Correlation of Isotope Count With Sentinel Node Positivity in Vulvar Cancer

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Objective: Sentinel node biopsy (SNB) has become standard of care in early stage vulvar cancer. As the correlation of isotope count with the presence of metastases remains unclear, often several active nodes are excised per groin. This can result in increased morbidity in node-negative disease despite of SNB. In the current analysis, we assess whether resection of the hottest node could be sufficient to detect sentinel lymph node (SLN) metastasis.

Methods: Patients with primary vulvar cancer receiving an SNB with radioactive tracer at the University Medical Center Hamburg-Eppendorf between 2008 and 2015 were evaluated.

Results: A total of 145 patients with SNB were analyzed; thereof, 144 underwent bilateral SNB, resulting in 289 analyzed groins. A median of 2 SLNs (range, 1–7) per groin were removed. From 94 (32.5%) of 289 groins, more than 2 SLNs were excised. Median overall SLN isotope count was 1400 cps. In 50 groins, a positive SLN was detected (unilateral in 38 patients, bilateral in 6). The median number of positive SLN per groin was 1 (range, 1–4). The SLN with the highest isotope count carried metastases in 36 (78.3%) of 46 groins (in 4 cases, the highest count was unknown). In 10 (21.7%) of 46 positive groins, the SLN with the highest count was not the metastatic SLN (9/10 second highest count). Median count of these 10 SLN was 60% of the highest count with a range from 11.0% to 74.0%.

Conclusions: The highest isotope count does not reliably detect the positive SLN in vulvar cancer. To prevent mostly fatal groin recurrences, surgeons should continue to remove all SLN accumulating relevant radioactive tracer over background activity.

Key Words: Vulvar cancer, Sentinel, Groin, Isotope count, Metastases

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Since publication of the results of the Groningen International Study on Sentinel Nodes in Vulvar Cancer (GROINSS-V1) in 2008 and the Gynecological Oncology Group 173 trial in 2012, sentinel node biopsy (SNB) is a widely accepted and reliable surgical staging method for clinically node-negative early stage vulvar cancer.[1,2] In general, the sentinel lymph

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node (SLN) is defined as the first node draining lymphatic fluid from the tumor site. Consequently, if nodal metastases occur, the tumor cells travel to the SLN and can ideally be diagnosed via biopsy of this previously marked node.[3] Sentinel lymph node identification in patients with vulvar cancer has been first described by Levenback et al.[4,5] In the early 1990s, they demonstrated the feasibility of intraoperative lymphatic mapping by administration of isosulfan blue dye in 2 case series of 9 and subsequently 21 patients.[4,5] However, larger studies from other tumor entities such as cutaneous melanoma and breast cancer showed that the combination of intraoperatively administered blue dye and preoperatively injected radioactive tracer (Tc99m) with intraoperative gamma probe detection is superior to blue dye alone.[6–8] With the combined technique, the SLN identification rate is generally very high with a sensitivity of 95% (95% confidence interval [CI], 92%–98%) and a negative predictive value of 97.9.[9,10] Ideally, with the SLN technique, only 1 node should be identified for excision to keep morbidity low. In vulvar cancer however, often multiple, up to 7 SLN nodes per groin are identified[11] and are consequently excised to guide further groin treatment.[12,13] Thereby, morbidity potentially is increased with recurrent seroma, lymphedema, wound breakdown, or erysipelas despite of node-negative disease. Theoretically, the SLN with the highest isotope count ("hottest" SLN) should be most likely to harbor tumor cells. However, in breast cancer, the false-negative rate for detection of SLN metastases was shown to be very variable if only the hottest SLN is removed (range, 9%–29%).[14–20] Currently, the 10% rule is the most widely accepted in breast cancer, meaning that all nodes with more than 10% of the ex vivo isotope count of hottest SLN should be removed, lowering the false-negative rate to 2.5% to 6.4%.[14,19,20] The 10% rule was also applied in 1184 early stage cutaneous melanoma patients subjected to SNB.[21] The false-negative rate significantly decreased from 13.9% to 0.4% by using the 10% rule compared with removing the hottest SLN only.

In vulvar cancer, the correlation of the isotope count with the presence of metastases remains unclear. The aim of this analysis was therefore to assess whether resection of the hottest node would be sufficient to reliably detect SLN metastases in vulvar cancer.

MATERIALS AND METHODS

Patient Population

Consecutive patients with squamous cell vulvar cancer undergoing successful SNB with radioactive tracer from 2008 to 2015 at the Gynecological Cancer Center in Hamburg, Germany were included in the analysis. Informed consent had been obtained from all included patients to review their medical records according to our investigational review board and ethics committee guidelines (Ethics Committee of the Medical Board Hamburg reference number 190504). The institutional approach to the surgical treatment of vulvar cancer during the investigational period consisted of radical excision of the primary tumor; the SLN procedure was offered to patients with locally restricted disease and clinically negative groin nodes according to the inclusion criteria of the GROINSS study published in 2008.[1] Three patients originally planned for SNB were subsequently excluded from the analysis. In the first patient, bilateral SNB was not successful owing to prior surgery in 1 groin. Instead she received an inguinofemoral lymphadenectomy (LAE) in that groin. In the other 2 patients, no SLN could be identified in planar lymphoscintigraphy preoperatively. Intraoperatively, lymph nodes were suspicious and metastatic in frozen section so that bilateral inguinofemoral LAE was performed. All pathological studies were performed by specialized gynecopathologists. Clinical outcome was followed from the date of primary surgery to the date of death or until June 2016. For tumor staging, the International Federation of Gynecology and Obstetrics stage groupings as well as the International Union Against Cancer tumor-node-metastasis classification sixth edition were used for homogeneity.[22,23]

Sentinel Node Detection

The day before surgery, all patients received a conventional planar lymphoscintigraphy of the groins. For that purpose, 4 peritumoral intradermal deposits (0.2–0.3 mL volume each) at 3, 6, 9, and 12 o'clock were placed, using a 27-gauge needle. An overall mean ± SD dosage of 85 ± 12 MBq 99mTc-nanocolloid was injected. One hour after injection, anterior and lateral static views were obtained for planar lymphoscintigraphy. The sites of the SLNs were marked on the skin. Images were made using a large field-of-view dual-head gamma scintillation camera (ECAM, Siemens Medical Solutions, Hoffman Estates, Ill) equipped with a low-energy high-resolution parallel-hole collimator with the following imaging parameters: matrix size of 256 × 256 and energy window at 141 keV with 15% width, 5 minutes each.

On the following day, a handheld gamma counter (Navigator GPS, RMD Instruments) was used intraoperatively to identify the SLNs. After removal of the first SLN, the groin was reexamined for residual radioactivity, and potentially further SLNs were removed. Radioactivity was measured, and the respective activity was documented for every single node in the study. Identified SLNs were sent for frozen section to the gynecopathologists; if they came back negative, no further node dissection was performed in the majority of patients. Ultrastaging of all negative SLNs obtained was performed postoperatively according to the GROINSS-VI study to identify further metastases as described previously.[1] In case of positive SLN in ultrastaging, a systematic unilateral or bilateral LAE was performed during a second surgical intervention.

Statistical Analysis

Difference in median isotype count was calculated by Wilcoxon rank sum test. Survival probabilities were calculated by Kaplan-Meier curves and log-rank test. An α level of 0.05 was used to assess statistical significance.

RESULTS

Patient Characteristics

A total of 145 patients with successful SNB (144 bilateral and 1 unilateral SNB) during the investigational period

TABLE 1. Patient characteristics

Characteristics	Total (n = 145)	Total (%)	N0 (n = 100)	N0 %	N+ (n = 45)	N+ (%)
Age at FD, median, y	57	n.a.	57	n.a.	56	n.a.
(range)	(20–88)		(20–85)		(26–88)	
Tumor stage						
pT1b	116	80	84	84	33	73.3
pT1a	5	2.7	4	4	0	0
pT2	20	13.7	11	11	9	19.6
pT3	4	2.7	1	1	3	6.5
Unilateral groin metastases	39	26.9	n.a.	n.a.	39	86.7
Bilateral groin metastases	6	4.14	n.a.	n.a.	6	13.3
Unilateral SLN metastases	38	26.2	n.a.	n.a.	38	84.4
Bilateral SLN metastases	6	4.14	n.a.	n.a.	6	13.3
Median tumor diameter, mm	18	n.a.	14	n.a.	21	n.a.
(range)	(1.2–75)	n.a.	(1.2–65)	n.a.	(2–75)	n.a.
Unknown	10	n.a.	9	n.a.	1	n.a.
Median depth of invasion, mm	3	n.a.	2.6	n.a.	4	n.a.
(range)	(0.4–27)	n.a.	(0.4–18)	n.a.	(1.1–27)	n.a.
Unknown	9	n.a	6	n.a.	3	n.a
Median size of lymph node metastasis, mm	2.5	n.a.	n.a.	n.a.	2.5	n.a.
(range)	0.15–26	n.a.	n.a.	n.a.	0.15–26	n.a.
Unknown	6	n.a	n.a	n.a	6	n.a
Resection status of vulvar primary						
R0	140	96.6	97	97	43	95.6
R1	0	0	0	0	0	0
VIN in margin	4	2.7	2	2	2	4.35
Unknown	1	0.68	1	1	0	0
Grading						
G1	6	4.1	6	6	0	0
G2	99	68.3	71	71	28	62.2
G3	38	26	21	21	17	37
Unknown	2	1.4	2	2	0	0
Vulvar surgery						
Vulvectomy	11	7.5	5	5	6	13
Radical local excision	133	91.7	94	94	39	86.7
No local surgery	1	0.68	1	1	0	0
Pelvic node dissection	1	0.68	0	0	1	2.2
Adjuvant therapy						
Radiotherapy	15	11	0	0	15	34.8
Chemoradiation	4	2.8	0	0	4	8.9
Laser	2	1.37	2	2	0	0
Radiation field						
Vulva/±groin/±pelvis	17	11.7	1	1	17	37.8
Groin only	3	2.1	0	0	3	6.7
No adjuvant therapy	124	85	99	99	25	54.4

(continued on next page)

TABLE 1. *(Continued)*

Characteristics	Total (n = 145)	Total (%)	N0 (n = 100)	N0 %	N+ (n = 45)	N+ (%)
Neoadjuvant RTX	1	0.68	1	1	0	0
Median FU, mo	30	n.a.	31.5	n.a.	28.5	n.a.
(range)	(0.1–101)	n.a.	(0.1–101)	n.a.	(1–73)	n.a.

n.a., not applicable; FD, first diagnosis; RTX, chemoradiation.

(2008–2015) were included in this analysis, resulting in 289 analyzed groins. Median follow-up (FU) was 30 months (range, 0.1–101 months). Detailed patient characteristics are listed in Table 1. Four patients received bilateral SNB despite a pT1a vulvar cancer due to lymphangioinvasion diagnosed in the primary tumor; the excised SLNs of these were all negative. Ten patients presented with a primary tumor size greater than 40 mm (range, 41–75 mm; median, 55 mm) in definitive histology; 4 of these had a positive SLN and received a consecutive inguinofemoral LAE (3 bilateral, 1 unilateral). After a median FU of 21 months, none of those 10 patients had developed a groin recurrence. However, 1 patient suffered from distant metastases to the lungs and died 19 months later.

Groin Characteristics

The majority of patients in this analysis received an SNB only (n = 101, 69.2%). Of these, 100 were node negative and did not require further treatment (Table 2). Median number of excised SLNs per groin was 2 (287 groins; range, 1–7; in 2 groins, the number of excised SLN was unknown) (Table 2). However, in 94 (32.5%) of 289 groins, more than 2 SLNs were excised. In 50 (17.3%) of 289 groins, a positive SLN was detected (unilateral in 38 patients, bilateral in 6). The median number of positive SLN per groin was 1 (range, 1–4). A total of 45 patients presented with lymph node metastases (39 patients unilateral, 6 bilateral); thereof, 1 had a non-SLN metastasis only. Of 44 SLN-positive patients, 36 patients had a second bilateral and 7 patients had a unilateral inguinofemoral LAE owing to small strictly unilateral tumors or ipsilateral micrometastasis with contralateral negative SLN. None of these 7 patients presented with a groin recurrence after a median of 21 months. One patient presented with a 1.5-mm SLN metastasis but did not receive a consecutive inguinofemoral LAE owing to comorbidities. This patient presented in a poor general condition and died owing to a stroke 11 months after diagnosis. In patients undergoing a complete groin dissection, a median of 10 lymph nodes per groin were dissected (range, 1–23; 83 groins). The median number of positive lymph nodes per groin was 1 in node positive patients (range, 1–5; 51 groins).

Three patients suffered from isolated groin recurrence after 5, 17, and 25 months. Thereof, 1 patient initially presented with microinvasive disease (pT1a) and negative bilateral SLN. She suffered from groin recurrence 25 months after initial diagnosis and received only unilateral complete groin dissection and radiation owing to comorbidities. She died from vulvar cancer 5 months after groin recurrence. The second patient was 66 at first diagnosis and presented with a pT1b squamous cell tumor (14 mm, G3). She received a bilateral SNB (0/4SLN) and wide excision of the tumor. Unilateral groin recurrence occurred after 5 months. The third patient was 48 at initial diagnosis and also presented with a pT1b, 10 mm squamous cell vulvar cancer. She received a wide excision and SNB (0/3SLN). Groin recurrence occurred 17 months after first diagnosis. Both patients received bilateral radical groin dissection and consecutive chemoradiation with cisplatin (40 mg/m^2) to the groins and pelvis. They were still alive with no evidence of disease at 22 and 49 months after groin recurrence, respectively. Both patients received bilateral complete groin dissection and consecutive chemoradiation to the groins with cisplatin. They were still alive at last FU 22 and 49 months after groin recurrence, respectively.

Two- and 3-year disease-free survival was 86.1% (95% CI, 0.79–0.94) and 80.7% (95% CI, 0.72–0.9) in node-negative patients with SNB only (n = 100) and 77.5% (95% CI, 0.65–0.92) and 74.4% (95% CI, 0.61–0.89) in node-positive patients (n = 45), respectively (P = 0.33 for 3-year disease-free survival; Fig. 1). Five-year OS was 90.9% (95% CI, 0.84–0.98) in node-negative and 82.6% (95% CI, 0.69–0.99) in node-positive patients.

Sentinel Isotope Count

Median overall SLN isotope count was 1400 cps. There was no difference of median count in negative compared with positive SLN (median count 1396 cps vs 1614 cps; P = 0.90). The SLN with the highest isotope count carried metastases in 36 (78.0%) of 46 groins (in 4 cases the highest count was unknown). In 10 (25.0%) of 46 positive groins, the SLN with the highest count was not the metastatic SLN (in 9/10 second highest count). Median count of these 10 SLN was 60% of the highest count with a range from 11.0% to 74.0% (Supplementary Table 1, http://links.lww.com/IGC/A752).

DISCUSSION

Despite numerous studies that confirmed the accuracy of SNB for assessment of the nodal status, the optimum technique of SNB in vulvar cancer still remains a matter of debate. In vulvar cancer, high background activity often leads to removal of greater than 2 SLNs and therefore might increase morbidity despite node-negative disease. The present analysis shows that the highest isotope count correlates with SLN metastases in the majority of cases (75%); however, it does not reliably predict SLN metastases in all patients. With the application of the 10% rule suggested by other authors primarily for breast cancer or malignant melanoma,[14,19–21] no SLN metastases would have been missed in our cohort (lowest

TABLE 2. Groin characteristics

Groin Characteristics	N = 145 Patients	%
Groin surgery		
Full groin dissection (unilateral or bilateral)		
SLN^+ followed by unilateral groin dissection	7	4.8
SLN^+ followed by bilateral groin dissection	36	24.8
SLN^+ no groin dissection due to comorbidity	1	0.69
Non-SLN positive followed by bilateral groin dissection	1	0.69
SLN		
Patients with unilateral excised SLN	1	0.69
Patients with bilateral excised SLN	144	99.3
Patients with unilateral positive SLN	38	26.2
Patients with bilateral positive SLN	6	4.1
SLN counts per groin (46 groins plus 4 unknown count)		
Groins with positive SLN highest count	36	78.3
Groins with other positive SLN	10	21.7
Groins with SLN count unknown	4 (4/50 groins)	8
SLN counts		
Counts SLN^+ (median) range	1614 (74–10,721)	n.a.
Counts SLN^- (median) range	1396 (4–17,412)	n.a.
Excised lymph nodes per groin (only pat. with full groin dissection)		
Median (range)	10 (1–23)	n.a.
No. groins	81	n.a.
Positive lymph nodes per groin (only pat. with full groin dissection)		
Median (range)	1 (1–5)	n.a.
No. groins	51	n.a.
Excised sentinel nodes/groin		
Median (range)	2 (1–7)	n.a.
No. groins	287	n.a.
No. excised sentinel nodes unknown	2	n.a.
Positive sentinel nodes/groin		
Median (range)	1 (1–4)	n.a.
No. groins	50	n.a.
Isolated groin recurrence	3	2.1

SLN^+, positive sentinel lymph node; SLN^-, negative sentinel lymph node; n.a., not applicable.

isotope count of the SLN that contained metastases: 11% of the highest count). In our cohort, only 1 patient presented with an SLN metastases in the third hottest SLN node, which still showed 25% of the isotope count of the hottest SLN (269/1073 cps). There is no clear consensus in national guidelines regarding the number of SLN nodes that should be excised, and intraoperatively surgeons are often faced with the decision to remove a fourth or fifth mildly active node. However, the 10% rule was already part of the sentinel node detection protocol in the GROINSS-VI studies. After removal of the sentinel nodes, the biopsy bed was reexamined for radioactivity, and if higher than 10% of the first excised lymph node, the dissection was continued in search of additional sentinel nodes. Protocols for the absolute dose of 99Tc nanocolloid injection have largely been adapted from breast cancer and melanoma. They differ between countries and institutions (50–100 MBq total dose per injection spot). In vulvar cancer, the SLN procedure has initially been performed combining radioactive tracer and blue dye and therewith yielded highly accurate detection rates (98%–100%).[24] New guidelines do not mandatory require blue dye.[25,26] Compared with breast cancer and melanoma, the SLN detection rate in vulvar cancer is even more accurate. In a meta-analysis of 8059 patients with breast cancer, 96% had successfully mapped SLNs. However, in more than 50% of included breast cancer studies, detection rates of greater than 90% were reported.[27] A possible explanation for the higher identification rate in vulvar cancer[24] might be the anatomy of the lymphatic channels to the inguinofemoral

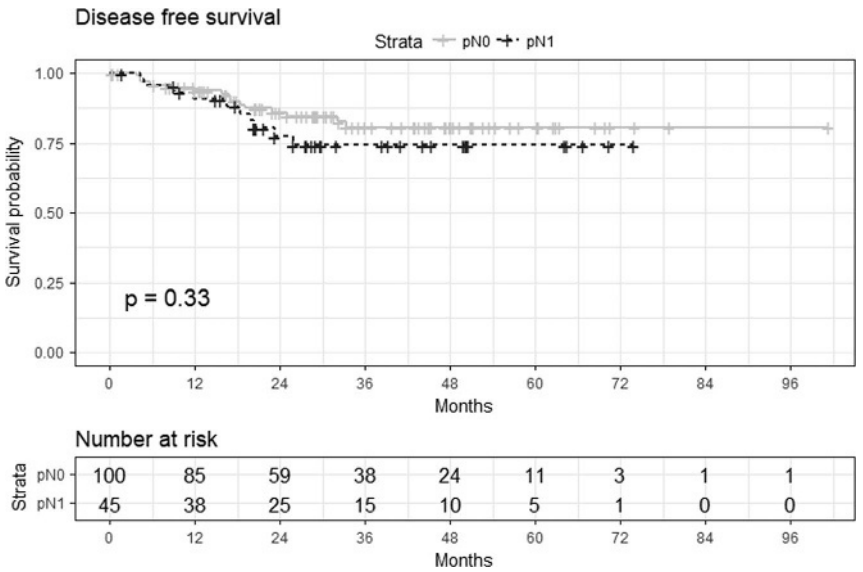

FIGURE 1. Kaplan-Meyer curve of disease free survival of pN0 versus pN1 patients.

nodes, which seem to vary only minimally, and the SLN is almost always located above the cribriform fascia of the fossa ovalis. The nodal recurrence risk, however, seems less favorable in vulvar cancer when compared with breast cancer (2.3% vs 0.1%–0.3%).[28] This might be partly explained by the fact that the majority of breast cancer patients will receive adjuvant treatment, whereas node-negative vulvar cancer patients will not. Therefore, the accuracy of SLN detection technique is even more important, as lethality of nodal recurrence in vulvar cancer is high. Some authors proposed the use of bigger particles like colloidal albumin or (99m)Tc-phytate in comparison with Technetium. Sentinel lymph node number is closely related to the size of the radiocolloid used,[29] and bigger particles might not get so easily into other nodes than into the sentinel node. New surgical techniques to identify the SLN may be of additional value for intraoperative imaging of the SLN in vulvar cancer. Recently, intraoperative near-infrared fluorescence (NIRF) imaging was introduced as a new technique for SLN detection. This approach is based on the intraoperative injection of a fluorescent agent around the primary tumor, which will flow through the lymphatic vessels and accumulate in the SLN(s). Upon excitation with a laser beam, the agent emits light of a longer wavelength, which is captured and processed by a fluorescence camera. Real-time images can be displayed on monitors in the operating theatre. Currently, the fluorescent agent of choice is indocyanin green, a Food and Drug Administration–approved agent with little toxicity that has been used for decades for ophthalmic angiography and evaluation of liver perfusion. In different pilot studies, this technique has been investigated for gynecological malignancies, for example, vulvar cancer.[30–34] In 70 patients with vulvar cancer that have been described in 5 clinical studies, 96.6% of SLN detected by 99mTc nanocolloid were also fluorescent in vivo. This was in contrast to blue dye, which stained only 70.6% of the SLNs ($P < 0.001$). However, in a pilot study by Crane et al,[30] detection rates in obese patients were limited, owing to the limited penetration depth of NIRF. In total, 29 SLNs were detected by radiocolloid, of which 26 were also detected by fluorescence and 21 were blue. The authors conclude that these first clinical results indicate that intraoperative transcutaneous lymphatic mapping using fluorescence is technically feasible in a subgroup of lean vulvar cancer patients. Currently, indocyanin green is the only Food and Drug Administration–approved NIRF agent. Future research on intraoperative fluorescence imaging for SLN detection may benefit from fluorescent agents with stronger penetration properties. Results of larger clinical studies especially concerning the oncologic safety (false-negative rate) and number of removed SLNs are still pending.

This retrospective analysis shows that the hottest SLN has the highest likelihood of harboring the tumor cells in vulvar cancer. In all other cases except for one, the second hottest SLN contained the tumor cells. However, because groin recurrences in vulvar cancer are almost always fatal, oncologic safety has to be balanced carefully against the potentially increased morbidity of a fourth or fifth excised sentinel. Based on the results of this study, it is not sufficient to remove the hottest sentinel node only and surgeons should follow the 10% rule known from other entities as a guidance.

REFERENCES

1. Van der Zee AG, Oonk MH, De Hullu JA, et al. Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. *J Clin Oncol*. 2008;26:884–889.
2. Levenback CF, Ali S, Coleman RL, et al. Lymphatic mapping and sentinel lymph node biopsy in women with squamous cell carcinoma of the vulva: a gynecologic oncology group study. *J Clin Oncol*. 2012;30:3786–3791.
3. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;127:392–399.

4. Levenback C, Burke TW, Gershenson DM, et al. Intraoperative lymphatic mapping for vulvar cancer. *Obstet Gynecol*. 1994;84:163–167.
5. Levenback C, Burke TW, Morris M, et al. Potential applications of intraoperative lymphatic mapping in vulvar cancer. *Gynecol Oncol*. 1995;59:216–220.
6. Albertini JJ, Lyman GH, Cox C, et al. Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. *Jama*. 1996;276:1818–1822.
7. Krag DN, Meijer SJ, Weaver DL, et al. Minimal-access surgery for staging of malignant melanoma. *Arch Surg*. 1995;130:654–658; discussion 9–60.
8. Kapteijn BA, Nieweg OE, Liem I, et al. Localizing the sentinel node in cutaneous melanoma: gamma probe detection versus blue dye. *Ann Surg Oncol*. 1997;4:156–160.
9. de Hullu JA, Doting E, Piers DA, et al. Sentinel lymph node identification with technetium-99m-labeled nanocolloid in squamous cell cancer of the vulva. *J Nucl Med*. 1998;39:1381–1385.
10. Meads C, Sutton AJ, Rosenthal AN, et al. Sentinel lymph node biopsy in vulval cancer: systematic review and meta-analysis. *Br J Cancer*. 2014;110:2837–2846.
11. Woelber L, Eulenburg C, Grimm D, et al. The risk of contralateral non-sentinel metastasis in patients with primary vulvar cancer and unilaterally positive sentinel node. *Ann Surg Oncol*. 2016;23:2508–2514.
12. Mahner S, Jueckstock J, Hilpert F, et al. Adjuvant therapy in lymph node-positive vulvar cancer: the AGO-CaRE-1 study. *J Natl Cancer Inst*. 2015;107. doi: 10.1093/jnci/dju426.
13. Klapdor R, Hillemanns P, Wolber L, et al. Outcome after sentinel lymph node dissection in vulvar cancer: a subgroup analysis of the AGO-CaRE-1 study. *Ann Surg Oncol*. 2017;24:1314–1321.
14. Bourgeois P, Nogaret JM, Veys I, et al. How 'hot' is the pathologically positive sentinel lymph node in breast cancer patients? *Nucl Med Commun*. 2003;24:513–518.
15. Wong SL, Edwards MJ, Chao C, et al. Sentinel lymph node biopsy for breast cancer: impact of the number of sentinel nodes removed on the false-negative rate. *J Am Coll Surg*. 2001;192:684–689; discussion 9–91.
16. Tafra L, Lannin DR, Swanson MS, et al. Multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue dye. *Ann Surg*. 2001;233:51–59.
17. Borgstein PJ, Pijpers R, Comans EF, et al. Sentinel lymph node biopsy in breast cancer: guidelines and pitfalls of lymphoscintigraphy and gamma probe detection. *J Am Coll Surg*. 1998;186:275–283.
18. Rink T, Heuser T, Fitz H, et al. Lymphoscintigraphic sentinel node imaging and gamma probe detection in breast cancer with Tc-99m nanocolloidal albumin: results of an optimized protocol. *Clin Nucl Med*. 2001;26:293–298.
19. Martin RC 2nd, Edwards MJ, Wong SL, et al. Practical guidelines for optimal gamma probe detection of sentinel lymph nodes in breast cancer: results of a multi-institutional study. For the University of Louisville Breast Cancer Study Group. *Surgery*. 2000;128:139–144.
20. Camp ER, Cendan JC, Feezor R, et al. The hottest sentinel lymph node is not always the positive node. *Am Surg*. 2004;70:475–478; discussion 8.
21. McMasters KM, Reintgen DS, Ross MI, et al. Sentinel lymph node biopsy for melanoma: how many radioactive nodes should be removed? *Ann Surg Oncol*. 2001;8:192–197.
22. Beller U, Quinn MA, Benedet JL, et al. Carcinoma of the vulva. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet*. 2006;95(suppl 1): S7–S27.
23. Benedet JL, Bender H, Jones H 3rd, et al. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet*. 2000;70:209–262.
24. de Hullu JA, Hollema H, Piers DA, et al. Sentinel lymph node procedure is highly accurate in squamous cell carcinoma of the vulva. *J Clin Oncol*. 2000;18:2811–2816.
25. Schnürch HG, Ackermann S, Alt CD, et al. Diagnosis, therapy and follow-up care of vulvar cancer and its precursors. Guideline of the DGGG and DKG (S2k-Level, AWMF Registry Number 015/059, November 2015. *Geburtshilfe Frauenheilkd*. 2016;76:1035–1046.
26. ESGO Guidelines Vulvar Cancer. 2016. Available at: https://guidelinesesgoorg/media/2016/08/ESGO-Vulvar-cancer-Complete-report-fxd2pdf. Accessed September 13, 2016.
27. Kim T, Giuliano AE, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: a metaanalysis. *Cancer*. 2006;106:4–16.
28. de Hullu JA, Oonk MH, Ansink AC, et al. Pitfalls in the sentinel lymph node procedure in vulvar cancer. *Gynecol Oncol*. 2004;94:10–15.
29. Wilhelm AJ, Mijnhout GS, Franssen EJ. Radiopharmaceuticals in sentinel lymph-node detection — an overview. *Eur J Nucl Med*. 1999;26:S36–S42.
30. Crane LM, Themelis G, Arts HJ, et al. Intraoperative near-infrared fluorescence imaging for sentinel lymph node detection in vulvar cancer: first clinical results. *Gynecol Oncol*. 2011;120:291–295.
31. Schaafsma BE, Verbeek FP, Peters AA, et al. Near-infrared fluorescence sentinel lymph node biopsy in vulvar cancer: a randomised comparison of lymphatic tracers. *BJOG*. 2013;120:758–764.
32. Matheron HM, van den Berg NS, Brouwer OR, et al. Multimodal surgical guidance towards the sentinel node in vulvar cancer. *Gynecol Oncol*. 2013;131:720–725.
33. Hutteman M, van der Vorst JR, Gaarenstroom KN, et al. Optimization of near-infrared fluorescent sentinel lymph node mapping for vulvar cancer. *Am J Obstet Gynecol*. 2012;206:89 e1–5.
34. Handgraaf HJ, Verbeek FP, Tummers QR, et al. Real-time near-infrared fluorescence guided surgery in gynecologic oncology: a review of the current state of the art. *Gynecol Oncol*. 2014;135:606–613.