodes. When routine H&E staining of SLN was negative for metastases, 3 further step sections (6- to 7-μm thickness) were cut from all pieces at an interval of 0.5 to 1 mm. One of these further sections was used for immunohistochemical detection of epithelial cells. The rest of the sections from each piece were stained again with H&E. Therefore, 2 extra sections were stained by pancytokeratine in lymph nodes that were smaller than 0.5 cm; and 3 extra sections, for lymph nodes that were 0.5 cm or larger. Immunohistochemistry was carried out using a monoclonal antibody directed against cytokeratins AE1 to AE3 (Neomarkers, Fremont, Calif). Appropriate negative and positive controls were done in each case. All non-SLNs excised during the operation were examined by routine H&E staining, and neither step sectioning nor immunochemistry was performed for them.

S-100, CD1a, and CD83 Staining

To find out whether the SLNs were immunomodulated compared with the non-SLNs, 56 representative SLNs (7 metastatic and 49 tumor-free) and 56 representative non-SLNs (4 metastatic and 52 tumor-free) were selected. One SLN and 1 non-SLN node were chosen from each patient for DC analysis. The SLN with the largest diameter was selected as the representative SLN. One of the lymph nodes excised from the para-aortic region that is far away from the tumoral side was randomly selected as the representative non-SLN. The rationale behind the selection of the para-aortic region as the non-SLN location was its relatively far location from the tumor site, assuming that the effect of the tumor on this location in immunomodulation would be less compared with its effect on adjacent locations.

Formalin-fixed and paraffin-embedded tissue blocks were sectioned at 2-μm thick from selected SLNs and non-SLNs, then they were stained with antibodies against S-100 (Novocastra, Newcastle, United Kingdom) as a marker for DCs. The mean number of the S-100–positive (+) DCs in the paracortical areas of the lymph nodes were between those of the SLN and the non-SLNs. The maturation status of the DCs was assessed by the expressions of CD1a and CD83. Monoclonal antibodies against CD1a (Neomarkers) and CD83 (Immunotech, Marseille, France) were used at appropriate dilution in immunohistochemistry. For each marker, the number of positive cells per high-power field (×400) was counted in 10 paracortical areas. Paracortical areas are expected to be the sites where the most prominent changes occur regarding the immunomodulation of DCs. This was the logic behind this selection.

Dendritic cells were counted at the 10 most densely infiltrated areas. Mean population densities in each group were counted. A total of 49 CD1a (25 SLNs and 24 non-SLNs), 49 S-100 (25 SLNs and 24 non-SLNs), and 48 CD83 samples (25 SLNs and 23 non-SLNs) from patients with endometrial cancer and a total of 77 CD1a (39 SLNs and 38 non-SLNs), 76 S-100 (39 SLNs and 37 non-SLNs), and 76 CD83 samples (39 SLNs and 37 non-SLNs) from patients with cervical cancer were evaluated.

Unfortunately, because of technical difficulties in some sections, staining was not successful and the results could not be evaluated (CD1a in 8, S 100 in 9, and CD83 in 10 sections). In a total of 3 CD1a (1 SLN and 2 non-SLNs), 3 S-100 (1 SLN and 2 non-SLNs), and 4 CD83 sections (1 SLN and 3 non-SLNs) in patients with endometrial cancer and a total of 5 CD1a (2 SLN and 3 non-SLNs), 6 S-100 (2 SLNs and 4 non-SLNs), and 6 CD83 sections (2 SLNs and 4 non-SLNs) in patients with cervical cancer, staining was not successful.

Statistical Analysis

Statistical analyses between the SLNs and the non-SLNs were performed using the Mann-Whitney U test. A $P < 0.05$ was considered significant.

RESULTS

Patients With Cervical Cancer

The median age of the 32 patients was 46 years (range, 38–80 years). A total of 15 (47%) of 32 patients were with FIGO stage IB1 cancer; 10 (31%), stage IB2; 4 (12.5%), stage IIA; and 3 (9.5%), stage IA2.

TABLE 1. Number of lymph nodes excised during surgery

	Tumor-Free	Metastatic	Total
SLN	102	14	116
Non-SLN	1680	7	1687
Total			1803

Patients With Endometrial Cancer

The median age of the 24 patients was 54 years (range, 32–69 years). A total of 17 patients were with FIGO stage I cancer (4, stage IA; 7, stage IB; and 6, stage IC), and 7 patients were with FIGO stage IIA cancer.

In both groups of patients with cervical and patients with endometrial cancers, the most sensitive method for SLN detection was gamma probe with a detection rate of 100%, followed by LS (87.5% and 90.9%, respectively). The mean numbers of SLNs detected by gamma probe in each patient with cervical and patient with endometrial cancers were 2.09 and 2.04, respectively.[1–5] The most common localization of the SLN was in the external iliac area (47.8% and 47%, respectively). There was no patient with a para-aortic–only SLN localization in both tumor groups of patients.

In patients with endometrial cancer, during the operation, 74% of all SLNs were found in obturatory and external iliac localizations. Sentinel lymph nodes were detected in pelvic localization in 19 patients and in para-aortic and pelvic localizations in 5 patients. More than 1 SLN was excised in 16 (66.6%) of 24 patients. A total of 49 hot SLNs were found during the operation. Bilateral SLNs were found in 11 patients (45.8%), and unilateral SLNs were found in 13 patients (54.2%). The para-aortic SLNs could also be detected by pericervical injection technique, and 7 (14%) of 49 SLNs were found to be located at the para-aortic region. There was no patient with a para-aortic–only SLN localization.

The SLN localizations were variable among patients with cervical cancer, including the external iliac area (47.8%), the obturatory region (32.8%), the paracervical area (6%), the common iliac area (9%), and the para-aortic lymph node groups (4.4%). More than 1 SLN was excised in 20 (62.5%) of 32 patients. A total of 67 hot SLNs were found during the operation. Bilateral SLNs were found in 16 patients (50%), and unilateral SLNs were found in 16 patients (50%). Pelvic SLNs were detected in 30 of 32 patients, whereas para-aortic and pelvic SLNs were detected in 2 of 32 patients.

In patients with endometrial cancer, 3 external iliac SLNs and 1 obturatory non-SLN were metastatic. In patients with cervical cancer, 11 SLNs and 6 non-SLNs were metastatic. Seven SLNs were in the obturatory region, and 4 SLNs were in the external iliac region. Three non-SLNs were found in the parametrial region, 1 was found in the obturatory region, and 2 were in external iliac localization. Micrometastases not found by routine H&E staining were found by immunohistochemistry and step sectioning in 2 patients (n = 1, cervical; n = 1, endometrial cancer). On the basis of the histopathological analysis, the negative predictive value for predicting metastasis in patients with cervical and patients with endometrial cancers were 100% and 95%, respectively, and false negative results were 0 and 1, respectively.

TABLE 2. Number of lymph nodes studied for immunomodulation

	Tumor-Free	Metastatic	Total
SLN	49	7	56
Non-SLN	52	4	56
Total			112

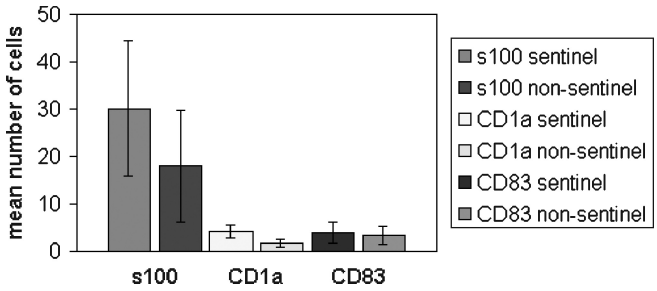

FIGURE 1. Distribution of S-100(+), CD1a(+), and CD83(+) DCs in tumor-free SLNs versus tumor-free non-SLNs.

Dendritic Cell Analysis

At surgery, a total of 1803 (1034, cervical cancer; 769, endometrial cancer) pelvic and para-aortic lymph nodes were excised from all patients. The mean number of pelvic and para-aortic lymph nodes was 32 in each patient with cervical and patient with endometrial cancers (range, 13–50 and 15–53, respectively). By routine H&E examination, it was revealed that 1782 of them were tumor-free lymph nodes (102 SLNs and 1680 non-SLNs), whereas 21 of them were metastatic lymph nodes (14 SLNs and 7 non-SLNs). All positive non-SLNs were within the patients with a positive SLN. Details of the lymph nodes excised during surgery are illustrated in Table 1.

In total, the number of SLNs was 116 (102 negative SLNs and 14 metastatic SLNs). The mean numbers of SLNs detected by gamma probe in each patient with cervical and patient with endometrial cancers were 2.09 and 2.04, respectively.[1–5] In patients with endometrial cancer, more than 1 SLN was excised in 16 (66.6%) of 24 patients, and in patients with cervical cancer, more than 1 SLN was excised in 20 (62.5%) of 32 patients. One SLN and 1 non-SLN nodes were chosen from each patient for DC analysis. Details of lymph nodes studied for immunomodulation are illustrated in Table 2.

The mean (SD) numbers of S-100(+) DCs in tumor-free SLNs and tumor-free non-SLNs were 30.12 (14.20) and 17.98 (11.86) ($P < 0.05$), respectively, whereas those of the CD1a(+) immature DCs were 4.19 (1.49) and 1.7 (0.90) ($P < 0.05$), respectively, and those of the CD83(+) mature DCs were 3.91 (2.14) and 3.4 (1.91) ($P > 0.05$), respectively. The distribution of S-100(+), CD1a(+), and CD83(+) DCs in tumor-free SLNs versus tumor-free non-SLNs are shown in Figure 1.

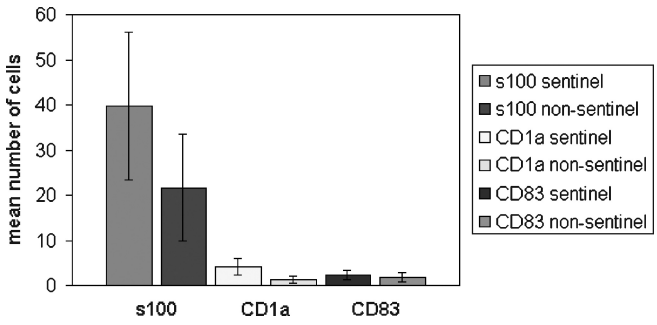

FIGURE 2. Distribution of S-100(+), CD1a(+), and CD83(+) DCs in metastatic SLNs versus metastatic non-SLNs.

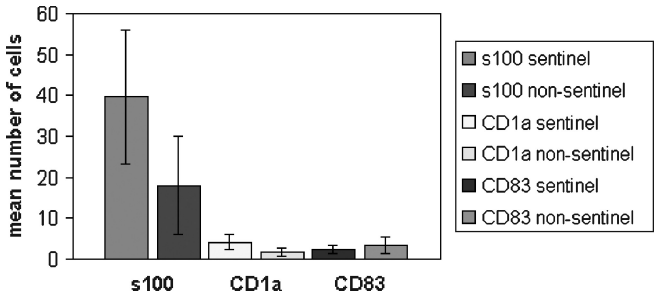

FIGURE 3. Distribution of S-100(+), CD1a(+), and CD83(+) DCs in metastatic SLNs versus tumor-free non-SLNS.

The mean (SD) numbers of S-100(+), CD1a(+), and CD83(+) cells in metastatic SLNs and metastatic non-SLNs were 39.70 (16.30) versus 21.63 (11.76) ($P < 0.05$), respectively; 4.10 (1.80) versus 1.29 (0.75) ($P < 0.05$), respectively; and 2.26 (1.00) versus 1.81 (1.04) ($P > 0.05$), respectively, which are shown in Figure 2.

When metastatic SLNs were compared with tumor-free non-SLNS, the mean (SD) numbers of cells stained with S-100, CD1a, and CD83 were 39.70 (16.30) versus 17.98 (11.86) ($P < 0.05$), , respectively; 4.10 (1.80) versus 1.70 (0.90) ($P < 0.05$), respectively; and 2.26 (1.00) versus 3.40 (1.91) ($P > 0.05$), respectively. Distribution is displayed in Figure 3.

Finally, when the metastatic SLNs were compared with the tumor-free SLNs, the mean (SD) numbers of S-100(+), CD1a(+), and CD83(+) cells were 39.70 (16.30) versus 30.12 (14.20) ($P > 0.05$), respectively; 4.10 (1.80) versus 4.19 (1.49) ($P > 0.05$), respectively; and 2.26 (1.00) versus 3.91 (2.14) ($P < 0.05$), respectively, which are shown in Figure 4.

DISCUSSION

Sentinel lymph node biopsy is still under investigation in endometrial cancers. Although several methods including blue dye, preoperative LS, and intraoperative gamma probe or a combination of these methods have been used to detect SLN in these particular group of cancers, there are still controversies for the optimal method to be used and the most appropriate injection site of either blue dye or radiocolloid. In an ideal SLN procedure, peritumoral injection of blue dye or radiocolloid is preferred. Because the endometrium is clinically nonvisible and nonpalpable and has multiple lymphatic drainage patterns, endometrial cancer may not be an ideal target for SLN biopsy. Subserosal intraoperative myometrial blue dye injection has been used in previous studies.[1,2] By using this injection technique, the anatomic drainage of the corpus uteri could be reflected. However, by this technique, it is not possible to use a combination of radiocolloid and blue dye, and the number and the localization of myometrial injection sites have not been standardized. In a study of 15 patients with endometrial cancer, Burke et al[1] reported an SLN detection rate of 67% by using a subserosal myometrial injection of blue dye. Echt et al[2] could not identify any SLN in 8 patients by subserosal injection. Holub et al,[3] by using a laparoscopic SLN procedure with blue dye alone, identified SLNs in 72% of 25 patients. Peritumoral subendometrial injection with the guidance of preoperative hysteroscopy has been introduced as a promising method. This injection technique permits targeted injection and mimics the natural lymphatic drainage of endometrial cancer. Niikura et al[8] injected radiocolloid under hysteroscopic guidance and reported the SLN detection rate of 82%. They claimed that hysteroscopy accurately showed superficial tumor extension and permitted targeted injection. However, previous studies have shown that hysteroscopy is less reliable for evaluating endometrial cancer extension, particularly to the uterine corns and internal os of the cervix.[14] Moreover, there will be risks of tumor cell dissemination and metastasis into the abdominal cavity through the fallopian tube after hysteroscopy.[15] Cervical dilation with laminaria for 15 hours, followed by hysteroscopic injection, is far from being a minimal invasive surgical procedure. On the other hand, the pericervical injection technique as a simple procedure has been introduced as an alternative approach allowing the use of combined radiocolloid and blue dye. The criticism of the pericervical injection technique is that the pericervical area does not reflect all of the uterus, and the detection rate of para-aortic drainage is low. Barranger et al[9] used pericervical injections of blue dye and radiocolloid, and SLNs could be identified in 16 (94.1%) of the 17 patients. In another study by Raspagliesi et al,[10] 18 patients with endometrial cancer were submitted to hysteroscopic injection of radiocolloid and blue dye. Using this technique, in 24% of cases, SLNs were found in the para-aortic area. Pericervical injection has also been preferred in our study, with a technical success rate of 100% with gamma probe. The para-aortic SLNs could also be detected by this technique, and 7 (14%) of 49 SLNs were found to be located at the para-aortic region.

It is well known that regional lymph nodes have a crucial role in developing the immune response to cancer cells. Sentinel lymph nodes, as the first tumor-draining lymph nodes, deserve extra investigation on this matter. Recently, while the studies of SLNs were increased, the immunological status of SLNs have been researched. There have been few studies on breast cancer, malignant melanoma, and oral tumors.[16–18] In our study, DC markers (S-100) in SLNs versus non-SLNs were compared. CD1a and CD83 were used as markers for immature and mature DCs, respectively, and a comparison between metastatic and tumor-free SLNs was made in DC maturation. The mean numbers of the S-100(+) and the CD1a(+) DCs in the tumor-free SLNs were found significantly higher compared with that of the tumor-free non-SLNs. A similar result was found in the comparison of metastatic SLNs and metastatic non-SLNs, which clearly showed that SLNs, whether metastatic or tumor-free, are immunologically more active sites than non-SLNs, which may be attributed to their being the first lymph nodes to drain the tumor.

We also put some effort to explain whether the state of tumor infiltration may reflect a change in the immune response of SLNs in DC maturation. For this reason, a comparison between the metastatic SLNs and the tumor-free SLNs were made. Our analysis showed that the CD83(+) mature DCs were more abundant in tumor-free SLNs than in metastatic ones. This result was in concordance with the study by Sakakura et al,[16] which attributed this finding to the presence of more potent immunosupression in metastatic SLNs compared with that in tumor-free nodes.

To find out whether the SLNs were immunomodulated compared with the non-SLNs, the SLN that was largest in diameter was selected as the representative SLN. One of the lymph node

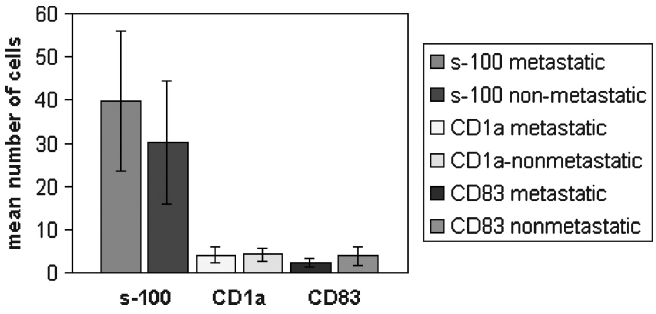

FIGURE 4. Distribution of S-100(+), CD1a(+), and CD83(+) DCs in metastatic SLNs versus tumor-free SLNs.

excised from the para-aortic region that is far from the tumoral side was randomly selected as the representative non-SLN. The rationale behind the selection of the para-aortic region as the non-SLN location was its relatively far location to the tumor site, assuming that the effect of the tumor on this location in immunomodulation would be less compared with its effect on adjacent locations.

One of the limitation of this study is the limited number of patients studied. Therefore, data from endometrium and cervical cancers were combined and not analyzed individually. Additional larger studies are needed to further explore the role of DCs in the metastatic disease in each group of patients. The second limitation is the selection of only 1 representative SLN and 1 non-SLN for DC analysis. More than 1 SLN and non-SLN could have been analyzed, but this would have caused extra labor to our pathology laboratory. Another study may be planned including all SLNs and non-SLNs for analysis of DCs.

In conclusion, significantly higher numbers of S-100(+) and CD1a(+) DCs in SLNs compared with those in non-SLNs may indicate that SLNs are the first sites of immunostimulation. Immunosupression may be the underlying factor for the metastatic involvement of SLNs, which might be secondary to the significantly decreased number of mature DCs in metastatic SLNs compared with tumor-free SLNs.

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