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REITA NYBERG

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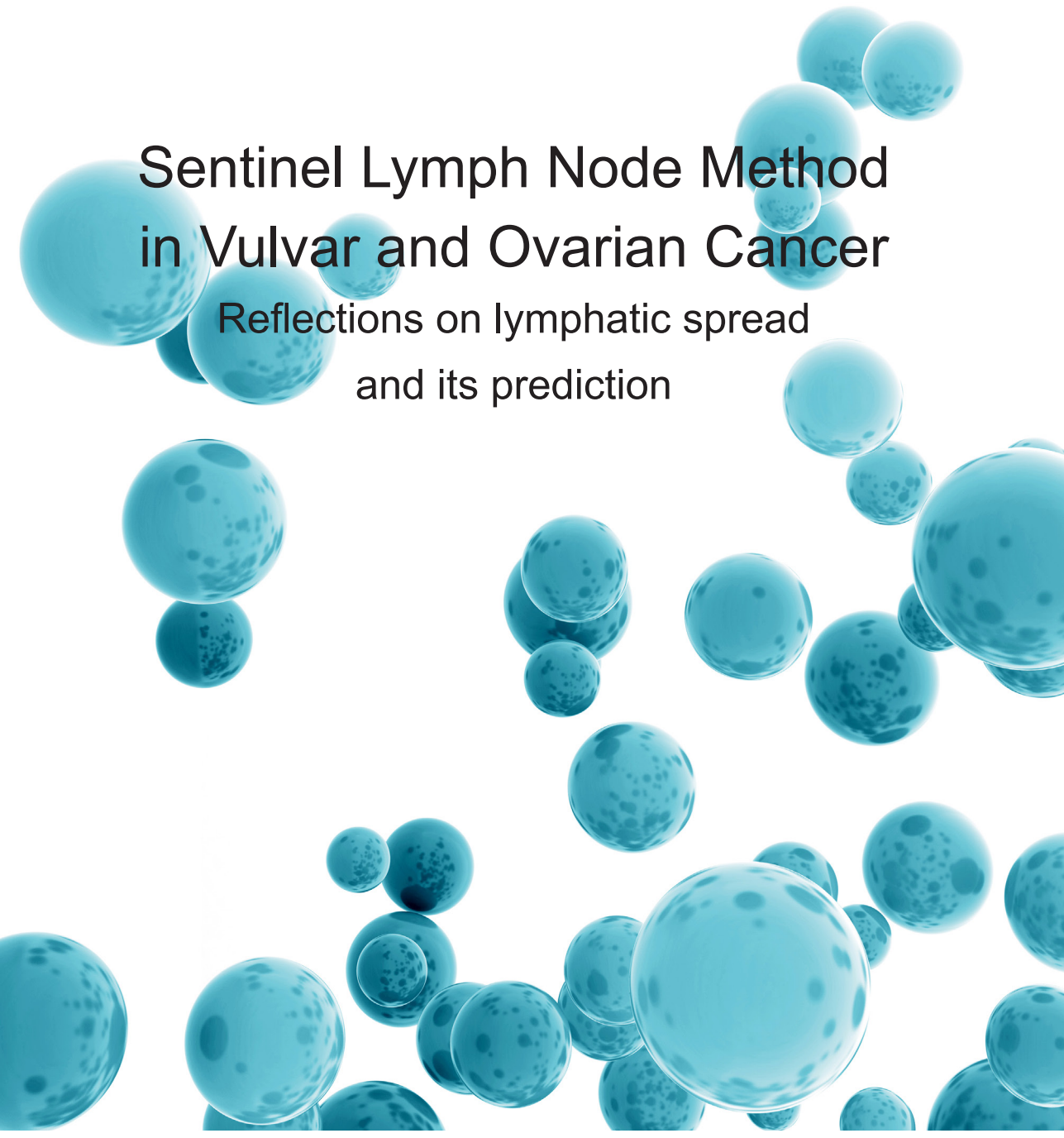
Reflections on lymphatic spread and its prediction



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Sentinel Lymph Node Method in Vulvar and Ovarian Cancer

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ACADEMIC DISSERTATION
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of the University of Tampere,
for public discussion in the small auditorium of building M,
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UNIVERSITY OF TAMPERE

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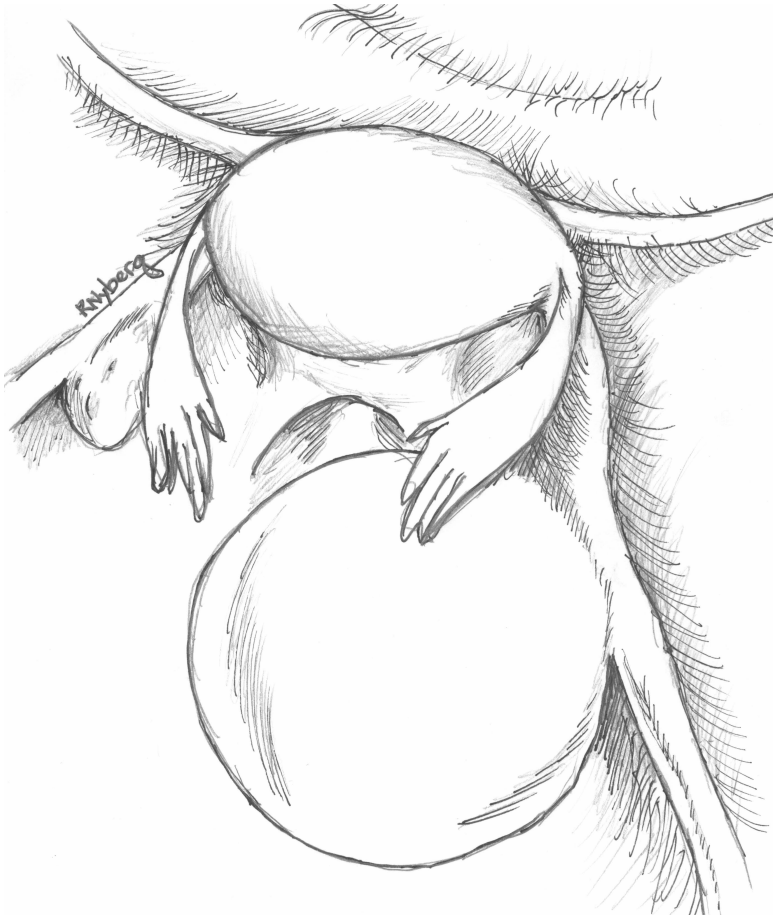

ABSTRACT

Lymphatic spread if one of the most important prognostic factors in gynecological cancers. Therefore, nodal staging is an essential part of cancer diagnosis and treatment planning. The sentinel lymph node (SLN) is the first lymph node to receive lymphatic fluid from the tumor and is at greatest risk of metastasis. According to the SLN concept, with locating and retrieving the SLN for analysis it is possible to predict the stage of the regional lymph nodes in relation to cancer metastasis without extensive surgical procedures.

In 2001-2004, the SLN method was adopted for assessment of vulvar cancer in the Tampere University Hospital. The SLNs of 47 patients, regardless of clinical stage, were located with blue dye and radiocolloid injections and dissected separately for analysis, and a complete lymph node dissection was then performed. In the first part of this thesis, the surgical reports and results of histopathological analysis of SLNs and other regional lymph nodes were retrospectively compared to determine the detection rate and reliability of the SLN method. In early stage vulvar cancer, the detection rate with the combined method was 100 % and there were no false-negative SLNs. Thus, the SLN method accurately predicted the nodal stage of patients with early vulvar cancer.

In the next stage, the paraffin blocks of samples from the same vulvar tumors were used for evaluating associations between lymphangiogenesis, SLN metastasis, surgical stage and clinical course of the disease. Forty-four tumor samples and 17 metastatic SLNs were available for retrospective immunohistochemical analysis. 67 % of the malignant vulvar tumors expressed vascular endothelial growth factor C (VEGF-C) in their invasive edges. This expression was also seen in 76 % of SLN metastases. Positive tumoral VEGF-C expression did not significantly associate with higher surgical stage, presence of SLN metastasis, higher recurrence rate or poorer prognosis, although some trends were observed. Negative VEGF-C expression in SLN metastasis might serve as an indicator of metastasis-free non-SLN, but that should be verified in a larger study.

The other objective of this study was to establish a SLN technique for intraoperative use in ovarian cancer. Sixteen women with high-risk endometrial cancer and scheduled for laparotomy were enrolled to a prospective pilot study. Blue

dye and radiocolloid were injected into a healthy ovary at the beginning of laparotomy. After removal of uterus and adnexa, the blue and hot SLNs were mapped during lymph node dissections (LND). In 94 % of patients, 1-3 SLNs were detected with the combined method. All SLNs were located in the para-aortic area; those related to the left ovary were mostly (64 %) detected above inferior mesenteric artery (IMA), whereas almost all right-ovary-related SLNs (94 %) were located under the IMA level ($p=0.001$). One allergic reaction to blue dye was encountered and managed during the study. The pilot study confirmed that it is feasible to use conventional tracers intraoperatively for mapping of ovarian SLN and a feasibility study followed, conducted in an authentic patient population with ovarian tumors.

20 patients with ovarian tumors and no suspicion of malignant spread were scheduled for laparotomy and enrolled into this prospective study. At the beginning of each operation, blue dye and radiocolloid were injected next to the ovarian mass into the mesovarium. If the mass was benign and radical surgery was not required, the SLNs were mapped transperitonally. When LND was performed, all SLNs were mapped and removed separately for analysis after opening of the retroperitoneum. The final histopathology of the SLNs and non-SLNs were compared. The SLN detection rate with the combined method was 100 %, and 1-3 SLNs per patient were detected. Most of the SLNs (90 %) were located in the para-aortic area; in 60 % of the cases no SLNs were detected in other regions, and in 30 % there were also pelvic SLNs. Isolated pelvic SLNs were rare (10 %). In three women, LND was indicated due to the early ovarian cancer; one patient had nodal metastasis, and a positive SLN predicted correctly her nodal stage. SLN concept deserves further investigation in relation to the surgical treatment of early ovarian cancer.

TIIVISTELMÄ

Imusolmukelevinneisyys on yksi tärkeimmistä ennusteeseen vaikuttavista tekijöistä gynekologisissa syövissä. Siksi sen kartoittaminen on tärkeä osa niin syövän diagnostiikkaa kuin hoidon suunnitteluakin. Vartijaimusolmuke on ensimmäinen imusolmuke, joka ottaa vastaan kasvaimesta tulevan imunesteen, ja on siksi suurimmassa riskissä syövän etäpesäkkeiden suhteen. Vartijaimusolmukemenetelmässä paikallistamalla ja poistamalla vartijaimusolmuke tutkittavaksi on mahdollista määrittää alueellisten imusolmukkeiden tila syövän levinneisyyden suhteen ilman laajoja imusolmukepoistoja.

Vuosina 2001-2004 Tampereen yliopistollisessa sairaalassa oltiin ottamassa käyttöön uutta vartijaimusolmukemenetelmää ulkosynnytinsyövän hoidossa. Vartijaimusolmuke pyrittiin paikantamaan sinivärin ja radioisotoopin avulla 47 potilaalta syövän kliinisestä levinneisyydestä huolimatta. Vartija poistettiin näytteeksi erikseen, minkä jälkeen tehtiin täydellinen imusolmukkeiden poisto. Väitöskirjatyön ensimmäisessä osiossa leikkauskertomukset käytiin takautuvasti läpi, ja vartijaimusolmukkeiden ja muiden imusolmukkeiden PAD-vastauksia verrattiin toisiinsa löytymisosuuden ja menetelmän luotettavuuden määrittämiseksi. Yhdistetyn menetelmän löytymisosuus alkuvaiheen ulkosynnytinsyövässä oli 100 %, eikä vääriä negatiivisia vartijaimusolmukkeita ollut. Vartijaimusolmukemenetelmä ennusti imusolmukemetastaasit oikein alkuvaiheen ulkosynnytinsyövässä.

Saman potilasryhmän kudosnäytteitä käytettiin takautuvassa analyysissa, jossa pyrittiin selvittämään pahanlaatuisten ulkosynnytinkasvainten ja vartijaimusolmuke-etäpesäkkeiden lymfangiogeneesin yhteyttä kirurgiseen levinneisyyteen ja taudinkulkuun. 44 kasvainnäytettä ja 17 metastaattista vartijaimusolmuketta tutkittiin immunohistokemiallisin menetelmin. 67 % pahanlaatuisista ulkosynnytinkasvaimista ilmensi invasiivisilla reuna-alueillaan imutiekasvutekijä VEGF-C:tä ja sama ilmiö havaittiin myös 76 %:ssa vartijaimusolmukemetastaaseja. VEGF-C:n ilmentyminen kasvaimissa ei yhdistynyt merkitsevästi korkeampaan levinneisyysluokitukseen, vartijaimusolmuke-etäpesäkkeiden esiintymiseen, korkeampaan uusiutumisriskiin tai huonompaan ennusteeseen, vaikkakin joitakin trendejä havaittiin. VEGF-C:n ilmentymisen puuttuminen vartijaimusolmuke-etäpesäkkeessä saattaisi toimia

merkkinä puhtaista alueellista imusolmukkeista, mutta tämä löydös tulisi varmistaa suuremmassa aineistossa.

Toinen aihepiiri tutkimuksessa oli vartijaimusolmukemenetelmän kehittäminen leikkauksenaikaiseen käyttöön munasarjasyövän hoidossa. Kuusitoista korkean riskin kohtusyöpäleikkaukseen tulevaa naista rekrytoitiin ensin prospektiiviseen pilottitutkimukseen. Leikkauksen alussa siniväriä ja isotooppia ruiskutettiin toiseen normaaliin munasarjaan. Kohdun ja sivuelinten poiston jälkeen siniseksi värjäytyneet ja säteilevät imusolmukkeet paikannettiin imusolmukepoistojen aikana. Yhdistetyllä menetelmällä pystyttiin paikantamaan 1-3 vartijaa 94 %:lla potilaista. Kaikki vartijat löytyivät aortan vierusalueilta. Vasemman munasarjan vartijat sijaitsivat useimmiten alemman suolilievevaltimon (IMA) tason yläpuolella (64 %), kun taas lähes kaikki oikean munasarjan vartijat (94 %) sijaitsivat tämän tason alapuolella ($p=0.001$). Yksi potilas sai leikkauksen aikana siniväristä allergisen reaktion, joka hoidettiin. Pilottitutkimus vahvisti, että munasarjan vartijaimusolmukkeet olivat paikannettavissa käyttämällä tavanomaisia merkkiaineita leikkauksen aikana. Nämä tulokset johtivat jatkotutkimukseen varsinaisessa kohdeväestössä.

Viimeistä osatyötä varten prospektiiviseen tutkimukseen rekrytoitiin 20 naista, joilla oli todettu avoleikkausta vaativa munasarjakasvain eikä maligniteetin leviämistä kasvaimen ulkopuolelle epäilty. Siniväriä ja isotooppia ruiskutettiin munasarjakasvaimen viereen munasarjaliepeeseen joka leikkauksen alussa. Jos kasvain osoittautui hyvänlaatuiseksi eikä radikaalia leikkausta tarvittu, vartijaimusolmukkeet paikannettiin vatsakalvon läpi. Jos imusolmukepoistoja tarvittiin, kaikki vartijaimusolmukkeet paikannettiin ja poistettiin erikseen näytteeksi vatsakalvon takaisen tilan avaamisen jälkeen ennen imusolmukepoistoja. Vartijaimusolmukkeiden PAD-vastauksia verrattiin muiden imusolmukkeiden vastauksiin. Löytymisosuus yhdistetyllä menetelmällä oli 100 % ja joka potilaalta löydettiin 1-3 vartijaimusolmuketta. Suurin osa vartijoista sijaitsi aortan vierusalueilla joko yksin (60 %) tai yhdessä lantiosta löytyneiden vartijoiden kanssa (30 %). Vain 10 % vartijoista sijaitsi yksinään lantion imusolmukealueilla. Kolmelle potilaalle tehtiin täydelliset imusolmukepoistot, ja näistä yhdellä oli etäpesäkkeitä poistetuissa imusolmukkeissa. Positiivinen vartijaimusolmuke ennusti oikein hänen imusolmukelevinneisyytensä. Tulosten perusteella vartijaimusolmukemenetelmän tutkimista alkuvaiheen munasarjasyövän hoidossa kannattaa ehdottomasti jatkaa.

TABLE OF CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original publications. In the text, they are referred to by their Roman numerals (I-IV).

I Nyberg RH, Iivonen M, Parkkinen J, Kuoppala T, Mäenpää JU, 2007. Sentinel node and vulvar cancer: a series of 47 patients. *Acta Obstetricia Gynecologica Scandinavica*, 86(5), pp.615-619.

II Nyberg RH, Laurila M, Staff S, Mäenpää JU, 2017. Can vascular endothelial growth factor C expression be of use in predicting surgical stage or prognosis in vulvar cancer? *Journal of Cancer Research & Therapy*, 5(8), pp.50-55.

III Nyberg RH, Korkola P, Mäenpää J, 2011. Ovarian sentinel node: is it feasible? *International Journal of Gynecological Cancer*, 21(3), pp.568-572.

IV Nyberg RH, Korkola P, Mäenpää J, 2017. Sentinel node and ovarian tumors: a series of 20 patients. *International Journal of Gynecological Cancer*, 27(4), pp.684-689.

 In addition, some unpublished data are presented in this thesis.

ABBREVIATIONS

BMI	Body-mass index
BOT	Borderline ovarian tumor
BSO	Bilateral salpingo-oophorectomy
CI	Confidence interval
CRT	Chemoradiotherapy
CT	Computed tomography
CK19	Cytokeratin 19
DR	Detection rate
DSS	Disease-specific survival
dVIN	Differentiated vulvar intraepithelial neoplasia
EC	Endometrial cancer
EOC	Epithelial ovarian cancer
FIGO	International Federation of Gynecology and Obstetrics
5-FU	5-fluorouracil
FNR	False-negative rate
FSS	Fertility sparing surgery
GOG	Gynecologic Oncology Group
GROINSS-V	GROningen INternational Study on Sentinel nodes in Vulvar cancer
H&E	Hematoxylin and eosin
HPV	Human papillomavirus
HR	Hazard ratio
ICG	Indocyanine green
ICG:HSA	Indocyanine green adsorbed to human serum albumin
ICRP	International Commission on Radiological Protection
IHC	Immunohistochemistry
IF LND	Inguino-femoral lymph node dissection
IFP	Interstitial fluid pressure
IMA	Inferior mesenteric artery
ITC	Isolated tumor cells
LAVC	Locally advanced vulvar cancer

LEC	Lymphatic endothelial cell
LND	Lymph node dissection
LSG	Lymphoscintigraphy
LVD	Lymphatic vessel density
LVI	Lymphovascular invasion
MBq	Megabequerel
MRI	Magnetic resonance imaging
mGy	Milligray
mRNA	Messenger-RNA
NACT	Neoadjuvant chemotherapy
NCCN	National Comprehensive Cancer Network
NICE	National Institute of Health and Care Excellence
NIR	Near-infrared
NRP-2	Neuropilin-2
OC	Ovarian cancer
OR	Operation room
OS	Overall survival
OSNA	One step nucleic acid amplification
PlGF	Placental growth factor
PCR	Polymerase chain reaction
PET-CT	Positron emission tomography–computed tomography
qRT-PCR	quantitative reverse transcriptase-polymerase chain reaction
SBR	Signal-to-background ratio
SCC	Squamous cell carcinoma
SLN	Sentinel lymph node
SPECT	Single-photon emission computed tomography
STIC	Serous tubal intraepithelial carcinoma
RCT	Randomized controlled trial
RT	Radiation therapy
^{99m}Tc	Technetium-99m
TAM	Tumor-associated macrophage
TNM	Tumor – node – metastasis
TAH	Total abdominal hysterectomy
US	Ultrasound
U.S.	United States
uVIN	Vulvar intraepithelial neoplasia of usual type
VC	Vulvar cancer

VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VIN	Vulvar intraepithelial neoplasia

1 INTRODUCTION

In gynecologic malignancies, lymphatic spread to regional lymph nodes is one of the most important prognostic factors. It affects substantially the extent of surgery and the need of adjuvant therapy. Standard anatomic imaging such as computed tomography (CT) and magnetic resonance imaging (MRI), lack sensitivity and specificity in detecting metastatic nodal disease. Therefore, the evaluation of the regional lymph nodes with regard to metastasis is a substantial part of cancer diagnostics and staging (Paño et al. 2015).

Traditionally, nodal staging has been performed either by taking a fine- or core-needle biopsy from the regional lymph nodes, excising them for histological evaluation or by performing complete lymph node dissection (LND). Of these, complete LND is the most accurate way of detecting nodal metastasis, but it predisposes the patient to short-term and long-term morbidity including bleeding, injuries to vital structures, wound infections and dehiscence, lymphedema and lymphatic cysts. Since most patients with early-stage tumors do not have nodal metastasis, there has been an urgent need for alternative methods of exploring the regional lymph node status (Uren et al. 2016).

It is now known that metastatic lymphatic spread is not just a passive drift of cancer cells, but a complex interaction of molecular factors expressed and initiated by tumor cells, alterations in the tumor microenvironment and mechanical forces within the tissues (Nathanson et al. 2015). Even before metastasis actually takes place, the tumor draining lymph nodes undergo remodelling processes: lymphangiogenesis, changes in structure and lymphatic flow, increases in chemokine and cytokine production and alterations in immune cell composition. This creates a premetastatic niche (Pereira et al. 2015). After locoregional metastasis, metastatic nodes can act as potential mediators of metastasis to distant nodes and organs (Hirakawa 2009).

The sentinel lymph node (SLN) is a lymph node that receives the lymphatic fluid directly from the tumor. These lymph nodes are at greatest risk of metastatic involvement, and therefore represent the status of all other regional lymph nodes. Locating and removing the SLN for analysis could offer a less radical way of surgical nodal staging, saving the patient from aforementioned morbidity. Frozen section

analysis of a SLN can offer information for intraoperative decisions about additional surgery. The SLN approach also enables selection of highest-risk lymph nodes for analysis with more accurate methods than traditional histopathology, revealing low-volume metastatic disease that otherwise would be missed. Other characteristics, such as SLN lymphangiogenesis and angiogenesis (Pastushenko et al. 2016) or the presence of certain immune cell types (Mansfield et al. 2012), can give information on the patient's individual risk of regional or distal metastasis.

The SLN approach has gained an established position in the treatment of breast cancer and melanoma (Motomura 2015; Madu et al. 2017), as in many other solid tumors (Saad & Buscombe 2015). In gynecologic cancer, the SLN concept has been a subject of a growing interest for over 20 years. With the surge of mini-invasive surgery, new imaging methods and tracers, the SLN concept has been revisited. It has been adopted into treatment of early vulvar cancer and has a strong potential in cervical and endometrial cancer surgery, although some questions remain still unanswered. In ovarian cancer, the approach has long been considered inconceivable until recently.

The purpose of this study was to investigate applications of the SLN concept for nodal staging in vulvar and ovarian cancers. The first objective was to assess the accuracy of the SLN method in vulvar cancer, in order to incorporate it to the daily practice. Since lymphangiogenesis in the primary tumor and SLNs has been associated with an increased risk of nodal metastasis and a poorer prognosis in many cancers, this association was also studied in the same population that underwent SLN biopsy for vulvar cancer. Finally, the feasibility of the SLN method in ovarian cancer was evaluated in two different studies: first in a pilot study to establish a SLN technique for intraoperative use, and then in a feasibility study involving an authentic population with suspicious ovarian tumors.

2 REVIEW OF THE LITERATURE

2.1 Role of lymphangiogenesis in lymphatic metastasis

Lymphangiogenesis involves the formation of new lymphatic vessels from pre-existing lymphatics. This process occurs normally during embryonic development, but also during wound healing or certain pathological processes, such as cancer or inflammation (Lohela et al. 2009; Stacker et al. 2014).

2.1.1 The lymphatic system and its normal development

The lymphatic system is made up of a network of lymphatic vessels and lymphoid organs. The primary lymphoid organs, namely bone marrow and thymus, are responsible for producing and selecting lymphocytes. The secondary lymphoid organs, including the lymph nodes, tonsils and mucosa-associated lymphoid tissue provide an environment for lymphocytes to encounter foreign antigens and initiate specific immune response. The lymphatic system has three main functions. Firstly, it maintains tissue and blood volume by transporting excess interstitial fluid back to the circulation and at the same time removes catabolic products from tissues and organs. Secondly, it is a crucial part of the immune system enabling the surveillance of and response to the antigens. Thirdly, it transports dietary fat from the gut to the liver (Albrecht & Christofori 2011; Stacker et al. 2014; Betterman & Harvey 2016).

The normal lymphatic vasculature is composed of blind-ending lymphatic capillaries, that drain into collecting lymphatic vessels via so-called pre-collector vessels. The collecting lymphatic vessels contain bicuspid valves that ensure a one-way flow of lymph from the peripheral part of the body towards the blood circulation. En route to the bloodstream, lymph is filtered through the lymph nodes, presenting antigens and antigen-presenting cells to the immune system. Finally, lymph drains into two main lymphatic vessels, the right lymphatic duct and the thoracic duct. Under low interstitial fluid pressure (IFP), the lymphatic capillaries are collapsed, but when the amount of extracellular fluid increases, the higher IFP leads to tissue swelling and gap formation in the lymphatic endothelial lining, allowing the fluid to enter the capillaries. The transport of lymphatic fluid is promoted by smooth

muscle contractility of the lymph vessels, skeletal muscle contractions, arterial pulsation and respiration (Albrecht & Christofori 2011; Betterman & Harvey 2016).

During embryogenesis, the lymphatic endothelial cells (LEC) bud off the cardinal veins, migrate into the surrounding tissue and proliferate to form primary lymphatic sacs. These sacs continue the formation of the primitive lymphatic vasculature by sprouting and proliferating. The leading edges of the sprouting lymphatic vessels contain specialized cells in their tips, that are able to sense and respond to chemoattractants. Vascular endothelial growth factor C (VEGF-C) acts as a major attractant. When binding to its target receptor vascular endothelial growth factor receptor 3 (VEGFR3), the chemoattractants induce lymphangiogenesis and promote migration and proliferation of LECs, regulating lymphatic vessel growth. Later during fetal development, the primitive lymphatic vasculature maturates by developing into a hierarchical network of capillaries and collecting lymph vessels. During this process, the collecting lymph vessels regenerate intraluminal valves and a smooth muscle coverage, while the capillaries remain porous as they have an intermittent basement membrane and are not covered by smooth muscle cells. This maturation is controlled by the VEGF-C/VEGFR3 -signaling route (Albrecht & Christofori 2011; Pereira et al. 2015; Betterman & Harvey 2016).

2.1.2 Vascular endothelial growth factors and their receptors

VEGFs and their target receptors (VEGFRs) shown in Figure 1 are best known for their part in tightly regulated and interconnected signaling pathways that control vasculogenesis, angiogenesis and lymphangiogenesis during embryonic and fetal development (Tammela et al. 2005; Lohela et al. 2009).

VEGF-A (also called VEGF) and its main target receptor VEGFR-2 are essential for both vasculogenesis and angiogenesis. VEGF-A synthesis is upregulated by hypoxia and a lack of nutrients. Furthermore, several growth factors, inflammatory cytokines, oncogenes and hormones induce VEGF-A. The VEGF-A/VEGFR-2 pathway activates angiogenesis by inducing proliferation, sprouting, migration and tube formation of endothelial cells and by increasing endothelial permeability. On the other hand, lymphangiogenesis is mainly controlled via the VEGF-C or D/VEGFR-3 pathway. The VEGF-A/VEGFR-2 pathway has a weaker effect on lymphangiogenesis. It can induce lymphatic vessel enlargement but only little sprouting or migration. One possible mechanism of the lymphangiogenic action of VEGF-A may be linked to the recruitment of inflammatory cells, which express

VEGFR-1 and secrete lymphangiogenic factors (Tammela et al. 2005; Lohela et al. 2009; Zheng et al. 2014).

VEGF-B and placental growth factor (PlGF) act as angiogenic modifiers via the VEGFR-1 pathway. VEGFR-1 is expressed in endothelial cells, monocytes and macrophages, some hematopoietic cells and pericytes. VEGF-B and PlGF induce angiogenesis in certain pathological conditions, such as ischemia, inflammation, wound healing and tumor growth (Tammela et al. 2005; Lohela et al. 2009).

VEGFR-1, VEGFR-2 and VEGFR-3 are a family of receptor tyrosine kinases. VEGFR-1 is mainly expressed by blood vessels, while VEGFR-2 and VEGFR-3 are expressed by LECs. Full-length VEGF-C binds to VEGFR-3 with high affinity and, when proteolytically processed, it is also capable of interacting with VEGFR-2. Neuronal guidance molecule Neuropilin-2 (NRP-2) acts as a coreceptor for VEGFR-3 during lymphatic sprouting induced by VEGF-C. NRP-2 is expressed in veins and LECs (Tammela et al. 2005; Coso et al. 2014).

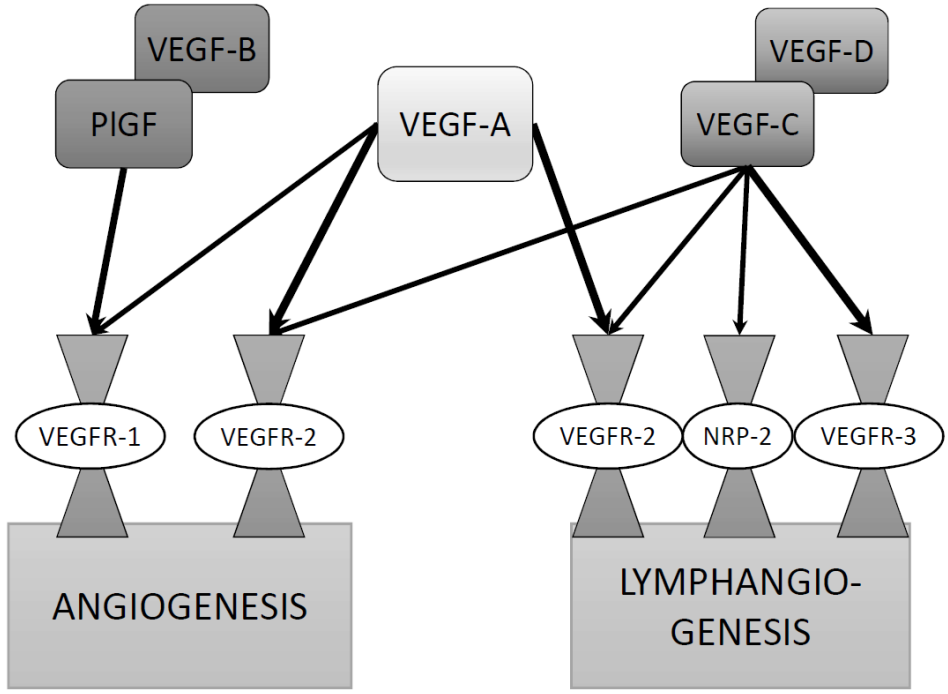

Figure 1. Diagram of the vascular endothelial growth factor (VEGF) ligand and receptor family in mammalians (Hirakawa 2009). The cellular reactions downstream following ligand-receptor activation differ depending on whether the cell membrane if from a blood or lymphatic endothelial cell. PlGF = Placental growth factor, NRP-2 = Neuropilin-2.

2.1.3 Mechanisms of tumor-induced lymphangiogenesis

In growing tumors, the hypoxic conditions and other stimulating factors provoke tumor cells to express a variety of angiogenic and lymphangiogenic growth factors, including VEGF-C, VEGF-D, VEGF-A, platelet-derived growth factor BB and Angiopoietin-1 and -2. They all can promote vessel sprouting, LEC proliferation and hyperplasia, forming both new blood and lymphatic vessels in the proximity of the tumor and enlarging the pre-existing ones. VEGF-C and VEGF-D are the most specific lymphangiogenic growth factors, often expressed by tumor cells, tumor-associated macrophages and tumor-associated fibroblasts in the primary tumors and their stroma. Animal experiments show that it is possible to prevent lymph node metastasis and distant metastasis by blocking either VEGF-C or its receptor VEGFR3 (Albrecht & Christofori 2011; Podgrabinska & Skobe 2014; Stacker et al. 2014).

Tumor cells also express several molecules that suppress host immunity, such as programmed death ligand 1 (PD-L1), indoleamine-2,3-dioxygenase (IDO) and siglec-9. In addition, they can recruit and educate tumor-associated immune cells to promote tumor progression via upregulation of the same immunosuppressive factors (Kuol et al. 2017).

In the past, the lymphatics were regarded mainly as a passive transport system for the metastatic cells. Tumor-induced lymphangiogenesis was thought to increase the possibility of lymphatic metastasis simply by providing more opportunities for tumor cells to enter lymphatic vessels. Recent research shows that cytokine-induced hyperpermeability of the tumor blood vessels leads into increased IFP in the tumor, which, in turn, results in higher lymph flow towards regional lymph nodes, despite the fact that the newly-formed intratumoral lymphatics are not functional but mostly collapsed due to the high IFP (Hoon et al. 2006; Albrecht & Christofori 2011). Experiments with mouse models show that intratumoral lymphangiogenesis or lymphatic vessels are not necessary for lymph node metastasis. Functional lymphatics do exist in the tumor margins, and in the presence of VEGF-C overexpression, their diameter increases, which also increases the surface area for lymphovascular invasion (LVI) and promotes lymphatic entry of the tumor cells (Stacker et al. 2014). Recently it has been found that tumor cells can also be guided into the lymphatic vessels by LEC-secreted chemokines (Podgrabinska & Skobe 2014).

Increased lymph flow from the tumor induced by VEGF-C carries growth factors, chemokines and other signaling factors upstream to the first tumor-draining lymph node, i.e. the SLN. Exposure to a variety of tumor-derived factors creates a

"metastatic niche" in the SLN. This concept refers to a microenvironment that supports the survival and outgrowth of disseminated tumor cells. Results of animal models and human cancer studies show that lymphangiogenesis in the SLN precedes lymph node metastasis independently of the lymphangiogenesis at the tumor site. In addition to remodeling of its vasculature, the SLN sustains the alterations to its immune and stromal cell populations and their function, leading to an immunosuppressive cytokine environment and SLN immunosuppression. In animal models, the level of immunosuppression of a SLN is associated with the primary tumor size. Once the tumor cells have invaded the SLN, they serve as a major source of lymphangiogenic factors and promote secondary metastasis to non-SLNs and distant organs (Hirakawa et al. 2007; Albrecht & Christofori 2011; Chung et al. 2012; Liersch et al. 2012; Podgrabinska & Skobe 2014; Cochran et al. 2015; Pereira et al. 2015; Sleeman 2015; Wakisaka et al. 2015; Pastushenko et al. 2016).

2.1.3.1 VEGF-C induced lymphangiogenesis and its impact on the course of disease

The effect of tumor-associated VEGF-C overexpression on SLN metastasis has been studied especially in melanoma. According to four studies summarized Table 1, tumoral VEGF-C expression is associated with an increased risk of sentinel lymph node metastasis. The relative risk of tumor cells expressing VEGF-C to metastasize to the SLN is 2.72, when all studies on this topic are pooled (95 % Confidence Interval [CI] 1.71–4.37, $p<0.0001$) (Dadras et al. 2005; Boone et al. 2008; Gallego et al. 2011; Cianfarani et al. 2012). Boone et al. also observed VEGF-C expression by tumor-associated macrophages (TAMs), in which the staining intensity was even stronger than in melanoma cells and was strongly associated with the SLN status ($p=0.003$). Gallego et al. reported VEGF-C expression by fibroblastic stromal cells around the tumor, not by the tumor cells themselves, which was clearly more frequent in metastatic SLNs than in SLNs not invaded by metastasis (90 % vs. 17.5 %, $p<0.0001$). Thus, it seems that not only tumor cells but also stromal and tumor-associated inflammatory cells affect the origin of SLN metastasis.

Studies of various human cancer types have shown that overexpression of VEGF-C by the primary tumor is associated with a poorer prognosis. In patients with melanoma, it associates with shorter progression free survival (PFS), poorer overall survival (OS) and lymph node metastasis (Boone et al. 2008), and is a poor prognostic factor in non-small-cell lung cancer and adenocarcinoma of the lung (Kilvaer et al. 2015; Kojima et al. 2005). High levels of VEGF-C expression in gastric cancer tissue imply a poorer overall prognosis than low VEGF-C levels (Cao et al. 2014). Primary tumor VEGF-C expression has been reported to associate with the

likelihood of lymph node metastasis in lung, esophageal, prostate, thyroid and colorectal cancer (Stacker et al. 2014).

Table 1. Summary of studies on the association between VEGF-C expression and SLN metastasis in melanoma. N.S. = non-significant. The statistical significance of the distribution of VEGF-C expression in all patients was analyzed with the 2-sided Fisher's exact test.

Publication	Number of patients	Number of samples with cytoplasmic VEGF-C expression (total number of SLN-positive tumors)	Number of samples with cytoplasmic VEGF-C expression (total number of SLN-negative tumors)	p value
Dadras et al. 2005	45	16 (18)	12 (27)	0.0082
Boone et al. 2008	113	20 (25)	48 (88)	0.022
Gallego et al. 2011	50	6 (10)	23 (40)	N.S.
Cianfarani et al. 2012	62	30 (36)	9 (26)	<0.0001
Total	**270**	**72 (89)**	**92 (181)**	**<0.0001**

A recent meta-analysis of 21 eligible breast cancer studies and 2828 patients demonstrated that high tumoral VEGF-C expression is significantly associated with poor survival. The combined hazard ratios (HRs) were 1.87 (95 % CI 1.25–2.79, p=0.001) for PFS and 1.96 (95 % CI 1.15–3.31, p=0.001) for OS. For the subgroup of non-Asian patients, the pooled HRs were even higher, 2.04 (95 % CI 1.36–3.05, p=0.001) for PFS and 2.61 (95 % CI 1.51–4.52, p=0.001) for OS (Zhang et al. 2016). A meta-analysis of 27 colorectal cancer studies showed a statistically significant association between high VEGF-C expression and shorter OS (1428 patients; HR=1.95; 95 % CI 1.31–2.92, p=0.007). VEGF-C overexpression was also associated with nodal metastasis (3212 patients; OR=4.21; 95 % CI 3.49–5.08; p=0.004) and LVI (1471 patients; OR=2.18; 95 % CI 1.65–2.88; p=0.000) (Zong et al. 2016).

2.2 Principles of the sentinel lymph node method

The first report of the existence of "a specific lymph center, so-called sentinel lymph node" and the surgical technique for its biopsy in the treatment of penile cancer was published in 1977 (Cabanas 1977). In the early 1990's, the sentinel lymph node (SLN) technique was adopted into the surgical treatment of malignant melanoma and breast cancer (Alex et al. 1993; Krag et al. 1993). Since then, 26 randomized controlled trials (RCTs) have proven that SLN biopsy is a safe and beneficial alternative for axillary lymph node dissection for patients with operable primary breast cancer (Bromham et al. 2017). In the surgical treatment of malignant melanoma, the SLN technique improves the outcome of patients with occult disease by preventing the development of clinical regional nodal involvement. It also helps to identify node-negative patients who would not benefit from complete lymphadenectomy (Tardelli et al. 2016).

2.2.1 Definition of a sentinel lymph node

Initially, a sentinel (= guard) lymph node was defined by Morton et al. as the first lymph node in the lymphatic chain that receives drainage from the primary tumor, thus protecting the regional nodal field. Therefore, the SLN carries the highest likelihood of containing metastases. The SLN represents the metastatic status of all lymph nodes in the same lymphatic basin; if the SLN is free from metastasis, so should also the rest of the regional lymph nodes be (Morton et al. 1992).

Later, the definition of SLN has been extended to any lymph node that receives drainage directly from the tumor. When there are more than one lymphatic pathway from the primary tumor, every first node along each individual lymphatic channel is a SLN (Thompson & Uren 2000).

Furthermore, if the SLN harbors metastasis, it can be bypassed because of lymph flow stasis. This may result a non-sentinel lymph node being identified as a SLN and an incorrect conclusion about the regional nodal status. Therefore, during surgery, all suspicious or enlarged lymph nodes should be removed and considered to be a SLN (De Hullu et al. 2004).

2.2.2 General aspects of primary tumors and utility of the sentinel lymph node concept

For the SLN method to be clinically feasible and safe, some preconditions must be met:

1) the primary tumor should be relatively easy to reach for the injection of tracer(s)

2) the lymphatic drainage of the primary tumor can be predicted

3) the SLNs are not located too close to the primary tumor, in order to be distinguished from the tumor and injection site

4) the SLNs can be located and removed without greater risk than the risk of a complete LND

5) there are reliable and accurate methods for histopathologic assessment of SLN(s)

6) relying on the SLN method and omitting a complete LND should not pose more risks to the patient than LND and its possible side effects, i.e. risk of recurrence in the nodal area in case of a false-negative SLN.

2.2.3 Evaluating feasibility: Essential methodological concepts

During a feasibility study, the SLNs and the other regional nodes are removed for analysis and examined separately, and their status regarding metastasis is compared. When evaluating the results, certain concepts must be taken into consideration.

Detection rate (DR) refers usually to a portion of patients, in whom the identification of SLN(s) is successful during a feasibility study. Ideally, the SLN(s) can be detected in all patients, resulting in a DR of 100 %.

A false-negative SLN refers to a node detected by the studied method, where the metastasis is not identifiable, although some other node in the same lymphatic basin does harbor metastasis, see Table 2 (Kataria et al. 2016). The proportion of false-negative SLN findings in a study population is usually given as a percentage calculated from the number of procedures performed. This variable is often confused with the variable false-negative rate. In clinical settings, a false-negative SLN could lead into cancer recurrence in that nodal area. The causes of a false negative SLN are discussed below.

False-negative rate (FNR) is the rate of occurrence of negative test results in patients known to have the disease for which they are being tested, in this case nodal metastasis. In other words, it indicates the probability of having metastases in non-SLNs if SLN is negative. The FNR is calculated by dividing the number of false-negative results by all procedures that give a node-positive result (both in SLNs and in non-SLNs) as follows (Krag 1998; Estourgie et al. 2003):

$$\text{FNR} = \frac{B}{B + C + D} \times 100\%$$

The FNR is therefore one of the most important figures when evaluating the safety of the method. The lower it is, the safer is the method for a patient.

Negative predictive value (NPV) refers to the probability of a negative test result correctly indicating the absence of disease, in this case, no lymph node metastasis. It is calculated as follows:

$$NPV = \frac{A}{A + B} \text{ x } 100\%$$

Table 2. Interpretation of results of a histopathological analysis of sentinel lymph nodes and regional nodes.

Histopathology	No metastatic cells in other regional nodes	Metastatic cells in other regional cells
No metastatic cells in a sentinel lymph node	True negative (A)	False negative (B)
Metastatic cells in a sentinel lymph node	Metastatic cells only in a sentinel lymph node (C)	Metastatic cells both in sentinel and non-sentinel lymph nodes (D)

2.2.4 Tracers for labeling the sentinel lymph node

SLN detection requires a tracer injection into the proximity of the primary tumor. For optimal SLN detection, an ideal tracer has the following features (Schauer et al. 2005b; Cousins et al. 2014):

- it is easy to inject and economical to use
- it is readily resorbed into the lymphatic network and transported to the target node(s) but has minimal absorption into veins and capillaries
- it stays in the target node long enough to be clinically detected
- during this time, there is minimal spillage of the tracer to the lymphatic upstream. This prevents unnecessary collection of second echelon lymph nodes
- it can easily be distinguished from the background
- it imposes little risk to patient's health

The most important factor controlling lymphatic transport is particle size, usually reported as the hydrodynamic diameter. Particle size affects the uptake into the lymphatic channels, the speed of transport to the target node and the time of retention inside the draining node. The smaller the particle size of the tracer is, the

quicker it is taken up and transported to the node of interest. On the other hand, the time frame for SLN detection is also shorter, because the tracer moves forward along the drainage labeling also to second echelon nodes. Tracers with small-sized particles (for dyes, diameter less than 5–10 nm) can also diffuse from the lymphatic vessel, reducing the likelihood of identification in low concentrations (Cousins et al. 2014).

Medium-sized tracers (nanoparticles, diameter 50–200 nm) are slower to be transported to the SLNs, but also stay there longer providing a longer-lasting window for detection and imaging. However, medium-sized tracers might not migrate outside the injection area or they may circumvent the SLN if it contains metastatic cells. Large-sized tracers (microparticles, diameter over 500 nm) migrate to the lymphatics by phagocytosis via macrophages and dendritic cells, and therefore their transport to the SLN is very slow (Figure 2) (Cousins et al. 2014).

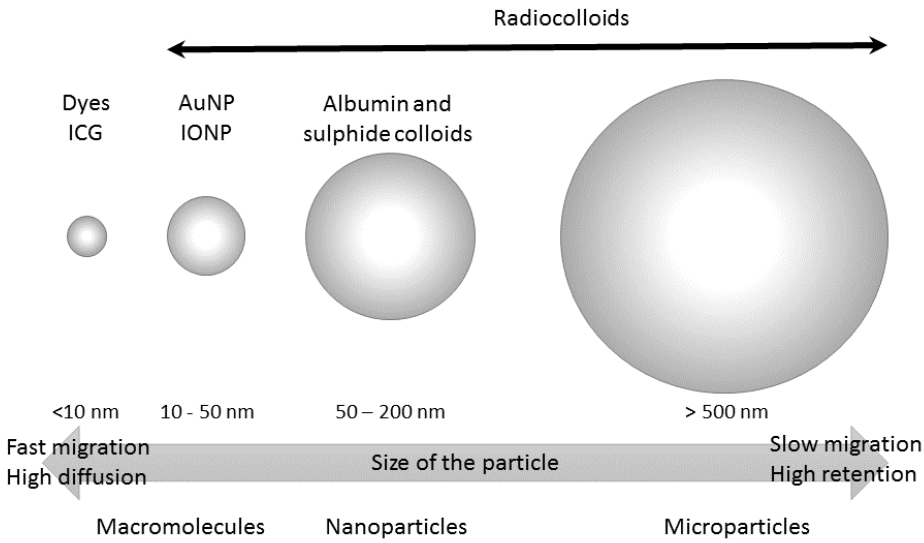

Figure 2. Effect of the tracer size on transport into the lymphatics. ICG = indocyanine green, AuNP = gold nanoparticle, IONP = iron oxide nanoparticle. Reprinted from Biotechnology Advances, Vol. 32, Cousins et al., "Clinical relevance of novel imaging technologies for sentinel lymph node identification and staging", pp. 269–279, Copyright (2014), with permission from Elsevier.

Because of the differences in the reabsorption and transportation of different tracers, it is imperative that the surgeon and the whole team are familiar with the used tracer(s). A summary of different tracer features is presented in Table 3.

Table 3. Summary of features of available tracer types for clinical use. +++ = excellent, ++ = good, + = acceptable. * needs more clinical studies.

Tracer type	Availa-bility	Economy	Timing of injection	Safety for patient	Safety for staff	Need for preope-rative imaging	Need for special equip-ment	Ease of detection	Effective-ness in clinical use
Vital blue dyes	+++	+++	++	++	+++	no	no	++	+
Radio-isotopes	+	+	+	+	+	yes	yes	+	+++
Fluoro-phores	++	++	+++	+++	+++	no	yes	+++	++*
Hybrid tracers	+	+	++	+	+	yes	yes	+++	++*

2.2.4.1 Vital blue dyes

The vital blue dyes were the first tracers to be utilized for the detection of SLNs. They are inexpensive, easy to store up and harmless for the medical staff. They have a small particle size and low molecular weight, which causes rapid transport to the target node but short retention in the node of interest. Because of this, dyes are typically injected to the patient in the operation room (OR) after induction of anesthesia or even intraoperatively. Timing is of essence. If injected too soon, several other nodes in same lymphatic basin can be stained, when the blue dye travels upstream. If injected too late, the injection site is intensively stained, but the dye has not yet reached the lymph nodes. The short time window for detection may cause problems with obese patients whose SLNs are located deep inside the tissue, and puts demands on the surgeon's technical skills (Schauer et al. 2005a; Cousins et al. 2014; Vidal-Sicart et al. 2014).

Isosulfan blue, also called lymphazurin, was used by Morton and his colleagues during the initial studies of SLN concept (Morton et al. 1992). It is still the most common blue dye used in the United States (U.S.). After injection, isosulfan blue binds to local proteins, especially albumin, and is rapidly absorbed by the lymphatics. The blue-stained nodes can easily be visualized within the surrounding tissue, but only for a short time. Isosulfan blue is a triphenylmethane-based dye, a member of the rosaniline family of dye compounds, which are also commonly used to color commercial products like textiles, cosmetics and paper. Patients may previously have been exposed to these dyes in their daily lives, which makes sensitization to isosulfan blue possible. Moderate and severe allergic reactions to this compound during SLN mapping have been reported in up to 2 % of patients. The reactions can range from urticarial reactions to blue hives and to severe anaphylaxis. A little less than half of the patients who develop an anaphylactic reaction need resuscitation. Preoperative prophylaxis with glucocorticoids, diphenhydramine and famotidine may reduce the severity of allergic reactions, but not the overall incidence (Masannat et al. 2006; Bézu et al. 2011; Thevarajah et al. 2005; Kelley & Holmes 2011).

Patent blue V is an isomer of isosulfan blue with similar lymphotropic qualities, and in use especially in Europe. It is also used commercially as a food colorant (E131), causing sometimes sensitization. The risk for allergic reactions seems little lower than with isosulfan blue; minor reactions after patent blue V injections happen in 0,9 % of the cases and severe reactions in 0,07 % of the cases (Bézu et al. 2011). It has no mutagenic activity, DNA damaging capabilities or reproductive toxicity, but after chronic exposure, reduced values of hemoglobin, hematocrit and red blood cell count have been reported (Amchova et al. 2015).

Both patent blue V and isosulfan blue may cause falsely low pulse oximetric readings. Blue dyes absorb the light of wavelengths near to 660 nm and 940 nm, thus interfering with the pulse oximetric readings. When used in large quantities, the patient's face and neck can temporarily turn bluish. The urine typically becomes blue-stained for the next couple of days. Transient methemoglobinemia has been reported (Lai et al. 2011).

Since both patent blue V and isosulfan blue can cause anaphylaxic reactions, **methylene blue** has been tested as an alternative tracer for SLN mapping. It is commonly used in diagnostic and surgical procedures because of its its color, e.g. for delineating fistulae in urologic surgery. It is used to treat methemoglobinemia. It acts as an antagonist to vasodilatation by directly inhibiting nitric oxide synthase, and is therefore useful also in the treatment of patients in the stages of distributive shock. Methylene blue is as effective as the aforementioned blue dyes for mapping of the SLN, but causes fewer and milder allergic reactions, although severe anaphylactic shock has been reported. Other adverse events include skin reactions which are fairly common. In intradermal injections, up to 21 % of patients suffer from erythema, superficial ulcerations and even necrosis. If the injection site is excised during surgery, this risk is eliminated (Masannat et al. 2006; Bézu et al. 2011; Kelley & Holmes 2011; Hosseinian et al. 2016).

2.2.4.2 Radiocolloids

Radiopharmaceuticals consist of a non-radionuclide portion, which acts as a carrier, and a radionuclide portion, which emits photons that can be detected by special imaging equipment. The most common medical radionuclide to label these radiopharmaceuticals is technetium-99m (^{99m}Tc) that has a suitable half-life of six hours. ^{99m}Tc is the decay product of molybdenum-99 (^{99}Mo), which is produced by irradiating a target of uranium (^{235}U) foil with neutrons and then separating the ^{99}Mo from the other resulting fission products. The half-life of ^{99}Mo is 66 hours, which prevents its storing for long time periods. ^{99}Mo is currently produced in six reactors worldwide, and their output varies according to their maintenance schedules. In 2008, coincidental and simultaneous closure of all reactors for maintenance resulted in a worldwide shortage of 99mTc, raising significant concerns about its continuous availability (Jain et al. 2009; WNA 2017; Kelley & Holmes 2011).

For SLN mapping, ^{99m}Tc is attached to nanoparticles like sulfur or albumin colloid, which after transportation to the SLN are mostly retained there, allowing detection by preoperative imaging or a handheld gamma detector. A radiocolloid

must reflect the best compromise between fast lymphatic drainage and optimal retention in the SLN (Jain et al. 2009).

^{99m}Tc-sulfur colloid, mostly used in US, has an average particle size of 200 nm (range 50–1000 nm), to decelerate its absorption to the lymphatic system. **^{99m}Tc-antimony trisulfide colloid**, mostly used in Canada and Australia, has the smallest particle size of 3–30 nm. It has a rapid migration to the SLN level, but continues to migrate even higher in the lymphatic chain with the risk of sampling second-echelon nodes. **^{99m}Tc-albumin colloid** has quite an ideal particle size, since 95 % of the particles are less than 80 nm (range 5–100 nm) in diameter. Therefore, it is swiftly absorbed and transported into SLNs where it is trapped by phagocytosis into macrophages which yields a prolonged residence time, allowing the detection even 24 hours after injection (Borgstein et al. 1998; Jain et al. 2009; Giammarile et al. 2014; Vidal-Sicart & Valdés Olmos 2016).

The problem with current ^{99m}Tc-labeled radiocolloids are low rates of clearance and accumulation; only 5 % of the injected dose is cleared away from the injection site during first 60 minutes and less than 2 % accumulated in the SLN after same time. This can partly be overcome by using simultaneously blue dye with ^{99m}Tc-labeled radiocolloid to provide visual guidance. The combination of blue dye and isotope has produced the best results in most studies (Borgstein et al. 1998; Jain et al. 2009).

Use of a radioactive isotope creates a need for radiation protection of the patient and medical staff in the nuclear medicine department, OR and pathology department. The injected activity required for detection in the SLN ranges usually from 10 to 150 MBq, depending on the tumor, the interval between the injection of the tracer and the procedure and duration of the procedure. The absorbed dose to the patient depends on the injected activity, the interval between the injection and the removal of the SLN(s), and whether the injection site (primary tumor) is also removed during the procedure. Because the radiocolloid hardly migrates outside the lymphatic system, it is estimated that less than 20 % of the injected activity is absorbed by the patient. The absorbed radiation dose – ranging usually from 20 to 40 mGy – is far below the threshold for undesired deterministic radiation effects. The equivalent radiation dose to the patient corresponds to 12–36 days of background radiation. If any kind of scanning is used to locate SLNs, the additional radiation exposure from imaging is considerably higher. Compared to conventional PET-CT and CT imaging at the time of cancer diagnosis, the radiation exposure from SLN mapping is trivial (Giammarile et al. 2014; Suutari 2017).

The medical staff that handles the radiocolloid is also exposed to radiation. The occupational exposure of the nuclear medicine staff caused by a SLN procedure is very low compared to other diagnostic procedures. The person who gives the injection of the radiotracer is exposed to the highest dose, which is, however, considerably lower than the ICRP (International Commission on Radiological Protection) threshold for the annual hand dose of radiation workers. The surgical staff's exposure during the surgical procedure is minimal; whole-body dose is below 1 μSv per operation. The highest whole-body dose falls on the surgeon performing the SLN procedure, below 2 μSv per operation. Therefore, no radiation monitoring or special shielding is required in the OR. Regular disposable protective garments, like jackets, cloves, hats and goggles, are enough to shield from possible stains and spatters. Pregnant staff members are often advised not to participate in SLN procedures, if any radiotracers are used, although it would require participation in more than 100 procedures to exceed the safety limits of radiation exposure calculated for a pregnant woman (Giammarile et al. 2014). According to the most conservative estimates, it would be safe for a pregnant surgeon to perform less than 100 SLN procedures during the pregnancy (Saha et al. 2016). The radiation exposure of the staff in the pathology department is even less than that in the OR, and well below the limits to general population (Giammarile et al. 2014).

2.2.4.3 Fluorophores

Fluorescent organic molecules (fluorophores) are a compromise between the uncertain visualization of the blue dyes and the pervasive signal of the radioisotopes. With fluorophores, the identification of SLN is based on the fluorescence emitted upon exposure to an excitation light source (near-infrared light, NIR, 700–900 nm), not on the staining of the lymph node. Depending on the wavelength, NIR fluorescent light penetrates the tissue from millimeters to even centimeters, thus showing structures that are not yet surgically exposed. However, it does not alter the surgical field, because the NIR fluorescent light is invisible. Not only are the SLNs localized, but also the draining lymphatic channels can be visualized. The use of NIR light is safe compared to ionizing radiation, since the amount of light is small and the instrument can be kept relatively far away from the target (Frangioni 2003; Tanaka et al. 2006; Polom et al. 2011; Schaafsma et al. 2011; Cousins et al. 2014).

Indocyanine green (ICG), the most common fluorophore in use, is a water soluble, low-molecular-weight, tricarbocyanine dye. It absorbs and emits light in the NIR spectrum (approximately 820 nm). It was introduced 60 years ago as a photographic dye but was later rediscovered as a tracer to facilitate SLN mapping.

ICG has an excellent safety profile; since its approval by the U.S, Food and Drug Administration (FDA) in 1958, anaphylactic reactions have been reported only rarely. The estimated incidence of allergic reaction is 1:10.000, as reported by the manufacturer. The agent itself is cheap and requires no specific patient preparation before injection. The costs consist of the imaging system required to detect the fluorescence (Frangioni 2003; Schaafsma et al. 2011; Cousins et al. 2014; Handgraaf et al. 2014).

ICG has a moderate affinity to human serum albumin. It is rapidly taken up by the lymphatics and transported to the SLN. Extravasation is negligible, and uptake by the liver is fast, followed by excretion into the bile. Therefore, it can very well be injected intraoperatively, also saving the patient from a painful experience. Due to the small molecular size of ICG, its retention time in the SLN is not very long. This can be resolved by conjugating ICG with human serum albumin, which increases its hydrodynamic diameter up to 7.3 nm, also improving its retention to SLN and contrast to the background (signal-to-background ratio, SBR). The fluorescence of ICG is reported to be visible through 0.5–1.0 cm of soft tissue. Still, obesity of the patient is still related to a higher false-negative rate even when using NIR fluorescence guided SLN mapping (Ebert et al. 2011; Polom et al. 2011).

The concentration of a fluorophore affects its visibility. Both ICG and **albumin adsorpted ICG (ICG:HSA)** exhibit intense quenching as their concentrations are increased (Gioux et al. 2010). Dilution will occur upon injection and uptake to the lymphatics, which, in theory, counteracts the quenching effect. Several dose-escalation trials in different cancer types have been conducted to assess the optimal concentration of ICG:HSA. In breast cancer, the influence of the ICG:HSA concentration on the SBR was normally distributed, indicating that a concentration of 600 µM would be optimal compared to 200–500 µM and 800 µM, and that a higher concentration than 800 µM causes a decline in the NIR fluorescence signal and SBR (Mieog et al. 2011). A similar effect has been reported in vulvar cancer and melanoma, although the differences between the concentrations were not significant (Hutteman et al. 2012; van der Vorst et al. 2013).

2.2.4.4 Hybrid tracers

A combination of blue dye and radiocolloid has yielded better SLN DR than blue dye or radiotracer alone. However, the differences in particle sizes and tracer migration can result in a discrepancy between preoperative imaging results and optical mapping of SLN during the surgical procedure, leading to uncertainty about real SLNs. A direct integration of preoperative and intraoperative imaging can be

achieved by using a hybrid tracer that contains both a radioactive and a fluorescent label (Valdés Olmos, Vidal-Sicart, et al. 2014; van den Berg et al. 2014).

Several potential tracers for hybrid SLN mapping have been tested in preclinical studies (van den Berg et al. 2012). Intraoperative, **ICG-^{99m}Tc-nanocolloid** has been tested in prostate cancer, head- and neck melanoma, oral cavity carcinoma, breast, penile and vulvar cancers, and shows similar draining pattern to its parental compound ^{99m}Tc-nanocolloid. This tracer combination is based on the ability of ICG to interact with fatty acid binding pockets in albumin-based colloids. The preparation of ICG-^{99m}Tc-nanocolloid does not differ much from the preparation of regular nanocolloids. ICG is added to the nanocolloid solution, which is almost instantly ready for injection. By using a hybrid tracer, one single injection would be enough to enable both preoperative nuclear imaging and intraoperative radio- and fluorescence-guided SLN mapping (Valdés Olmos, Vidal-Sicart, et al. 2014; van den Berg et al. 2014). However, this hybrid tracer has not gained much popularity in the clinical work.

2.2.5 Techniques for sentinel lymph node imaging and detection

2.2.5.1 Preoperative imaging for locating the sentinel lymph node

Since the first report on the use of radiocolloid and a handheld gamma probe during surgery to locate SLN in melanoma (Alex & Krag 1993), it did not take long for Uren et al. to figure out that the standard lymphoscintigraphy (LSG) technique could be modified to help in detecting the location and number of SLNs and to mark them on the patient's skin before operation (Uren et al. 1993). This new approach allowed new and unexpected drainage pathways to be described and all possible SLNs to be detected, especially if the primary tumor was located near the central line or its lymphatic drainage was ambiguous. It gave an opportunity to tailor the surgery individually, facilitated the procedure by minimizing surgical dissection needed and led to a shorter training period for surgeons to accurately excise the SLN. Preoperative LSG and intraoperative use of blue dye and gamma detector soon became a golden standard for SLN mapping in several cancers (Alazraki et al. 1997; Valdés Olmos et al. 1999; Czerniecki et al. 2001; Uren et al. 2016).

However, planar bidimensional LSG images are not informative enough, when the location of the SLN is deep inside the tissue or in complex anatomical areas like the head and neck area or the pelvis. Single-photon emission computed tomography (SPECT) is a tomographic version of conventional LSG and has higher contrast

resolution and intrinsic sensitivity than LSG. When emission of gamma radiation (SPECT) and low-dose transmission of X-rays (CT) are combined, one can create a fused SPECT/CT image overlying both anatomic landmarks and functional imaging. This hybrid technique offers a more precise anatomic localization of SLNs, shows also the SLNs near the injection site or in aberrant lymphatic basins, improves the overall DR and lowers the FNR. SPECT/CT has special advantages in mapping of SLNs in cancer types draining into the pelvis or neck, and in obese patients (Even-Sapir et al. 2003; Madeddu & Spanu 2004; Van Der Ploeg et al. 2008; Bockisch et al. 2009; Valdés Olmos, Rietbergen, et al. 2014; Navalkissoor et al. 2015).

The use of preoperative imaging has its disadvantages. First, imaging and OR logistics must be carefully planned to ensure both optimal intraoperative detection of the gamma radiation without a need for additional radiotracer injections. Second, SPECT/CT involves a small extra dose of radiation to the patient because of the addition of CT imaging. Third, the preoperative imaging comes with an additional cost. In breast cancer, it has been suggested that SPECT/CT should be used in problematic cases only, in order to let the advantages to prevail (Van Der Ploeg et al. 2008). However, Stoffels et al. showed in melanoma patients, that adding SPECT/CT to the SLN procedure did not increase the total costs substantially, while the costs of the surgical procedure and hospital stay decreased significantly, resulting in a 30 % reduction in total costs. Also, the cost-effectiveness of SPECT/CT was excellent because it detected more positive SLNs and was associated with a lower rate of surgical morbidity (Stoffels et al. 2014). Evidently, the value and cost-effectiveness of SPECT/CT should be evaluated separately in each cancer type.

2.2.5.2 Perioperative detection of the sentinel lymph node

Blue dyes cause staining of the lymphatic channels and SLNs, thus allowing them to be visually detected during operation: the surgeon is able to follow the blue lymph channels to the blue-stained SLN. This requires usually some dissection of the perilymphatic tissue. However, if the SLNs are located close to the injection site, which will also become blue-stained, they might be difficult to distinguish. The diffuse staining on the operation field can disturb the overview. On the other hand, the blue dye is not too visible through a thick layer of surrounding tissue, and therefore performs poorly on overweighed patients (Schauer et al. 2005c; Verbeek et al. 2015).

The gamma radiation emitted by injected radiotracer can be detected intraoperatively with a handheld gamma probe either through up to 5 cm of skin and subcutaneous tissue or within the exposed surgical cavity. The surgical incision does

not have to be as wide as with the use of blue dye, in which case the visual detection of the draining lymph channels is an important part of the localization of the SLN. The probe is placed into a sterile bag for use inside the surgical field. The control unit shows count rates (count per time unit) and gives an acoustic signal. The probe has a collimator, which allows radiation from one direction only to be transmitted, helping to determine the exact location of the radiation source. During surgery, the background activity taken far from the injection site and possible SLN locations is recorded first. After that, the probe is used to locate the highest activity, always pointing the tip away from the injection site. The SLN has usually at least a tenfold activity compared to background activity, but this depends on the injected activity and the distance between the injection site and the SLN. After the hot node(s) is (are) removed, the surgical bed is checked for any residual activity (Zanzonico & Heller 2000; Schauer et al. 2005a; Giammarile et al. 2014).

To improve the intraoperative detection of the radioisotope, portable gamma cameras have been developed. This equipment allows real-time scintigraphic imaging with an overview of all hot spots in the surgical field. Portable gamma cameras allow a larger field of view than handheld probes, which helps to detect all SLNs and to verify whether they have been completely resected. They can also distinguish between the injection site and the SLN close to it. Gamma cameras can be divided into two categories according to their own size and field of view size they provide. A proper scintigraphic image requires 10 to 60 seconds to be achieved. Small gamma cameras can be positioned and held manually during that time. Larger cameras need a support system. A small-size field of view means diameter under 5 x 5 cm^2. Large field of view size cameras exceed that (Vidal-Sicart et al. 2014; Valdés Olmos & Vidal-Sicart 2016). Portable gamma cameras are not frequently used in daily practice.

The human eye, the surgeon's most important mapping tool, is insensitive to NIR light. The use of NIR fluorescence in SLN mapping requires a specific imaging system that not only emits and receives NIR light but also converts it into visible information on a video screen. The modern intraoperative NIR imaging systems can detect both white light and NIR wavelengths, thus providing real-time simultaneous information of the anatomical structures (color video) and NIR fluorescence signal (function). Several imaging systems are currently available, and they can be adopted to open or endoscopic surgery alike (Tanaka et al. 2006; Gioux et al. 2010; Schaafsma et al. 2011; Handgraaf et al. 2014).

2.2.6 Assessment of sentinel lymph nodes for metastasis

The chance of detecting a lymph node metastasis depends on the size of the node and the amount and distribution of tumor cells inside the node. Lymph node metastases can be divided into macrometastases (tumor deposits more than 2 mm in diameter), micrometastases (tumor deposits of 0.2–2 mm in diameter) and isolated tumor cells (ITC; single cells or isolated clusters of cells with a maximum diameter of 0.2 mm or less than 200 cells in a single histological cross-section). The impact of very low-volume nodal disease, especially ITC, on patient survival and optimal treatment are controversial in many cancer types. Therefore, when choosing the SLN assessment method, the clinician should consider what kind of findings provide necessary information on patient's prognosis and which method gives the most reliable guidance for treatment decisions about complete LND and/or adjuvant treatment (van Diest et al. 1999; Treseler 2006; Messina & Rosa 2015; Sobin et al. 2015).

When a complete LND is warranted after a positive SLN finding, the evaluation of a SLN as for cancer metastasis would ideally take place intraoperatively, enabling the complete LND during the same surgical procedure, if necessary. With this approach, the patient would avoid a second operation. It would also save costs and time to diagnosis and right adjuvant treatment. Since a false-negative SLN analysis leads to omitting the complete LND and/or adjuvant treatment, it may have a devastating effect on patient's survival. With each assessment technique, one must compromise between sensitivity and practicality. Most of the time, the appropriate approach is to perform both intraoperative and comprehensive postoperative analysis (van Diest et al. 1999; Treseler 2006).

2.2.6.1 Intraoperative analysis

The most common method for intraoperative tissue analysis is **frozen sectioning**. The technique includes bisecting the SLN and freezing either half or all of it, or sectioning the SLN into 2–3 mm sections and freezing either one suspicious section, all the pieces or random pieces if nothing suspicious is noted. The sections are then stained with hematoxylin and eosin (H&E) and analyzed by microscopy. Immunohistochemistry can also be used (for the technique, see below). The frozen sections are morphologically inferior to paraffin sections. Care must be taken to freeze the tissue flat and cut it cautiously, because step sectioning results in unavoidable tissue loss, which might cause missing some micrometastasis. Any leftover tissue is saved for postoperative analysis. The thinner the sections are, the higher is the sensitivity for detecting metastasis. However, the more sections there

are to be analyzed, the longer it takes to perform the analysis. At some point, the waiting time in the OR becomes impractical and the costs of the intraoperative analysis too high. In early breast cancer studies, the sensitivity of frozen sectioning ranged from 57 to 87 % (van Diest et al. 1999; Layfield et al. 2011). According to a review by Creager & Geisinger, the overall accuracy of SLN method was better when the SLNs are cut into 2–3 mm sections and all sections are examined intraoperatively than if the SLNs are bisected or if only some of them are examined (Creager & Geisinger 2002).

Although the specificity of frozen sectioning is high, one must bear in mind that false-positive findings do exist. According to a report by Strien et al., in a re-evaluation, 1 % of negative SLNs were originally deemed to be metastatic. False-positivity can arise from some keratin material inside CD68–positive, EMA-negative macrophages (so called pseudometastasis), from a subtype of dendritic cells that presents faint staining or from benign intracapsular nevi resembling metastasis (Strien et al. 2012).

Imprint cytology is a simple and rapid technique to assess SLNs intraoperatively. The slides are produced by simply pressing the cut side of each section firmly against the glass slide, then fixed, stained and examined. The imprints can be made without significant loss of nodal tissue, which may be a real problem in frozen sectioning (Treseler 2006). The accuracy of the technique depends on the experience of the pathologist, the use of serial sectioning when examining the SLNs and the proportion of micrometastasis in the slides. According to a meta-analysis by Tew et al., the technique performs well for detecting macrometastasis and has a pooled sensitivity of 81 % (95 % CI 74–86 %), but its pooled sensitivity for micrometastasis is considerably lower (22 %, 95 % CI 14–33 %). When comparing to frozen section, imprint cytology was less sensitive in three out of four direct comparisons. Its specificity was similar to frozen sectioning (Tew et al. 2005). Similar results have later been reported in a review by Layfield et al. (Layfield et al. 2011).

Molecular techniques offer a better possibility to analyze the entire SLN volume. The nodal tissue is homogenized and examined for messenger-RNA (mRNA) of marker genes that are overexpressed in the primary tumor but not in normal cells. The **quantitative reverse transcriptase-polymerase chain reaction** (qRT-PCR) allows differentiation between a high level of mRNA expressed by tumor cells and a low expression of non-cancerous cells. The quantity of real-time production of a target genetic material produced during polymerase chain reaction (PCR) is calculated using fluorescence and compared to a threshold level that would be the upper limit of the expression of non-neoplastic cells. Excess expression indicates

presence of a metastasis. This method is somewhat susceptible to contamination, which could lead to false-positive or false-negative results. To prevent this, a meticulous surgical technique and minimizing the amount of extranodal tissue during sample preparation is necessary (Layfield et al. 2011; Messina & Rosa 2015).

Another molecular technique is **one step nucleic acid amplification** (OSNA), in which the target gene mRNA is searched for from a homogenized SLN tissue, as in qRT-PCR. It is based on loop-mediated RT-PCR for amplification of cytokeratin 19 (CK19) mRNA, a metastatic molecular marker, at isothermal temperature without a need for simultaneous amplification of genomic DNA. In contrast to conventional RT-PCR, neither extraction nor purification of mRNA is required. The process is faster and simpler than qRT-PCR, taking only 16 minutes to be completed. However, homogenization of the nodal tissue takes additional time. Therefore, the total assay time depends on the number of SLN to be analyzed, varying between 32–33 minutes for one SLN and 37–40 minutes for two SLNs. In OSNA, six primers are required to bind the same gene compared to one in qRT-PCR, so it is relatively immune to genomic pseudogene interference. CK19 is a sensitive and specific epithelial marker, expressed in 98 % of malignant breast tumors, and can be applied to assess SLN metastasis in a variety of other cancers. It has been studied successfully also in endometrial cancer. The number of tumor cells in SLN correlates with copy number of CK19 mRNA, which allows establishing critical levels of copy numbers equivalent to the TNM classification system (Hoon et al. 2014; Tamaki 2015; Tamaki 2016; López-Ruiz et al. 2016).

Since tissue and cellular morphology is lost in tissue homogenization process, OSNA has some limitations. First, it cannot identify nodal lesions not related to epithelial cancers, like lymphoma or lymphadenitis. Second, metastatic capsular infiltration or extranodal growth cannot be evaluated, except by carefully dissecting the surrounding adipose tissue off the SLN and examining it by conventional histopathologic methods, which is a time-consuming and laborious task. Third, low expression of CK19 in metastatic tissue can lead into a false-negative result and understaging of the patient. On the other hand, benign epithelial inclusions are sometimes present in SLNs, causing a false-positive result. In endometrial cancer, their incidence in pelvic and para-aortic lymph nodes has been estimated to be as high as 5 %, but a recent paper by Lopéz-Ruiz et al. reported a rate of only 0.2 % (López-Ruiz et al. 2016). Under these circumstances, OSNA is not yet deemed to be cost-effective in the intraoperative diagnosis of SLN metastasis, until some information on clinical outcomes comparing different intraoperative diagnostic tests is available (Cserni 2012; Hoon et al. 2014; Huxley et al. 2015).

2.2.6.2 Postoperative assessment

Classic **histopathology** is regarded as the golden standard for SLN assessment, to which all the other diagnostic tests are compared. Not only can it differentiate metastatic SLNs from non-metastatic ones, but also the size, location and capsular invasion of the metastasis are described. Small nodes (less than 4 mm in diameter) are usually submitted in their entirety, and larger nodes are serially sectioned at 2–3 mm intervals along their longest axis, embedded in paraffin and stained with H&E and other staining methods (van Diest et al. 1999; Messina & Rosa 2015).

When the detection of micrometastasis and ITC are essential with respect to the patient's prognosis, the sectioning should be performed at shorter intervals if the normal pathologic assessment is negative for metastasis. This extended histopathologic examination, so called **ultrastaging**, involves serial sectioning at 50–400 µm intervals through the SLN and application of various tumor-specific antibodies (**immunohistochemistry**; IHC) to diagnose occult metastasis. Ultrastaging with IHC produces dozens of sections for microscopic analysis, thus being time-consuming and costly. Therefore, it is clinically applicable to only 1–2 nodes per patient. However, it improves the accuracy of the SLN method by detecting, for example, melanoma metastases in 35% and colorectal carcinoma metastasis in 21% of SLN negative by conventional histopathologic examination (Messina & Rosa 2015).

2.2.7 Pitfalls of sentinel lymph node technique

2.2.7.1 Impact of a learning curve

The experience of the surgeon has a vast impact on the safety of the SLN method. A certain learning curve for detecting the SLN has been reported by several authors. In breast cancer surgery, Morrow et al. reported a likelihood of identifying a SLN being 73 % during the first ten procedures, and increasing to 91 % after 30 procedures. The surgeons performing the greatest number of procedures also had the highest DRs. They did not observe any advantage from the use of either blue dye alone or the combined dye and radioisotope technique (Morrow et al. 1999). Chagpar et al. reported a significantly higher failure rate in SLN detection during breast cancer surgery if the surgeon had performed less than ten SLN procedures compared to those who had performed ten or more SLN procedures (7.8 % vs. 4.2 %, $p<0.001$). They also observed, that the type of SLN technique influenced the failure rate. When using only blue dye, inexperienced surgeons failed to identify

SLNs in 17.2 % of the cases, but only 7.2 % when using the combination of radioisotope and blue dye ($p<0.001$) (Chagpar et al. 2005). It has been hypothesized, that the use of fluorophores could further shorten the surgeon's learning curve (Polom et al. 2011).

In vulvar cancer surgery, ten performed cases of SLN procedure have repeatedly been specified as a learning curve (Meads et al. 2014). More complex SLN algorithms and/or surgical anatomy might require larger case load to improve DRs and lower FNRs. In endometrial cancer, approximately 30 performed cases per surgeon are needed for achieving a DR of over 90 %, but this varies individually among surgeons (Khoury-Collado et al. 2009). To overcome the slowly rising surgical learning curve and to maintain the surgical skills needed for the SLN procedure, it is recommended to refer the patients with rare tumors to high volume centers (Levenback et al. 2009). Individual surgeons are also encouraged to determine and follow up their own DRs and FNRs, when they set out to perform SLN procedures (Khoury-Collado et al. 2016).

2.2.7.2 Reduced tracer uptake into the sentinel lymph node

The most alarming risk of failure in SLN procedure is caused by metastatic blockage of the lymphatic flow to SLN(s). When a node is totally or almost totally infiltrated with metastatic cells, its perfusion is severely reduced. In such case, the lymphatic flow carrying the tracer by-passes the tumor-infiltrated SLN and drifts to some other tumor-free lymph node, labeling it. As a result, this substitute node is unjustly identified as SLN and the metastatic status of the regional nodes is not revealed. The patient becomes down-staged and is left without further surgical and/or adjuvant treatment (Borgstein et al. 1998; De Hullu et al. 2004; Schauer et al. 2005a).

This can be avoided in several ways. CT, MRI or US (ultrasound) imaging is used preoperatively to detect suspicious, enlarged lymph nodes. If observed, the SLN procedure is cancelled and a complete LND is performed instead. If during surgery a blue-stained lymph channel is observed to lead to a non-stained lymph node, this node is biopsied and sent for pathological examination. The nodal basin should always be palpated during the procedure, and any firm and/or enlarged node collected together with the SLN. If a SLN cannot be located during the procedure, a complete LND of that area should be performed (De Hullu et al. 2004).

Reduced tracer uptake has also been reported in case of fatty degeneration of the SLN(s) in elderly people. It means that 80–90 % of the lymph nodes, mainly the central parts, have been substituted by fat cells. During this process, the normal lymphatic channels of the node will disappear, causing marked reduction in the

lymphatic flow and an inability to label this kind of nodes with any tracers available (Borgstein et al. 1998; Schauer et al. 2005a).

2.2.7.3 Proximity of a sentinel lymph node to injection site

One possible pitfall is the proximity of the primary tumor to the nodal basin, where the SLN is searched for. When dyes are in use, intensive staining of the injection site interferes with the surgical field and hampers visual detection of the stained SLNs. Dilution of the blue dye and waiting for a longer time could solve the problem. When using radiotracers, the activity of the SLN can be difficult to distinguish from the activity of the injection site and background both with conventional preoperative imaging and with handheld probes. Before surgery, the injection site can be covered with a radiation-blocking plate during imaging. Care must be taken not to cover the nodal basin at the same time. Another intraoperative solution is to add collimation to the probe and, unless too slow, to excise the primary tumor away before the search for the SLN(s). A practical and easy way to avoid the activity of the injection site is to angle the probe away from it. Preoperative SPECT/CT imaging and portable gamma cameras used intraoperatively perform better in this respect, as mentioned above (Schauer et al. 2005a; Valdés Olmos, Rietbergen, et al. 2014; Navalkissoor et al. 2015).

2.3 Vulvar cancer

Vulvar malignant tumors constitute approximately 4–5 % of all gynecological cancers. About 90–95 % of the tumors are squamous cell carcinomas (SCC), followed by malignant melanoma (less than 5 %), basal cell carcinoma (2 %) and sarcomas (1–2 %). Primary adenocarcinomas are rare (2 %), and include extramammary Paget's disease (1 %), sweat gland carcinomas, breast-like carcinomas, apocrine adenocarcinomas, and Bartholin's gland adenocarcinoma. Neuroendocrine tumors and metastases from other origins also exist (Alkatout et al. 2015; Chokoeva et al. 2015).

The incidence of vulvar cancer (VC) is highest in the older age groups, with the peak incidence between 60 and 74 years of age with more than half of the new cases occurring in women over 70 years of age. Since 2002, the incidence in Europe has been rising, the rise being more pronounced within age groups below 60 years of age. This is believed to reflect the increasing prevalence of high-risk human papillomavirus (HPV) infections and following vulvar precursors, so called vulvar intraepithelial neoplasia (VIN) (Dittmer et al. 2011; Schuurman et al. 2013).

Vulvar SCC develops via two different etiologic pathways. The first one is associated with HPV infection and the second one is not. HPV-associated SCCs arise from VIN of usual type (uVIN), which mainly occurs in younger women with a history of persistent HPV infection, and is associated with smoking, higher number of sexual partners and compromised immune status. HPV-independent SCCs arise from differentiated VIN (dVIN), which is usually seen in older women, is associated with inflammatory dermatosis like lichen sclerosus and relates to chronic oxidative genetic damages. uVIN has higher tendency of spontaneous regression and slower progression to invasive carcinoma that dVIN (Del Pino et al. 2013; Preti et al. 2014; Trietsch et al. 2015).

VC spreads by expanding first into the adjacent structures: the vagina, urethra and anus. While the tumors grows in size, metastatic spread to the regional lymph nodes follows, first to the inguinal, then the femoral nodes, and finally to the pelvic nodes. Ultimately, there is hematogenous spread to distant organs; liver, lungs and skeleton (Alkatout et al. 2015).

2.3.1 Diagnosis and evaluation before treatment

VC is rarely asymptomatic. Symptoms include especially pruritus, localized pain and burning, while discharge and/or bleeding are less frequent. A vulvar lump or mass is usually detected, which may be ulcerated, leukoplakic, fleshy or warty on presentation. The diagnosis of VC is made by a biopsy of a suspicious lesion. Multiple biopsies of multiple sites or repeatedly of the same lesions may be necessary. If VC is suspected, excision of the primary tumor before evaluation by a gynecologic oncologist is not recommended, as it hinders the accurate estimation of tumor size, location and surgical margins and may impede the use of SLN biopsy. Histopathologic assessment of the biopsy includes evaluation of the histologic type, grade and the depth of stromal invasion, which is defined from the epithelial-stromal junction of the most superficial adjacent dermal papilla to the deepest point of invasion (Deppe et al. 2014; Zweizig et al. 2014; Alkatout et al. 2015; Hacker et al. 2015).

Prior to treatment, the size of the primary tumor and its proximity to adjacent structures like the urethra, anus, vagina and clitoris are measured. Any palpable nodes in the groins and supraclavicular area or fixation to the bone are noted. The vulvar and perianal skin as well as vagina and cervix are examined for any coexistent neoplasia, in order to determine the boundaries of resection. This examination is

sometimes painful to the patient, and may require application of local anesthetic or even general anesthesia (Zweizig et al. 2014; Hacker et al. 2015).

With bulky tumors, MRI can be of use to determine the anatomic extent of the disease, especially if urethral involvement is suspected. Also, it is useful in evaluation for suspicious inguinal nodes. CT and PET-CT (positron emission tomography–computed tomography) can be used to evaluate distant metastatic disease. PET-CT is highly specific but fairly insensitive for detecting groin metastases, and its NPV varies between 57 % to 86 %. Therefore, it can be used for treatment planning (for example, omitting a SLN biopsy and performing a complete LND) but not to substitute surgical nodal staging (Cohn et al. 2002; Kamran et al. 2014; Oldan & Patel 2014; Zweizig et al. 2014; Alkatout et al. 2015).

Staging of VC is surgical, and relies on the classification of The International Federation of Gynecology and Obstetrics (FIGO) modified in 2009 (Mutch 2009). It is presented in Table 4.

2.3.2 First-line treatment strategies in vulvar cancer

2.3.2.1 Surgery

The primary treatment of VC is surgery. With en-bloc radical vulvectomy and inguino-femoral lymph node dissection (IF LND), the survival rates increased substantially in the past, but postoperative morbidity was high. The prevalence of wound breakdowns and infections was as high as 85 %. Lymphedema and chronic cellulitis of the lower extremities as well as psychosexual problems especially in younger patients were common long-term complications (De Hullu et al. 2002). Over the years, several modifications have been made to standard surgery in order to reduce morbidity without compromising prognosis. Surgical treatment can be individualized, emphasis being on performing the most conservative operation that provides the cure in each patient's situation. The main oncologic principle remains the same: adequate lateral and deep excision margins for the tumor are required (Deppe et al. 2014; Zweizig et al. 2014; Alkatout et al. 2015; Sznurkowski 2016; Hacker et al. 2015).

Table 4. FIGO 2009 staging of vulvar cancer. For comparison, the TNM staging is also presented.

FIGO Stage	TNM	Clarification
I		Tumor confined to the vulva or perineum with no nodal metastasis:
IA	T1a N0 M0	Lesions ≤2 cm in size, confined to the vulva or perineum and with stromal invasion ≤1.0 mm
IB	T1b N0 M0	Lesions >2 cm in size or with stromal invasion >1.0 mm, confined to the vulva or perineum
II	T2 N0 M0	Tumor of any size with extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with negative nodes
III	T2 N1-2 M0	Tumor of any size with or without extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus), with positive inguino-femoral lymph nodes:
IIIA	T2 N1a M0	i) one lymph node metastasis (≥5 mm)
	T2 N1b M0	ii) 1–2 lymph node metastasis(es) (<5 mm)
IIIB	T2 N2b M0	i) 2 or more lymph node metastases (≥5 mm)
	T2 N2a M0	ii) 3 or more lymph node metastases (<5 mm)
IIIC	T2 N2c M0	positive nodes with extracapsular spread
IV		Tumor invades other regional (2/3 upper urethra, 2/3 upper vagina), or distant structures:
IVA	T3 N0-2 M0	i) upper urethral and/or vaginal mucosa, bladder mucosa, rectal mucosa, or fixed to pelvic bone
	T1-2 N3 M0	ii) fixed or ulcerated inguino-femoral lymph nodes
IVB	T1-3 N0-3 M1	Any distant metastasis including pelvic lymph nodes

Less than 1% of patients with invasion under 1mm will have inguinal lymph node metastases. Therefore, **stage IA lesions** can be treated with wide and deep local excision with at least 1 cm margins around the tumor without inguinal lymph node dissection. **Stage IB tumors that are less than 4 cm in diameter** require radical excisions and lymph node evaluation, since up to 28 % of patients with stromal invasion of 1–5 mm have nodal metastasis. Local radicality means at least 2 cm lateral margins without stretching the tissue and tumor excision to the deep fascia layer. In case of lateral unifocal tumors (>1 cm from the midline) and unsuspicious groins, ipsilateral surgical evaluation of the lymph nodes is sufficient. If ipsilateral nodes are tumor-free, the risk of contralateral metastasis is less than 1 %. If a lymphadenectomy is carried out, both superficial inguinal and deeper femoral nodes should be removed for histopathological analysis, to avoid higher risk of groin

recurrence (Gordinier et al. 2003; Micheletti & Preti 2014). A complete IF LND can be replaced with SLN biopsy (Chapter 2.3.4). At present, if the SLN turns out to be metastatic, bilateral IF LND is recommended. In the future, GROINS-VVI/GOG 270 protocol hopefully answers the question whether all patients with SLN metastasis require complete IF LND or if it can be omitted and replaced with adjuvant radiation when SLN metastasis is less than 2 mm in diameter (Penick et al. 2017; Kole & Robison 2016). In case of midline tumors without any suspicion of groin metastasis, SLN biopsy is still controversial (Chapter 2.3.4.2), and bilateral IF LND is often recommended (Zweizig et al. 2014; Hacker et al. 2015; Sznurkowski 2016), see Chapter 2.3.4.3.

Stage IB tumors larger than 4 cm, multifocal and stage II tumors require more radical surgery. Radical vulvectomy refers to removal of the entire vulva to the level of the deep fascia of the thigh, the periosteum of the pubis, and the inferior fascia of the urogenital diaphragm. The procedure can be modified by removing only the anterior, posterior, left or right side of the vulva (hemivulvectomy). When needed, 1 cm of the urethra can be removed without major problems with continence. Reconstructive surgery with various flaps can be used to optimize tension-free closure of the wound, to improve healing and to secure functional and cosmetic result and postoperative quality of life. Complete IF LND is performed either uni- or bilaterally, depending on the location of the primary tumor in relation to the midline (Zweizig et al. 2014; Alkatout et al. 2015; Hacker et al. 2015; Sznurkowski 2016).

In **locally advanced disease (Stage II, III and IVA)**, primary vulvar surgery is preferred, if the resection is possible with clear surgical margins without a need for stomas. Radical vulvectomy with pelvic exenteration (total, anterior or posterior) and stomas can be considered in certain cases after a thorough discussion with the patient. However, most often the patient is referred to primary chemoradiation, if the surgery would lead into damage of the anal or urinary sphincters (Hacker et al. 2015; Sznurkowski 2016).

When **clinically positive (bulky) groin LNs** are discovered during the initial workup (palpation and imaging), they should be removed for frozen section. If metastatic, a selective pelvic LND should be performed if there are enlarged nodes on preoperative imaging, and the suspicious nodes removed for histopathological analysis (i.e. nodal debulking). If the suspicious groin nodes are negative, IF LND of that side should be performed (Hacker et al. 2015; Sznurkowski 2016). Surgical removal of bulky positive nodes presumably improves local control and enhances the curative potential of radiation therapy (RT), but the optimal management of

bulky nodes is still unresolved. In case of clinically apparent groin metastasis, nodal debulking with adjuvant radiation has led to similar disease-specific survival (DSS) as complete IF LND with adjuvant radiation, but has a significantly lower complication rate (Hyde et al. 2007). It is of notice that a combination of complete LND and groin radiation may result in lymphocysts and severe lymphedema (Hyde et al. 2007; Nooij et al. 2015). Implementation of adjuvant RT is discussed in Chapter 2.3.2.2.

In case of **fixed or ulcerated nodes**, all macroscopic nodes in the groin and pelvis can be resected, unless they infiltrate muscle or femoral vessels, and the patient is treated postoperatively by groin and pelvic radiation. If the lymph nodes are not resectable, they should be biopsied to confirm the diagnosis before treating them with radiation with or without concomitant chemotherapy. If the patient is treated with primary chemoradiation, all macroscopic residual tumor in the vulva or groins should be surgically excised after it (Hacker et al. 2015; Sznurkowski 2016).

2.3.2.2 Radiation and chemoradiation

Radiation has traditionally been used as an **adjuvant treatment** in VC, and it is offered to patients with inguinal node metastasis and positive surgical margins if reexcision is not possible to reduce loco-regional recurrence and improve survival (van der Zee et al. 2016).

The optimal **adjuvant treatment for positive LNs** still needs to be defined. The AGO-CaRE-1 study involved more than 1200 patients and showed that adjuvant radiation therapy (RT) improves significantly the prognosis of node-positive patients, although it still remained worse than for node-negative patients. This advantage was seen in patients with two or more lymph node metastasis (Mahner et al. 2015). The advantage of adjuvant RT in case of one intracapsular lymph node metastasis is controversial. Some studies have not been able to demonstrate any clear survival advantage in this group of patients (Fons et al. 2009; Mahner et al. 2015), but there are also opposite results. In a study by Woelber et al, the negative impact of nodal metastasis was present in patients with even only one metastatic lymph node, and adjuvant RT reduced the impact of an additional metastatic node to non-significant (Woelber et al. 2012). RT might improve the prognosis of patients with one nodal metastasis, if LND has been less extensive (12 or fewer nodes removed). Adjuvant radiation improved the five-year DSS from 55 % to 77 % of patients with less extensive LND ($p=0.035$), but this effect was of borderline significance by multivariate analysis ($p=0.06$) (Parthasarathy et al. 2006).

At present, since benefits of adjuvant RT in patients with one small lymph node metastasis are controversial, it is usually recommended to those with two or more lymph node metastases or one macrometastasis (>2–5 mm in diameter), if extracapsular spread is present or if there is gross residual nodal disease left. The preferred radiation dose is 45–50 Gy to both groins and pelvis (Sharma 2012; Deppe et al. 2014). Based on expert agreement, radiation fields include ipsilateral groin area (Oonk et al. 2017) and are extended to the level of the bifurcation of the internal and external iliac artery, if there are no suspicious pelvic nodes (NCCN 2016; van der Zee et al. 2016; Oonk et al. 2017). In a retrospective analysis of the National Cancer Data Base in the U.S., combining chemotherapy to adjuvant radiation in node-positive patients reduced the risk of death by 38 % (HR 0.62, 95 % CI 0.48–0.79, p<0.001) (Gill et al. 2015). The American guidelines strongly recommend chemoradiation for patients with two or more positive groin LNs or a single LN with a macrometastasis (>2 mm in diameter) (NCCN 2016).

Adjuvant treatment for vulvar disease. A multivariable analysis showed that the risk for local recurrence is significantly lower for patients who have tumor-free margins < 8 mm than tumor-positive margins (HR 0.21, 95 % CI 0.08–0.55, p=0.001) (Nooij et al. 2016). The impact of close surgical margins is controversial. Heaps et al. presented already in 1990 that pathological margins less than 8 mm, equivalent to 10 mm of surgical margins, were associated with a 50 % risk of local recurrence (Heaps et al. 1990). Since then, different distances from the tumor to the margin have been evaluated. A recent meta-analysis of the current literature including 1278 patients confirmed that a tumor-free margin less than 8 mm is associated with a higher risk of local recurrence than a tumor-free margin ≥ 8 mm (pooled risk ratio 1.99, 95 % CI 1.13–3.51, p=0.02), although the authors could not repeat this finding in their own retrospective cohort analysis of 148 VC patients (Nooij et al. 2016). Visvanathan et al. stated that the risk of local recurrence was highest, when the tumor-free margin was 5 mm or less (p=0.002) (Viswanathan et al. 2013). A German multicenter retrospective register study concluded that adjuvant RT improved OS of patients with positive or close surgical margins compared to those who did not receive radiotherapy ((HR 0.36, 95 % CI 0.14–0.94, p=0.038), so that their OS was comparable to that of the patients with negative margins. In that study, 10 mm was considered to be the cut-off distance between close and negative margins (Ignatov et al. 2016). Three earlier series have also recommended adjuvant RT for close margins (Heaps et al. 1990; Faul et al. 1997; Viswanathan et al. 2013). The current treatment guidelines in Europe and the U.S. recommend adjuvant RT as an alternative to re-excision in case of positive margins, if it would require exenteration.

Close margins are mentioned as a risk factor, but there is no consensus for the threshold of pathological margin distance below which adjuvant RT should be advised (van der Zee et al. 2016; NCCN 2016).

In case of locally advanced vulvar cancer (LAVC) with extension to the urethra/anus or bones, or with fixed/ulcerated nodes, **RT with concurrent chemotherapy** (CRT; chemoradiotherapy) is recommended in order to avoid bladder/bowel diversion. If any residual tumor after completion of CRT is present either clinically or in biopsies, it should be surgically resected (Oonk et al. 2017; NCCN 2016). The scientific evidence for this approach is scarce. According to a Cochrane review, based on two retrospective studies, OS and the occurrence of treatment-associated adverse events were similar in groups treated by primary CRT and primary surgery. No data on the patient's quality of life were available (Shylasree et al. 2011).

It is of notice that many of the studies have excluded elderly patient with lower performance scores and medical comorbidities, thus suggesting better results than could be expected in clinical practice. The definition of inoperable or operable VC varies, leaving the inclusion criteria obscure in many studies. All this makes it harder to determine the best concurrent chemotherapy regimen and radiation scheme with the least toxicity and most clinical benefit. Nor is it clear, whether CRT should be used in LAVC as a neoadjuvant treatment followed by radical vulvectomy or as definitive treatment with dose escalation. Since surgical morbidity often increases after adjuvant RT, definitive CRT would seem more appropriate. Clinically node-negative patients should undergo surgical staging of the groins on beforehand for determination of the radiation fields (Mahner et al. 2015).

2.3.2.3 Chemotherapy and targeted treatment

With the exception of the neoadjuvant setting, the role of chemotherapy alone without RT is limited in the treatment of VC. It has mainly been used in the **palliative management** of advanced or recurrent VC, and only few clinical trials have been conducted. Some activity has been demonstrated for bleomycin alone or in combination with mitomycin C (response rate of 50 %) or with methotrexate and lomustin (56 %), for cisplatin and vincristine (40 %) or single agent paclitaxel (14 %). Especially with bleomycin-containing combinations, toxicity limits their use in this often heavily pre-treated population of advanced age and many comorbidities (Deppe et al. 2013).

Neoadjuvant chemotherapy (NACT), on the other hand, has shown more promising results. Its purpose is to allow less invasive surgery and avoid exenteration.

NACT has some advantages over CRT. Radiation causes cutaneous toxicity that often leads to wound complications in the radiation fields and prolonged healing after surgery. In chemo-naive patients, VC seems to be more chemosensitive than after completion of CRT – the latter could be saved for local recurrence. The responses and results of NACT followed by surgery are comparable to CRT (Reade et al. 2014). Tested chemotherapy agents include cisplatin combined with 5-FU, paclitaxel, 5-FU and paclitaxel or vincristine and bleomycin, or single agent bleomycin (Aragona et al. 2012), and cisplatin and paclitaxel with or without ifosfamide (Raspagliesi et al. 2014).

Of the targeted treatments, erlotinib – an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor – has shown a clinical benefit (partial responses or stable disease) in 68 % of patients with VC, but the average duration of responses was only three months. Toxicity was tolerable, but resulted in 17 % of the study population being unevaluable because of discontinuation of treatment (Horowitz et al. 2012).

There is a lack of efficient and less toxic systemic treatment options for all age groups. The orphan status of VC makes RCTs difficult to carry out, and multicenter trials are recommendable. Biological prognostic markers deserve more attention, in order to find new treatment strategies for advanced and metastatic VC.

2.3.3 Impact of nodal metastasis on outcome

Relapses after treatment occur in 26–37 % of patients with VC, usually within two years of primary treatment. According to several studies, the strongest prognostic factor is lymph node status. The 5-year survival rates range between 70 % and 98 % in node-negative patients and between 12 % and 41 % in patients with metastatic nodes (Gadducci et al. 2012). The OS is shortest in those patients, whose first recurrence is in the groin (median survival time of 49 months) compared to those whose first recurrence is somewhere else (median survival time of 175 months, $p<0.001$) (Stehman et al. 2009).

The number of positive nodes might be an independent prognostic factor. In some studies, the prognosis is already impaired among patients with only one metastatic lymph node (Podratz et al. 1982; Woelber et al. 2012). In most reports, however, the negative impact on OS has been seen only among patients with 2–3 metastatic lymph nodes (Gadducci et al. 2012; Baiocchi et al. 2013).

Raspagliesi et al reported that patients with nodal metastasis in both the groin and pelvic areas had significantly lower OS rates than patients with only groin node

metastasis (5-year survival: 30 % and 46 %; 10-year survival: 10% and 41%, respectively). However, their paper does not mention if the mean number of metastatic nodes differed between the groups (Raspagliesi et al. 2006). Since then, several reports have concluded that the assumed negative prognostic impact of bilaterality of nodal metastasis mainly reflects the worse prognosis associated with multiple nodal metastasis (Baiocchi et al. 2013; van der Steen et al. 2010; Tabbaa et al. 2012).

The extension of the nodal metastasis is of importance. The presence of extranodal spread is clearly a negative prognostic factor. In a recent meta-analysis of 13 studies and more than 2400 patients, extranodal extension of nodal metastasis was associated with higher risk of disease recurrence (RR 2.69, 95 % CI 1.61–3.76, $p<0.0001$), death due to cancer (RR 2.03, 95 % CI 1.12–3.69, $p=0.02$) and all-cause mortality (RR 3.18, 95 % CI 2.02–5.00, $p<0.0001$) (Luchini et al. 2016).

The diameter of the metastasis is also crucial. In 1992, Origoni et al. reported progressively lower 5-year survival rates with increasing size of the nodal metastasis; 90 % for metastases less than 5 mm in diameter, 42 % for 5–15 mm in diameter and 21 % for more than 15 mm in diameter (Origoni et al. 1992). For patients with only one lymph node metastasis, the greatest diameter of the metastasis was the most important prognostic factor ($p<0.01$) (Paladini et al. 1994). In the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V), the DSS was lower even with only one SLN metastasis larger than 2 mm in diameter than with smaller metastases (70 % vs. 94 %, $p=0.001$), and the risk of non-SLN metastases increased with the size of SLN metastases (Oonk et al. 2010).

The number of resected lymph nodes at primary surgery may influence prognosis. Gordinier et al. suggested in 2003 that groin recurrences after superficial inguinal lymphadenectomy were caused by unresected metastatic disease in inguinal lymph nodes (Gordinier et al. 2003). Le et al. defined groin surgery as optimal when at least 10 lymph nodes were extirpated bilaterally. A smaller number of lymph nodes predicted a significantly shorter time to first progression (HR 12.88, 95 % CI 1.47–112.89, $p=0.021$) and shorter DSS (HR 11.41, 95 % CI 2.21–58.86, $p=0.004$) (Le et al. 2007). In a large retrospective study with more than 1000 patients, patients with stage II VC who had more than 10 lymph nodes removed at surgery had a 5-year OS of 74 % compared to 60 % for those who had 10 or fewer lymph nodes removed at surgery ($p=0.04$). For stage III patients, the 5-year OS was 72 % and 36 %, respectively ($p=0.03$). For stage I patients, the number of removed lymph nodes did not influence the 5-year OS (Courtney-Brooks et al. 2010). Van Beekhuizen et al. stated that a total nodal count of less than 9 resected lymph nodes was an

independent risk factor for groin recurrence ($p<0.05$) and also for shorter DSS when the tumor was poorly differentiated ($p=0.025$) (Van Beekhuizen et al. 2014). Stehman et al. failed to show that groin recurrence after superficial lymphadenectomy resulted from a low number of removed lymph nodes. They questioned the prognostic value of node counting and noted that variations in anatomy and other factors may impact on its reliability (Stehman et al. 2009). Probably more important than to rely on LN numbers relative to IF LND efficiency is to acknowledge the correct lymphatic anatomy of the inguino-femoral region, and to remove all nodal tissue from the area bounded by the inguinal ligament superiorly, the sartorius muscle laterally and the adductor longus muscle medially (Micheletti et al. 2005).

2.3.3.1 Risk factors for nodal metastasis

Several tumor-dependent prognostic factors for lymphatic spread in VC have been identified. In a retrospective study by Ayhan et al., the rate of nodal metastasis increased when tumor diameter was larger than 10 mm (44 vs. 14 %, $p=0.009$), tumor grade was II or III (54 vs. 30 %, $p=0.031$), the lesion was ulcerative (50 vs. 27 %, $p=0.024$) and lymphovascular space involvement was present (73 vs 29 %, $p=0.002$). The risk of lymph node metastasis almost doubled when the invasion depth exceeded 5 mm (42 vs. 26 %, $p<0.05$) (Ayhan et al. 2008).

Sznurkowski et al. observed a similar effect with increasing invasion; the probability of groin metastases increased steeply from less than 10 % when the invasion depth of 5 mm to 70 % when it was 10 mm. They also reported an inverse correlation between histologic tumor grade and lymph node status (Spearman's correlation coefficient -0.24, $p=0.037$) (Sznurkowski et al. 2013).

Woelber et al. reported a rising probability of groin metastases with increasing primary tumor size (HR for every 10 mm; 1.28, 95 % CI 1.16–1.42, $p<0.001$), deeper invasion (HR for every 5 mm; 1.58, 95 % CI 1.26–1.97, $p<0.001$), higher histologic grade (HR for G3 vs. G1; 3.58, 95 % CI 1.10–11.69, $p=0.034$), and older age (HR for every 10 years; 1.30, 95 % CI 1.08–1.54, $p=0.004$) (Woelber et al. 2012).

A clitoral involvement may increase the probability of the LN metastasis: in a retrospective analysis of 347 patients, LN metastases were present in 31 % of patients with clitoral tumors, compared to 22 % of patients with non-clitoral vulvar tumors ($p<0.05$). This was not only the matter of the central location, because the difference persisted when compared with perineal (17 %, $p<0.05$) or other central tumors without clitoral or perineal involvement (23 %, $p<0.05$). The authors suggested that a more direct or bilateral lymphatic drainage from the clitoris to the

groins could explain the observed higher occurrence of LN metastasis, although the clitoral tumors were also larger and invaded deeper than the non-clitoral tumors (Hinten et al. 2015).

The biologic and biomolecular prognostic factors and their relation to LN metastasis are not as well known in VC as the clinico-pathological factors. Näyhä et al. suggested that increased angiogenesis and altered vessel characteristics indicate poor survival. In their study, lower (below median) vessel endothelium CD34 staining intensity was more common in the tumors of patients with LN metastasis ($p=0.02$), and it was associated with shorter DSS (age-adjusted HR 9.5, 95 % CI 2.41–37.46, $p=0.001$) (Näyhä & Stenbäck 2007). CD34 has previously been described to stain intensively only mature, well-formed vessels (Suster & Wong 1994).

$CDK1^{Tyr15}$ and $pCDK1^{Thr161}$ are bioactive forms of cyclin dependent kinase 1 which is an important regulator in G2/M cell cycle. High levels of $CDK1^{Tyr15}$ in the cytoplasm and that of $pCDK1^{Thr161}$ in nucleus associated with the presence of LN metastasis ($p=0.022$ and $p=0.009$, respectively) (Wang et al. 2015).

2.3.4 Sentinel lymph node method in vulvar cancer

In many ways, malignant vulvar tumors are ideal candidates for sentinel lymph node biopsy. The primary tumor itself is visible and easily reached for tracer injection. Lymphatic spread in VC is consistent and predictable. SLNs can be removed with less radical surgery, which causes fewer short-term and long-term complications like wound breakdown, cellulitis, lymphedema of the lower extremities and recurrent erysipelas (van der Zee et al. 2008). However, as recurrences in the groin area are most often fatal, failure in the SLN technique is disastrous for the patient's prognosis. Therefore, the safety of SLN biopsy in VC should be comparable to the safety of more radical surgery.

2.3.4.1 Safety of sentinel lymph node biopsy in vulvar cancer

Since 1994, the SLN method has been investigated in VC (Levenback et al. 1994). Several phase 2 studies and institutional series evaluating the feasibility and sensitivity of SLN biopsy by comparing the status of SLN to the complete IF LND results were conducted in the 2000's and early 2010's. Based on 47 studies, Hassanzade et al. concluded that the pooled SLN DR per patient was 94 % (95 % CI 92–96 %) and per groin 85 % (95 % CI 81–88 %), pooled sensitivity per patient 92 % (95 % CI 90–95 %) and per groin 92 % (95 % CI 89–94 %), and pooled NPV per patient 97 %

(95 % CI 96–98 %) and per groin 98% (95 % CI 97–99 %). The DR and sensitivity of SLN were closely related to the method used (blue dye, radiotracer or combination) and location of the tumor (midline vs. lateral). Palpable inguinal nodes impaired both DR and sensitivity (Hassanzade et al. 2013). In another meta-analysis, the pooled sensitivity of SLN biopsy (combined method and ultrastaging of SLN with ICH) compared to IF LND was 95 % (95 % CI 92–98 %). The SLN DRs varied with the method used in locating the SLN: for ^{99m}Tc alone, the combined DR was 94 % (95 % CI 91–96 %); for blue dye alone, it was 69 % (95 % CI 63–74 %) and for the combination of both is was 98 % (95 % CI 97–99 %) (Meads et al. 2014).

Then, a large prospective multicenter study on the safety and oncological outcome was published in 2008. The GROINSS-V study was an observational study of patients, whose treatment of VC consisted of radical excision of the primary tumor in combination with the SLN procedure performed with radiotracer and blue dye. The primary vulvar tumors were all less than 4 cm in diameter. If no SLN metastasis was detected, no further treatment followed and the patients were referred to close follow-up at every two months for the next two years. In case of a positive SLN, an IF LND was carried out. Adjuvant RT was given in case of more than one metastasis or in the presence of extranodal growth. A total of 276 women with a negative SLN biopsy were eligible for the observational study and were followed for a median of 35 months (range; 2–87 months). In this cohort, isolated groin recurrences after a negative SLN biopsy were observed in 8 patients (2.9 %), two of which had had multifocal disease. For patients with unifocal vulvar disease and negative SLNs, the actuarial groin recurrence rate after two years was 2.3 %, and the 3-year DSS 97 %. In addition to this reassuring oncological outcome, the short and long-term morbidity was significantly decreased in patients with SLN biopsy only compared to those in whom IF LND was performed: The incidence of wound breakdown in the groin was 12 % vs. 34 % ($p<0.001$), of cellulitis 5 % vs. 21 % ($p<0.001$), of recurrent erysipelas 0.4 % vs. 16 % ($p<0.001$), and of lymphedema of the legs 1.9 % vs. 25 % ($p<0.001$), respectively (van der Zee et al. 2008).

The follow up data of the GROINSS-V study was updated last year: 377 women (253 SLN negative and 124 SLN positive patients) who underwent the SLN procedure were followed for a median of 105 months (range 0–179). 261 women completed the five-year follow up. The primary isolated groin recurrence rate in SLN negative patients was 2.5 %, and all isolated groin recurrences were diagnosed within 16 months of the primary treatment. There were no isolated distant recurrences. The local recurrence rates were 25 % and 36 % at 5 and 10 years, respectively. The 5- and 10-year DSS of SLN negative patients were 94 % and 91 %, respectively. This was

considerably higher than the 5- and 10-year DSS of the SLN positive patients, namely 76 % and 65 %, respectively. SLN positive patients had an isolated groin recurrence rate of 8 % and a distal recurrence rate of 6.8 % at 5 and 10 years. Local recurrences were observed in 33 % and 46 % at 5 and 10 years, respectively (Te Grootenhuis et al. 2016).

Last year, Klapdor et al. published a retrospective sub-group analysis of the AGO-CaRE-1 study; a comparison of recurrence rates and survival of a large patient cohort who underwent groin staging as a part of their primary treatment and had a primary tumor smaller than 4 cm in diameter. A total of 772 patients were included in the comparison; 69 node-negative patients with SLN biopsy alone (group 1), 487 node-negative patients with IF LND (group 2), and 216 patients with IF LND and metastatic LNs (group 3). During a median follow-up of 33 months (range; 0–156), the isolated groin recurrence rates for groups 1 and 2 were 3.0 % and 3.4 % ($p=0.845$), respectively. Among the node-negative patients (groups 1 and 2), there were no differences between the incidences of recurrences in other locations (vulva, vulva and groins, other), either. The survival rates were similar for both node-negative groups regardless of the groin surgery; 3-year PFS rates were 83 % (95 % CI 72–93 %) for group 1 and 78 % (95 % CI 73–82%) for group 2 ($p=0.230$), and the 3-year OS rates 93 % (95 % CI 86–100 %) and 92% (95 % CI 88–96 %), respectively ($p=0.314$). Group 3 had significantly lower PFS and OS compared with both node-negative groups ($p<0.001$). The authors concluded that SLN biopsy for groin staging was not associated with increased recurrence rates or reduced survival compared with radical groin dissection, when the primary tumor was less than 4 cm in diameter (Klapdor et al. 2016).

2.3.4.2 Controversies of the sentinel lymph node method

In spite of years of research, there is still much debate about certain aspects of SLN biopsy in VC.

Although it is widely accepted that the size of the tumor should not exceed 4 cm, a question about the safety of the SLN procedure on the treatment of **tumors involving the midline** remains. A primary tumor is usually categorized as lateral if it is located more than 1 cm from the midline, while midline tumors involve central structures. The lymphatic drainage has been described already in the 19th century and relates strongly to the laterality (Sappey 1874). The drainage is considered to be potentially bilateral with vulvar tumors in and within 1 cm of the midline (Burke et al. 1995). In studies assessing feasibility and safety, SLN biopsy ± complete LND has been performed bilaterally for primary tumors located within 1–2 cm of the

midline, and unilaterally for tumor was located more than 1–2 cm from the midline. In their meta-analysis, Hassanzade et al. took notice of the considerably lower SLN DR per groin vs. per patient in patients with midline tumors (73 % vs. 95 %, respectively), which indicated that more than 20 % of the patients with midline lesions had unilateral drainage. However, the sensitivity was higher per groin vs. per patient (94 % vs. 90 %). In other words, when calculating the outcome per groin, the negative unilateral SLNs despite the contralateral nodal involvement, were considered as mere detection failure, not as a false negative SLN, as they would be considered in per-patient analysis (Hassanzade et al. 2013). Care should be taken when interpreting such results, because each patient has two groins and a missed LN metastasis in either of them leads to impaired prognosis.

Louis-Sylvestre et al. published a feasibility study that enrolled 17 patients with T1-T2 tumors involving midline or being close to it. SLN biopsy was performed with radioisotope ± blue dye followed by bilateral IF LND. LSG was performed preoperatively. In 76 % of cases (13/17), LSG showed only unilateral drainage, and in three of these groin where the SLN could not be identified, IF LND revealed several metastatic lymph nodes. The authors concluded that in case of midline tumors, a unilateral drainage in a preoperative LSG should not result in a one-sided SLN biopsy and omission of complete contralateral LND (Louis-Sylvestre et al. 2005)

In a long-term follow up study, three groin recurrences occurred during a median follow up of 58 months of patients (n=33) with midline tumors, while none of the patients with lateral tumors (n=36) relapsed. The recurrence rate for midline tumors was therefore 9 %, indicating that FNR could be higher with midline tumors. All SLN biopsies were performed with combined technique and a preoperative LSG (Robison et al. 2014).

Similarly, Klapdor et al. reported their only isolated groin recurrences (2/30; recurrence rate 6.6 % per patient) in patients with midline tumors, even when the SLNs were detected bilaterally by LSG and SPECT/CT and combined tracer injections, resected and deemed free of metastasis. According to their earlier report, SPECT/CT showed aberrant lymphatic drainage in 7 out of 40 patients (18 %), and 71 % (5/7) of them suffered from midline tumors. During a median follow up of 62 months, three patients (43 %, 3/7) – all with midline tumors and aberrant drainage patterns – developed a groin recurrence and two of them died of the disease (Klapdor et al. 2017; Klapdor et al. 2015). Therefore, an unexpected lymphatic drainage in cases of midline tumors should raise concerns and get proper attention.

A subgroup analysis of the GOG 173 assessed the reliability of LSG in relation to primary tumor location. In this subgroup, all patients had undergone LSG prior surgery, and SLN mapping was performed with injections of radiocolloid and blue dye. Bilateral IF LND was performed in case of tumors less than 2 cm from the midline, and ipsilateral IF LND if the tumor was located more than 2 cm from the midline. It is of notice that the inclusion criteria of GOG 173 included T2 tumors with clinical N0 status up to 6 cm of the largest diameter, larger tumors than in most other studies. For subgroup analysis, the investigators categorized the primary tumors as midline, lateral (more than 2 cm from the midline) or lateral ambiguous (less than 2 cm from midline but not involving it). They reported the lymphatic drainage to be bilateral in 22 % of the lateral tumor cases, 58 % of the lateral ambiguous and 70 % of the midline tumor cases. Of the patients with lateral tumors (64 patients), all ipsilateral SLNs were detected (a DR of 100 %), but the FNR was 21.4 % (3/14). In addition, these patients did not undergo contralateral IF LND even if the LSG showed bilateral drainage, and due to the lack of follow up data, the clinical significance of bilateral drainage in lateral tumors remains unresolved. In the group of lateral ambiguous tumors (65 patients), all patients underwent bilateral IF LND. The total SLN DR was 98 %. In patients with unilateral drainage by preoperative LSG (27 patients), the FNR was 10.0 % (1/10). Not a single SLN or non-SLN metastases were found in the contralateral side. Of the patients with bilateral LSG drainage, bilateral SLNs were identified at surgery in 23 out of 38 patients (61 %) and the FNR was 0 %. In 14 out of 38 patients (37 %), SLNs were only detected unilaterally and the FNR was as high as 14.3 % (1/7). In one patient, no SLNs were identified during surgery and there were metastases in her non-SLNs. In the group of midline tumors (105 patients), 32 patients (30 %) had unilateral drainage in preoperative LSG. In all patients, an ipsilateral SLN was detected. The FNR was 6.3 % (1/16). In addition, four patients expressed metastatic non-SLNs on the contralateral side, where no SLN was detected. 73 patients (70 %) with midline tumors presented with bilateral drainage: the DR was 95 % (69/73) in this group, and in 91 % of the cases (63/69), the SLNs were detected bilaterally. In six cases (9 %), the SLN was unilateral. The FNR was 14.3 % (3/21). Two patients in whom the SLNs were not detectable, also harbored metastatic non-SLNs. The authors conclude that in patients with lateral ambiguous vulvar tumors and unilateral drainage in LSG, the contralateral IF LND would be safe to omit (Coleman et al. 2013). However, the FNR of 10 % in that group is more than three-fold compared to the groin recurrence rate of 2.9 % in GROINSS-V study over the first 35 months. Also, the group of patients with midline tumors and unilateral drainage in

preoperative LSG should be handled cautiously and not to omit the contralateral complete LND, with reference to the total failure rate of 15.6 % (5/32) in GOG 173 subgroup analysis.

In conclusion, it seems that after a SLN procedure in midline tumors, the FNR and recurrence rate are higher than with lateral tumors, and the patients should be informed of it when discussing the surgical treatment. The possibility of aberrant lymphatic drainage patterns should be kept in mind. There is no conclusive evidence that in case of midline tumors it would be safe to omit the contralateral LND when only a unilateral SLN is detected.

The proper postoperative **methods for assessment of SLN** and the **relevance of micrometastasis** for the prognosis in VC are also debated. Terada et al. presented a small series of nine patients who underwent radical local excision and the SLN procedure with the combined method including preoperative LSG. Fifteen SLNs were collected. In one patient, a bilateral SLN procedure was performed and one SLN was found to be metastatic by conventional H&E staining. She then received a complete LND to that groin and all non-SLNs were negative. The other SLN had been negative by the H&E staining, but the patient developed a recurrence in that groin. At that point, the negative SLN was re-evaluated with ultrastaging and IHC with cytokeratin staining, and a micrometastasis was indeed found in the subcapsular sinus. In spite of further treatment, she later died of systemic disease. Following this, all the other negative SLNs were submitted to ultrastaging and ICH; two additional positive SLNs were detected, and the patients were treated accordingly with completion LND without a need for adjuvant RT. They remained disease-free during a median follow up of 21 months (range 6-40). The authors pointed out that only one-third of the metastatic disease in the SLNs were detectable with conventional histopathology (Terada et al. 2000).

In the GROINSS-V study, ultrastaging of pathological samples was included in the protocol if the SLN was negative on routine H&E examination. Frozen sectioning was done in 78 % of the operated cases (315/405); its sensitivity for detecting SLN metastasis was 48% (95 % CI 38–57 %), specificity 100% (95 % CI 98–100 %) and NPV 78 %. In all, 135 SLNs were metastatic; routine H&E histology revealed 59 % of them (80/135) and ultrastaging 41 % (55/135). A pathological review of a portion of the SLN specimen was performed later to evaluate the association between the size of the SLN metastasis and the patient's prognosis. According to the review, routine H&E analysis detected all SLN metastases larger than 5 mm of diameter, but its sensitivity decreased with the reduction of the size of SLN metastasis. If the diameter was more than 2 mm but less than 5 mm, H&E

analysis revealed 93 % of the SLN metastases (14/15). Metastases ≤ 1–2 mm were identified with H&E staining in 48 % of all cases (12/25). Additionally, ITC were found with ultrastaging and IHC in 28 SLNs, but H&E analysis was not able to detect them. The risk of additional non-SLN metastasis positively correlated with the size of the SLN metastasis. It was not possible to determine a cut-off size of the SLN metastasis for close-to-zero risk of non-SLN metastasis, but the risk of non-SLN metastasis seemed low when the SLN only harbored ITC. The prognosis of the patient was related to the size of the SLN metastasis. However, the 5-year DSS was 97 % for patients with ITC in SLN metastasis, comparable to the prognosis of SLN-negative patients in the same study. For those with SLN metastases 2 mm or smaller, the 5-year DSS was 88 %, for those with metastases of 2–5 mm in diameter 70 %, and for those with metastases larger than 5 mm 69 % (p=0.012) (Oonk et al. 2010). Thus, routine H&E histology is not sensitive enough for final pathological evaluation of a SLN. Ultrastaging is much more sensitive and has the capacity able to reveal micrometastatic disease. The detection of ITC might not be necessary with regard to the clinical course of the disease.

What about a **SLN procedure after previous vulvar surgery**? In one of the early publications, Levenback et al. presented a concern that previous vulvar surgery could disrupt the lymphatic drainage from the vulva, leading to an inferior SLN DR. They recommended that confirmation of the histological diagnosis should rather be done with a simple punch biopsy and not with incisional biopsy or by excision of the lesion (Levenback et al. 2001). In their study, the SLN mapping was performed with isosulfan blue dye only, contrary to the later reports. In their series of 42 patients, Hauspy et al. reported seven patients, who had undergone total excision of a vulvar lesion prior to the SLN procedure. This did not seem to affect the detection or accuracy of the SLN procedure (Hauspy et al. 2007). Crosbie et al. reported a slight, but statistically non-significant, difference in favor of less invasive biopsies. The average number of detected SLNs was lower in patients with the tumor excised prior to the SLN procedure than in patients with the tumor *in situ* (1.8 vs. 2.6, p=0.03) (Crosbie et al. 2010). Ennik et al. concluded that previous surgery did not significantly affect the results nor the safety of the SLN procedure. They did observe that there was a trend for a longer scintigraphic appearance time (the time from injection of technetium to appearance of the first lymph node) among patients with previous vulvar excisions. It is of note, that 43 % of the study population did not fulfil the common requirements for SLN procedure (no palpable inguinal nodes, tumor less than 4 cm in diameter) (Ennik et al. 2011). Woelber et al. reported an

excellent DR and no groin recurrences after previous incomplete or close-margin excision of the vulvar tumor (Woelber et al. 2013).

A summary of the performance of the SLN procedure after prior vulvar surgery is shown in Table 5. In all studies, midline involvement of the tumor seemed to influence the detection and false negative rates considerably more than previous vulvar surgery, although, due to the small numbers of patients, multivariate analysis was not performed in any of these studies.

Table 5. Summary of results of SLN procedure after previous vulvar surgery. LSG = lymphoscintigraphy, DR = detection rate, FNR = false negative rate, GRR = groin recurrence rate, WLR = wide local resection, BD = blue dye, Tc = technetium, N.A = not available, * = Fisher's exact test (two-tailed), † = per groin, ‡ = per patient.

Reference	Tracer	LSG	DR, FNR and GRR after punch biopsy	DR, FNR and GRR after incisional biopsy	DR, FNR and GRR after excisional biopsy or WLR	p value*
Levenback et al. 2001	BD	–	84 % (43/51) 0 % N.A. †	44 % (11/25) 0 % N.A. †		0.007 1.000 N.A.
Hauspy et al. 2007	Tc ± BD	±	N.A.	N.A.	100 % (7/7) 0 % N.A. ‡	N.A.
Crosbie et al. 2010	Tc + BD	+	100 % (17/17) 0 % 0 % ‡		93 % (14/15) 6.7 % 0 % ‡	0.469 0.469 1.000
Ennik et al. 2011	Tc ± BD	+	95 % (36/38) 7.9 % 5.2 % ‡		93 % (25/27) 0 % 0 % ‡	1.000 0.260 0.507
Woelber et al. 2013	Tc	+	N.A.	N.A.	100 % (32/32) N.A. 0 % ‡	N.A.

A repeated SLN procedure for recurrent vulvar cancer is also under debate. The concerns are as the ones above – does prior surgery of the vulva and inguinal area, and possible RT, interfere with the lymphatic channels in these regions and prevent the drift of tracers to the SLN? One has to bear in mind, that lymphedema is a frequent long-term complication of surgery as well as RT.

De Hullu et al. presented a case of a patient treated four years earlier for a T2 N1 M0 right-sided squamocellular carcinoma and vulvar dysplasia by radical vulvectomy and right-sided IF LND on separate incisions. The patients also received adjuvant RT to both groins. Now a new 1.5 cm tumor had emerged on the right side near the urethra. As a part of a diagnostic accuracy study, a repeated SLN mapping was performed with radioisotope, LSG and blue dye. On the left side, one SLN and a second-echelon node were detected in LSG quite soon after radiocolloid injection in the groin region, but the lymphatic flow on the right side was slower. Finally, the radioisotope became deposited straight at the pelvic nodes via inguinal lymph channels. After identification and removal of one SLN on the left, a complete IF LND was performed and four non-SLNs were collected, all negative by consecutive histopathological examination. On the right, the two external iliac SLNs were removed via an opening near the inguinal ligament, and proved also to be negative. No follow up data was provided. The authors speculated that there may have been neoformation of the lymphatic channels directly from the vulva to the pelvis, since direct lymphatic pathways are not described in the literature. If so, the frequency of pelvic LN metastasis in case of recurrent vulvar cancer should be as high as the frequency of inguinal metastasis. It is, however, known to be lower, less than 10 % (de Hullu et al. 2001).

In a case report, Landkroon et al. described a patient who was treated for a T2 lateral tumor (SCC) of her left labium without a suspicion of groin metastases. Left-sided SLN mapping (combined technique) and hemivulvectomy were performed. The SLN was negative by the routine histopathology and ultrastaging, and the surgical margins adequate (> 1 cm). Two years later, the patient presented with a right-sided, *de novo* -tumor (SCC) with clinically non-suspicious groin. Radical excision and a SLN procedure by preoperative LSG and the combined method to the right were performed. The LSG disclosed two SLNs in the right groin, which were successfully removed and proved to be tumor-free. During a 23-month follow up period, there were no signs of recurrence. Unlike in the preceding case by de Hullu et al., a repeated, uneventful SLN procedure after a second vulvar tumor was performed on the intact groin, as was the left-sided mapping of the irradiated groin in the case by de Hullu et al. (Landkroon et al. 2006).

Last year, Van Doorn et al. published a retrospective series of 27 patients from five university hospitals in the Netherlands. The patients were thought to be too fragile for IF LND or had refused complete LND at the time of a vulvar cancer recurrence. All these patients had undergone prior radical local excision and either a uni- or bilateral SLN procedure for vulvar SCC. The groins were examined with

ultrasound or CT for metastases, and a fine needle aspiration (FNA) was taken if appropriate. If FNA confirmed LN metastasis, the SLN procedure was cancelled. Otherwise, a repeat SLN procedure with the combined technique and preoperative LSG were performed at the time of second surgery. The groins were explored first during surgery, but if the signal from the groin(s) was too weak, the vulvar tumor was resected first to reduce the background radiation. For central tumors (15 patients), a bilateral SLN procedure was planned and for lateral tumors (> 1 cm rom the midline; 12 patients whose tumors were located contralateral to the first operation), it was performed ipsilaterally. In case of a metastatic SLN, a complete IF LND was performed unless the patient refused, in which case the treatment was individualized in agreement with the patient. Planned SLN biopsies succeeded in 84 % of the groins and in 77 % of the patients. In case of midline tumors, SLN mapping successfully located bilateral SLNs in 9 patients (60 %). In the remaining patients, the LSG showed either only unilateral SLN (in 4 patients; 27 %), nothing at all (7 %), or the SLN was not found during surgical exploration of the groin (7 %). In case of lateral tumors, the expected ipsilateral SLNs were identified in 58 % of the patients, in 4 patients (33 %) they were unexpectedly bilateral. One patient received ipsilateral IF LND and underwent successful contralateral SLN mapping. No SLNs were located in the external iliac region. In one patient, the radioactivity accumulated to the skin bridge between the vulvar tumor and the groin, not in the area of ordinary IF LND; no SLN was identified during surgery, and thus an oval skin area was removed. In the histopathologic examination, a small LN was found inside the tissue. Four patients had metastatic SLNs; one of them underwent IF LND and no other LN metastasis were found, two received adjuvant RT and one refused all further treatment. After a median follow-up of 27 months (range; 2–96 months), no groin recurrences were observed after a SLN procedure. The authors concluded that the DR associated with repeated SLN procedure for vulvar recurrence is lower than what is usually seen in primary surgery (around 95 % in several studies); this is especially true for midline tumors. The SLN locations were not always easy to predict, and the repeated procedure was technically more demanding than primary SLN mapping; the saphenous vein was injured at least in three groins and needed ligation. The success of the blue dye was harder than usual to extract from the data, although at least some hot SLN were also blue-stained. The repeated SLN procedure seems feasible, but its oncological safety needs to be verified in a multicenter, prospective study (Van Doorn et al. 2016).

2.3.4.3 Recommendations for sentinel lymph node procedure

The current European Society of Gynaecological Oncology (ESGO) and National Comprehensive Cancer Network (NCCN) recommendations for the use of SLN procedure in vulvar cancer (van der Zee et al. 2016; NCCN 2016) are shown in Table 6.

2.4 Ovarian cancer

Worldwide, ovarian cancer (OC) is the seventh most common cancer in women, with a higher incidence in economically developed countries than in less developed countries. It is associated with the highest mortality rate of all gynecologic cancers, and overall it is one of the most frequent causes of fatal malignancy in women (Koshiyama et al. 2017). Most OCs are epithelial in origin, with high-grade serous carcinomas constituting 70–80 % of the cases. Less common types are endometrioid (< 5 %), clear cell (3 %) and mucinous carcinomas (< 3 %). The peak incidence of OC is in the age group of 50–70 years, and three-quarters of new diagnoses are given to patients over 55 years of age (Sundar et al. 2015).

Epithelial OC (EOC) can roughly be divided into two types. Type I tumors (endometrioid, clear cell, mucinous and low-grade serous carcinomas) are generally slow-growing, fairly indolent neoplasms that are more likely to be detected in an early stage than type II tumors. Type II tumors (high grade serous carcinomas) are clinically aggressive neoplasms that grow fast and spread early. Type I tumors have clearly idintifiable precursors, but type II tumors may develop *de novo* from the tubal and/or ovarian surface epithelium (Koshiyama et al. 2017; Sundar et al. 2015). Serous tubal intraepithelial carcinoma (STIC) on the tubal fimbria often precedes high-grade serous carcinoma. A p-53 signature lesion – a histologically normal but TP53-mutant fallopian tube lesion – is presumably a precursor of STIC. Endosalpingiosis is a source of tubal-type epithelium outside the fallopian tubes, and might act as another origin of ovarian and peritoneal neoplasia (Karnezis et al. 2016).

OC has its particular way of intraperitoneal spread, so called transcelomic dissemination. During this process, tumor cells are shed from the primary tumor, transported by the peritoneal fluid and implanted onto the peritoneal surface of other pelvic and abdominal organs such as the uterus, fallopian tubes, mesentery and omentum. Direct invasion from the implantation and tumor site is common (Weidle et al. 2016; Sahdev 2016). Two other ways of spread are hematogenous and lymphogenous. Circulating tumor cells have been demonstrated from the peripheral blood of OC patients and are thought to be able to settle into secondary organs like the liver, lungs and bone marrow (Gasparri et al. 2016).

Table 6. European and U.S. guidelines for the use of SLN procedure in vulvar cancer. EBRT = external beam radiation therapy.

	ESGO Guidelines	NCCN Guidelines
Candidates for SLN biopsy	Patients with unifocal disease less than 4 cm of diameter and without suspicious groin nodes	Patients with a primary unifocal vulvar tumor for less than 4 cm of size, and negative clinical groin examination and imaging. No previous vulvar surgery that may have impacted lymphatic flow to the inguinal region.
Tumors involving midline	Bilateral SLN detection is mandatory. A contralateral IF LND should be performed in case of unilateral SLN detection	Bilateral SLN biopsy is recommended
Tracers	Use of radiocolloid is mandatory, blue dye is optional	Use of both blue dye and radiocolloid is recommended
Preoperative detection of SLNs	Lymphoscintigram is advised	Lymphoscintigraphy is optional
Intraoperative detection of SLNs	No recommendations	Use of a gamma probe is recommended
If SLN is not detected	A complete IF LND should be performed	A complete IF LND is recommended
Method of SLN analysis	Frozen section in order to prevent a second surgical procedure. Pathologic evaluation of SLNs that are negative on standard H&E staining should include ultrastaging and IHC.	No recommendations
Management of a positive SLN	A complete IF LND of the groin. Postoperative EBRT to the groin is recommended in case of more than one metastatic lymph node and/or presence of extracapsular lymph node involvement.	A complete IF LND and/or administration of adjuvant radiation to the affected groin(s). Contralateral groin should be evaluated surgically and/or treated with EBRT.

LN metastasis in the pelvic and para-aortic regions is more common than in any other gynecologic cancer. Three routes of lymphatic drainage from the ovary have been described. The main route consists of lymphatic vessels along the suspensory ligament and terminates in the para-aortic LNs. The second route runs within the ovarian and broad ligaments towards lateral and posterior pelvic wall, ending in the uppermost external iliac and obturator LNs, from where the lymph drains along external, internal and common iliac vessels to the para-aortic area. The third route passes along the round ligaments, draining into the external iliac and inguinal LNs (Ushijima 2007; Kleppe et al. 2015).

2.4.1 Diagnosis and evaluation before treatment

Women with OC usually present with nonspecific symptoms like pelvic or abdominal pain, abdominal distension or bloating, a feeling of fullness, early satiety or loss of appetite, increased urinary urgency or frequency, changes in bowel habit, unexplained weight loss and fatigue. Since these symptoms are common in average female population, the diagnosis is often delayed until more severe symptoms appear: ascites, pleural effusions and bowel obstruction. According to Sundar et al., almost one-third of OC patients in the United Kingdom get their diagnosis at emergency departments and another third through cross-specialty referrals; 80 % of them are diagnosed in an advanced stage (Sundar et al. 2015).

For women repeatedly complaining of these symptoms, the National Institute of Health and Care Excellence (NICE) recommends sequential testing of serum CA125 followed by abdominopelvic sonography, if the CA125 level is 35 IU/l or more. If a pelvic mass or ascites is detected, the patient should urgently be referred to specialized care (Sundar et al. 2015). A combination of HE4 and CA125 has higher sensitivity for detection of OC than CA125 alone. HE4 is less frequently elevated in benign tumors and endometriosis than CA125, both in premenopausal and postmenopausal women (Nowak et al. 2015). Pelvic and sonographic examinations are the first and suggestive steps of pretreatment evaluation. The next steps depend on the degree of suspicion that the mass might be malignant. Factors to consider include, e.g., age, menopausal status, size, complexity and laterality of the mass, CA12-5 level, associated signs and characteristics of the mass and family history (Jelovac & Armstrong 2011). If the suspicion is strong, CT can help to evaluate the mass and the extent of the disease, and especially, to distinguish unresectable disease from resectable (Wasnik 2013; Sahdev 2016; Forstner et al. 2016). PET-CT is not

recommended as a modality for primary detection of OC. MRI can be used for differentiating benign masses from malignant, but due to its limited availability and high costs, it is mainly used in inconclusive cases where surgery is to be avoided (Wasnik 2013).

For confirming the diagnosis of OC, a tissue or cytologic sample for pathologic evaluation is required. Fine or core needle biopsies from a suspicious ovarian mass without any signs of metastasis is usually avoided out of fear of dissemination. In the case of apparently advanced disease, biopsies and/or paracentesis can be considered, especially if the patient is frail. Surgery is often needed to reach an accurate histopathologic diagnosis and staging. If there is no suspicion of advanced disease, a staging laparotomy is performed. It includes midline incision, total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy (BSO), careful evaluation of all peritoneal surfaces and washings of the peritoneal cavity, biopsy and/or resection of any suspicious lesions; random blind biopsies of normal peritoneal surfaces, including the undersurface of the right hemidiaphragm, bladder reflection, cul-de-sac, right and left paracolic recesses, both pelvic sidewalls, omentectomy; complete or selected lymphadenectomy of the pelvic and para-aortic lymph nodes. Appendectomy is performed for mucinous histotypes. For selected patients, a minimally invasive approach may be considered (Jelovac & Armstrong 2011; Hacker 2017). If advanced disease is suspected, laparoscopy can be used to collect the necessary tissue samples and to evaluate resectability to avoid laparotomy which would only result in suboptimal residual disease. In case of resectable disease, laparoscopy can be converted into laparotomy (Nick et al. 2016).

Staging of OC is surgical, and all women with apparent early stage ovarian cancer should undergo complete surgical FIGO staging (Hacker 2017). The staging classification was revised in 2013 (Prat 2014), and is shown in Table 7.

2.4.2 First-line treatment strategies in epithelial ovarian cancer

The recommendations for the treatment depend strongly on the stage of the disease and the extend of the surgical debulking.

Table 7. FIGO 2014 staging classification of ovarian cancer. The TNM staging is presented for comparison.

FIGO Stage	TNM	Explanation
I		Tumor confined to ovaries:
IA	T1a N0 M0	Tumor limited to one ovary (capsule intact), no tumor on ovarian surface, no malignant cells in the ascites or peritoneal washings
IB	T1b N0 M0	Tumor limited to both ovaries (capsules intact), no tumor on ovarian surface, no malignant cells in the ascites or peritoneal washings
IC		Tumor limited to one or both ovaries with any of the following:
IC1	T1c1 N0 M0	1) surgical spill
IC2	T1c2 N0 M0	2) capsule ruptured before surgery or tumor on ovarian surface
IC3	T1c3 N0 M0	3) malignant cells in the ascites or peritoneal washings
II		Tumor involves one or both ovaries with pelvic extension (below pelvic brim):
IIA	T2a N0 M0	Extension and/or implants on uterus and/or fallopian tubes and/or ovaries
IIB	T2b N0 M0	Extension to other pelvic intraperitoneal tissues
III		Tumor involves one or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes:
IIIA1	T1-2 N1 M0	Positive retroperitoneal lymph nodes only (cytologically or histologically proven) i) metastasis up to 10 mm in greatest dimension ii) metastasis more than 10 mm in greatest dimension
IIIA2	T3a2 N0-1 M0	Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes
IIIB	T3b N0-1 M0	Macroscopic peritoneal metastasis beyond the pelvis up to 2cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes
IIIC	T3c N0-1 M0	Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)

FIGO Stage	TNM	Explanation
IV **IVA** **IVB**	T1-3 N0-1 M1	Distant metastasis excluding peritoneal metastases: Pleural effusion with positive cytology Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

2.4.2.1 Surgery

The goal of primary surgery is to provide an accurate histopathological diagnosis, establish the FIGO stage and reach optimal cytoreduction, i.e. absence of residual disease. After surgery, adjuvant chemotherapy is obligatory in cases of suboptimal debulking, optimally debulked advanced disease and early stage disease with a high risk of recurrence (Pepa et al. 2015; Jayson et al. 2014).

In early EOC, **staging laparotomy** described in Chapter 2.4.1 also serves as efficient optimal surgical treatment. This procedure should be performed by a gynecologic oncologist. Problems tend to arise, when EOC is not suspected to be the cause for the patient's acute symptoms (e.g. torsion or ovarian cyst rupture), if an emergency procedure is needed and performed after office hours or if the pathologist is inexperienced in frozen section analysis. All these factors can cause a delay in appropriate staging and adjuvant treatment (Trimbos 2017).

Nowadays, **staging laparoscopy** is used in some centers instead of laparotomy in early EOC. The mini-invasive technique has raised some concerns. Is it possible to inspect comprehensibly all serosal surfaces in the abdominal cavity? Palpation is also limited. Is the risk of port metastasis and capsule ruptures higher than in laparotomy? No randomized controlled trials exist, but some evidence has accumulated from retrospective studies. Bogani et al. concluded in their meta-analysis of pooled data of more than 3000 patients that laparoscopy seemed to be equivalent to laparotomy in the oncologic point of view. The risk of upstaging (OR 0.81; 95 % CI 0.55–1.20) or capsule rupture (OR 1.32; 95 % CI 0.52–3.38) were the same. Survival outcomes did not differ by the route of surgery. The interval from surgery to adjuvant chemotherapy was shorter with laparoscopy than laparotomy (weighted mean difference -5.16 days; 95 % CI -8.68 to -1.64). The blood loss during surgery was lower (weighted mean difference -156.5 mL; 95 % CI -216.4 to -96.5) and the hospital stay almost 4 days shorter (weighted mean difference -3.7 days; 95 % CI, -5.2 to -2.1). There were also fewer postoperative complications (OR 0.48; 95 % CI 0.29–0.81) (Bogani et al. 2017). Due to a lack of randomized prospective trials,

a Cochrane group did not yet estimate risks and benefits of laparoscopy in routine clinical practice (Falcetta et al. 2016).

Fertility sparing surgery (FSS) in the context of EOC means unilateral salpingo-oophorectomy and complete surgical staging (see Chapter 2.4.1). The uterus and contralateral healthy ovary are saved for reproduction. Since its purpose is to promote subsequent fertility, it is not encouraged in women whose fertility is impaired for other reasons, like older age. According to European guidelines from 2011, conservative surgery should only be considered in adequately staged patients with stage IA grade 1 (and probably 2) serous, mucinous or endometrioid tumors; careful follow-up is compulsory. FSS may probably be discussed with patients with stage IC grade 1 disease, too (Morice et al. 2011). According to a recent review of FSS, the recurrence rate after conservative surgery in stage IA grade 1 was 7% and 11% in stages IA grade 2 and IC grade 1/2 disease, similar to radical surgery (Bentivegna et al. 2016).

In apparent advanced cases of EOS, the aim of **primary debulking surgery** is optimal cytoreduction to no macroscopic residual. It might require careful preoperative evaluation with imaging and laparoscopy, a multi-professional dedicated surgical team and procedures in addition to staging laparotomy, e.g. surgical treatment of pelvis en bloc, extrapelvic bowel resections, diaphragmatic stripping and splenectomy (Hacker & Rao 2016). The chances for optimal cytoreduction decrease if there is extensive upper abdominal disease, involvement of the porta hepatis, small bowel mesentery and diaphragm, marked ascites or spread beyond the abdominal cavity (Jayson et al. 2014). A validated laparoscopic predictive model helps in evaluating the chances for optimal cytoreduction (Fagotti et al. 2008).

Interval debulking surgery is a widely accepted way of treating patients whose disease is primarily non-resectable. Here, surgery is performed during the chemotherapy course typically after three cycles (NACT). In selected populations, primary chemotherapy with interval debulking surgery may be non-inferior to upfront surgery with respect to survival outcomes and adverse events (Morrison et al. 2012; Suh et al. 2016).

2.4.2.2 Chemotherapy

The cornerstone of EOC treatment is platinum-based chemotherapy. In early stage EOC, **adjuvant chemotherapy** is usually recommended after staging surgery, if there are cancer cells in peritoneal washings (stage IC), pelvic tumor extension (stage II) or if histology shows aggressive histology (high grade, clear cell). If the cancer is confined to the ovary (stage 1A or 1B) and of low histological grade, prognosis

following surgery is excellent and no adjuvant therapy is recommended. In advanced and completely debulked cases, similar adjuvant treatment is advised. In suboptimally debulked advanced disease, bevacizumab is incorporated in the chemotherapeutic regimen (Chapter 2.4.2.3). According to several studies, the most favorable and non-toxic regimen is a combination of carboplatin and paclitaxel administered every three weeks for six times or more in advanced cases. Weekly "dose-dense" administration of either agents has led to significantly improved PFS and OS in the Japanese population. Median PFS was 28 months compared to 18 months for conventional treatment (HR 0.76, 95 % CI 0.62–0.91, p=0.0037), but the toxicity of the treatment was considerable. These results have not been validated in Westerns population, partly because the GOG 262 study protocol permitted administration of bevacizumab. Intraperitoneal administration of standard chemotherapy might also improve first-line treatment outcomes for patients with advanced disease, but is associated with considerable toxicity and discontinuation of the treatment. The optimal use of the dose-dense and intraperitoneal therapies have not been defined (Webber & Friedlander 2016).

Patients that are not good candidates for radical surgery, usually because of advanced age, poor performance status and medical comorbidities or because of the extent of the disease, are given **NACT** (Wright et al. 2016). They receive three cycles of standard chemotherapy and are then, if there is evidence of response, offered interval debulking surgery followed by another three courses of chemotherapy (Webber & Friedlander 2016).

2.4.2.3 Targeted therapies

Two large prospective randomized studies, GOG-0128 and ICON 7 trials have showed that **incorporating bevacizumab**, a humanized monoclonal antibody against VEGF, into the standard chemotherapy regimen for advanced EOC and continuing it after completion of chemotherapy leads to improved PFS. In GOG-0128, the median PFS was 10.3 months in the control group, 11.2 months in the concomitant-only bevacizumab arm and 14.1 months in the concomitant and maintenance (total duration of 15 months) bevacizumab arms (HR 0.72, p<0.001). The PFS benefit was maintained in all subgroups (FIGO stage, residual disease, histological subtype, tumor grade, age and performance status). According to ICON 7, patients with stage III disease and residual tumor > 1 cm after primary surgery or with stage IV regardless of the surgical result benefited the most from this treatment; their median PFS was 15.9 months with bevacizumab vs. 10.5 months with chemotherapy alone (HR 0.68, p<0.001). In the bevacizumab arm, the anti-

angiogenic treatment was continued for a total of 12 months, and the beneficial effect between two arms was greatest at 12 months. These results show that bevacizumab should be continued as **maintenance therapy** after completion of chemotherapy to delay disease progression (Colombo et al. 2016).

2.4.3 Impact of nodal metastasis on outcome

EOC has the highest mortality rate of all gynecologic cancers, mainly because of late presentation of most patients. Even if patients have complete response to first-line treatment, most of them with advanced stage disease will relapse within 18 months. The prognosis is strongly associated with FIGO stage, residual tumor burden after initial debulking surgery and performance status (Jayson et al. 2014). Additional prognostic factors are for example histologic type, advanced age and high-volume ascites (Davidson & Tropé 2014).

Lymph node involvement is an important prognostic indicator. It shifts an apparently local tumor to a higher stage. Accurate surgical staging in apparent early stage (I–II) EOC is associated with a better prognosis, as it provides information that guides further treatment (Zhou et al. 2016), on condition that at least 10 nodes are harvested from different and specific retroperitoneal sites (Trimbos 2011).

In the new FIGO 2013 staging, the difference between the impact of lymphatic and intraperitoneal spread on outcome has been acknowledged more accurately. While in the earlier FIGO 1988 staging, positive retroperitoneal LNs without any peritoneal involvement were included in stage IIIC, they are now classified as Stage IIIA1. Patients that present with regional LN metastasis alone have a better prognosis than patients presenting with extrapelvic peritoneal involvement but no regional LN metastasis, or with both peritoneal and LN metastases (Prat 2015; Bakkar et al. 2014). This difference has been reported in several studies. In a retrospective survival analysis of 878 patients, there were clear survival benefits in stages IIIA1 and 2 in comparison to stage IIIC (Paik et al. 2015). Hoon Suh et al. analyzed data of 870 patients with the old and new FIGO classifications. Before stage reassignment, the 5-year OS of patients with stage IIIC was 39 %. After reassignment, the OS of patients with regional LN metastasis alone was 66 % compared to 36 % of those remaining in stage IIIC ($p=0.0005$) (Hoon Suh et al. 2013). In a recent series of 218 patients, there were striking difference in the estimated 5-year OS rates between three groups of optimally debulked stage III patients: those with LN involvement alone had an OS rate of 92 % compared to 47 % of patients with peritoneal involvement alone and 45 % for patients with both

nodal and peritoneal involvement (p=0.005). The same groups had 5-year PFS rates of 64 %, 19 % and 18 %, respectively (p<0.001) (Gasimli et al. 2016).

The new FIGO staging divides stage IIIA1 into two groups according to the size of the LN metastasis (IIIA1(i) ≤ 10 mm or IIIA1(ii) > 10 mm in greatest dimension). However, no retrospective data exists to support the quantification of the size of the metastasis at this stage or its impact on the prognosis (Prat 2015). There was no difference in OS in a recent analysis either, and even while there was a difference in 3-year PFS (90 % vs. 63 %), it was not statistically significant (p=0.297) (Gasimli et al. 2016). Instead, the anatomic dissemination of the LN metastases seems to matter. Optimally debulked patients in stage IIIA1 with para-aortic LN metastases alone had the longest median OS of 69 months, while patients with pelvic and para-aortic or pelvic LN metastasis alone had median OSs of 47 and 46 months, respectively (p=0.09). The difference in median OS between the first and the second group of patients was significant (p=0.02). The median PFS was 28, 18 and 16 months, respectively (p=0.02) (Gasimli et al. 2016).

EOC often spreads simultaneously in the peritoneal cavity and retroperitoneum. Ovarian tumors spreading mainly through lymphatic channels without intraperitoneal dissemination might have a more favorable biological behavior than tumors spreading in the peritoneal cavity (Hoon Suh et al. 2013). Bachmann et al. showed that the impact of LN metastasis on prognosis decreased as the volume of residual tumor after debulking surgery increased. After optimal cytoreduction, the nodal status seemed to be the next most important prognostic factor for patients with advanced EOC (Bachmann et al. 2012).

2.4.3.1 Risk factors for nodal metastasis in early stage ovarian cancer

In apparent early stage EOC, the risk of metastatic LNs rises with increasing stage. Morice et al. reported in a series of 100 clinically early stage patients that 13 % of patients with stage IA, 33 % with stage IB and 38 % with stage IC had LN metastasis in the pelvic and para-aortic areas. In all, 20 % of patients with apparent stage I tumors and 40 % with stage II tumors had LN metastasis (Morice et al. 2003).

Tumor grade influences the risk of LN metastasis. Several studies have reported higher grade being associated with a higher risk of lymphatic spread even in early stage. Kleppe et al. concluded in a comprehensive review that the incidence of LN metastasis was lowest with grade 1 tumors (4 %), but rose considerably with the grade: to 17 % in grade 2 tumors and to 20 % in grade 3 tumors (Kleppe et al. 2011). Similar results were reported by Powless et al.: the incidence of lymphatic spread was

24% in grade 3 tumors compared to 2.2% in grade 1–2 tumors ($p<0.001$). The RR was 9.55 (95 % CI 1.79–177.72, $p=0.004$) in their series (Powless et al. 2011).

Tumor subtype is of importance. Mucinous and endometrioid early stage EOC have the lowest incidence of LN metastasis; 3 % and 7 %, respectively. Undifferentiated and serous EOC have the highest incidence of 29 % and 23 %, respectively, clear cell tumors having an intermediate incidence (14 %) (Kleppe et al. 2011).

Also, in a multivariate analysis by Powless et al., the laterality of the ovarian tumor (bilateral vs. unilateral) gave a RR of 3.19 (95 % CI 1.26–8.31, $p=0.015$) and the presence of ascites a RR of 3.09 (95 % CI 1.15–8.20, $p=0.023$) for LN metastasis (Powless et al. 2011).

Lymphovascular space invasion in malignant ovarian tumors, regardless of extent, predicts LN metastasis after controlling for tumor stage and high grade serous carcinoma (OR 5.74, 95 % CI 1.13–29.2, $p=0.035$). Thus, it is an independent predictor of lymphatic spread (Matsuo et al. 2012). In a larger study involving 434 EOC patients with stage I disease, it was associated with an increased risk of hematogenous and lymphatic metastasis by multivariate analysis (HR 4.79, 95 % CI 1.75–13.2, $p=0.002$), although PFS and OS were not significantly affected (Matsuo et al. 2014).

2.4.4 Sentinel lymph node method in ovarian cancer

Ovarian cancer has not been a popular subject in the SLN research field. The target population extends to clinically local EOC only – a small proportion of all EOC cases. The injection of the tracers to or next to the ovarian mass has long been considered difficult and risky in relation to unwanted tumor dissemination. On the other hand, most patients with apparent early stage EOC will not benefit from systematic staging lymphadenectomies (El-Ghobashy & Saidi 2009). When surgically staged, the proportion of EOC patients with LN involvement has varied between 14 and 20 %. Half (45–50 %) of the LN metastasis are found in the para-aortic area, 20–25 % in the pelvic area and 29–30 % in both areas (Angioli et al. 2008; Kleppe et al. 2011). However, compared with lymph node sampling, it takes 90 minutes longer to complete a systematic LND ($p<0.0001$), the median volume of blood loss is 300 mL more in latter case ($p<0.0001$), the median hospital stay is 1 day longer ($p=0.003$) and 36 % of patients receive transfusion compared to 22 % with LN sampling only ($p=0.012$) (Maggioni et al. 2006). Thus, a less radical way of accurate LN staging in EOC would be valuable.

Before 2011, only two reports of experimental SLN mapping in relation to the ovary were published. The first one in 1991 included patients undergoing laparoscopy for benign causes and the lymphatic drainage and SLNs were identified with postoperative LSG. According to the second study from 2004, activated charcoal solution (CH40) was used to map ovarian lymphatics, a tracer that has not been applied in Europe or U.S. for this purpose (Vanneuville et al. 1991; Negishi et al. 2004). In 2011, we published a pilot study (III) about retroperitoneal ovarian SLN mapping with conventional blue dye and radioisotope. Since then, other groups have also taken interest in the subject (Chapter 6.4).

2.4.4.1 Controversies of the sentinel lymph node method

One big question in the of SLN concept with regard to EOC is the suitability of the currently used tracers for intraoperative use. It is nearly impossible to carry out the tracer injection safely and precisely before surgery. As described in Chapter 2.2.4.1, vital blue dyes are swiftly taken up to the lymphatics and transported to the SLN, which makes them good candidates for experiments in intra-abdominal surgery. However, they also tend to leak upstream into second echelon LNs, and when used alone, they are often not accurate enough to allow SLNs to replace systematic LND.

Radioisotopes on the other hand perform much better both alone and in combination with blue dyes, but they traditionally are injected hours before the actual operation, even one day before. Yet when nanocolloids are used, it is not necessary to allow the migration of the tracer to the SLNs for several hours after the injection. In breast cancer surgery, it was feasible to inject radiocolloid intraoperatively after induction of anesthesia without compromising the accuracy of SLN method, which saved also the patient from the discomfort of the injection (Dauphine et al. 2006). It could be more daunting that imaging with LSG or SPECT/CT would not be available and the surgeon would have to depend only on a handheld gamma detector if the radioisotope is injected intraoperatively.

The question of oncological safety of injection near the malignant ovarian mass needs also to be answered. Although accidental surgical spill is not considered as deleterious as capsule rupture before surgery since it does not seem to increase the risk of disease recurrence, it should be avoided to reduce the need for adjuvant chemotherapy (H. S. Kim et al. 2013). Even more disturbing is the thought of possibly disseminating malignant cells into the lymphatics or circulation by intratumoral tracer injection (El-Ghobashy & Saidi 2009).

2.5 Sentinel lymph node method in other gynecologic cancers

2.5.1 Endometrial cancer

In Western countries, endometrial cancer (EC) is the most common gynecologic malignancy (Mariani et al. 2008). According to the Finnish Cancer Registry, approximately 830 new cancer cases of uterine corpus were diagnosed per year in 2010–2014, 71 % of them early stage, excluding unreported stages (Malila et al. 2017).

The role of routine lymphadenectomy in EC and its benefit with respect to survival has been debated, although surgical staging is recommended by FIGO. Two large randomized phase 3 trials have failed to demonstrate survival benefit from systematic pelvic LND in early-stage EC. Panici et al. concluded that although LND significantly improved surgical staging, it had no impact on disease-free or overall survival compared to no LND. Morbidity was significantly higher in the LND than in no-LND arm, mostly due to lymphocysts and lymphedema (43 % vs. 12 %, respectively, $p=0.001$). The value of LND was considered to reside in determining prognosis and tailoring the adjuvant treatment (Panici et al. 2008). Similarly, the ASTEC trial did not show any difference for systematic LND in terms of OS, disease-specific and recurrence-free survival compared to no LND. Even when the risk of major surgical complication was generally low, the side effects of surgery were significantly more frequent in the LND arm than in the no-LND arm ($p<0.0001$); ileus was present in 3 % vs. 1 %, deep vein thrombosis in 1 % vs. 0.1 %, lymphocysts in 1 % vs. 0.3 % and major wound dehiscence in 1 % vs. 0.3 %, respectively (Barton et al. 2009).

When considered to be of "low risk" by Mayo Clinic criteria (grade 1 or 2 disease, < 50 % myometrial invasion, and tumor diameter < 2 cm), the risk of nodal metastasis or recurrence is less than 1 % compared to a 16 % risk in patients not meeting these criteria, and to a 40 % risk in patients with non-endometrioid histology. In this low-risk group, routine LND is therefore not beneficial. However, in case of higher risk, systematic pelvic and para-aortic LND up to the renal veins seemed necessary (Mariani et al. 2008). SEER analysis have since confirmed these results (Vargas et al. 2014). When considering the rise of mini-invasive surgical techniques, the higher surgical morbidity caused by systematic LND and its negligible effect on survival, it is no wonder that less radical ways of performing surgical staging are warranted.

Reports of the experimental use of the SLN technique in EC have been published since 1996. The first reports were usually of small, single institution patient series concentrating initially on experiments with different tracers and injection sites. The combination of blue dye and radioisotope has performed best in early studies, as in many other cancer types (Kang et al. 2011), although ICG seems to be the choice for the future (Sinno et al. 2014; Buda et al. 2016). Locating deeply inside the pelvis, the body of uterus is not easily reachable for tracer injection. Subserosal, cervical and hysteroscopically guided sub-endometrial injection sites have been suggested. The first route is associated with decreased sensitivity and is not always technically feasible due to the distortion of the fundus by myomas or tumor, even in laparotomy. Hysteroscopic injections have not led to higher DRs, and are also technically demanding. They require a separate procedure usually during the day preceding the operation, which creates a logistical challenge. The cervix, on the other hand, is almost always accessible for the tracer injection. This route has yielded improved pelvic DRs compared to the other injection sites, although mapping of the aortic area remains modest. It is currently the most favored injection route in EC (Khoury-Collado et al. 2016; Kang et al. 2011; Holloway et al. 2017).

There is no formal evidence to identify the best method for pathological evaluation of SLNs in EC. An initial evaluation by routine H&E staining is advised and, if negative, ultrastaging with serial sectioning and IHC with cytokeratin AE1/AE3. Although the full meaning of low-volume disease in EC is unclear, it may have relevance for determining suitable adjuvant treatment and a follow-up plan (C. H. Kim et al. 2013)

In 2012, a surgical algorithm for SLN mapping in EC was suggested (Figure 3). Barlin et al. found that applying this algorithm to SLN mapping with blue dye reduced the FNR rate from 15 % to 2 % (Barlin et al. 2012). Since then, other groups have reported a significant drop in FNR rate, an increase in NPV and high LN metastasis DR in combination with low number of removed LNs (Vidal et al. 2013; Ehrisman et al. 2016; Hagen et al. 2016). The use of SLN mapping with staging LND has significantly increased the DR of LN metastasis compared to staging LND alone (adjusted OR 3.29, $p<0.0001$) (Holloway et al. 2016).

Several reports support the concept that the oncologic results of SLN mapping in early EC are comparable to traditional surgical staging, even in high-risk groups (Holloway et al. 2017). How et al. even reported improved pelvic sidewall recurrence-free survival during a follow-up period of 48 months of patients who underwent SLN biopsy compared to those who underwent systematic LND (HR 0.32, 95 % CI 0.14–0.74, $p=0.007$) (How et al. 2017). Recent results of the FIRES trial showed that

the sensitivity of SLN mapping with cervical ICG injection for finding LN positive EC was 97.2 % with a NPV of 99.6 %. This level of accuracy seems safe enough to replace complete LND (pelvic or pelvic and para-aortic) in the treatment of clinically early EC (Rossi et al. 2017).

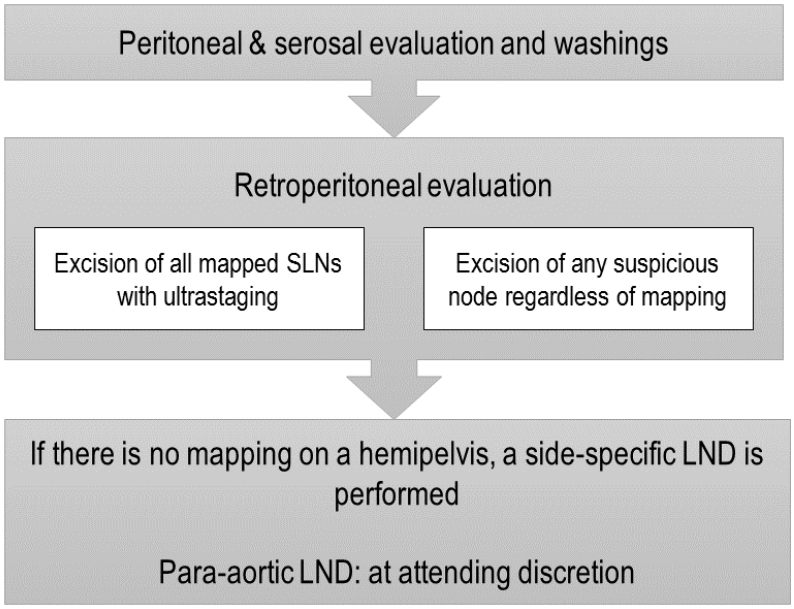

Figure 3. NCCN surgical algorithm for SLN mapping in early endometrial cancer. Decisions about completion para-aortic dissection should be at the attending surgeon's discretion based on individualized patient characteristics and tumor-based risk criteria (depth of invasion, histology and pelvic node status). Reprinted from Gynecologic Oncology, Vol. 125, Barlin et al., "The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: Beyond removal of blue nodes", pp. 531–535, Copyright (2012), with permission from Elsevier.

In summary, the Society of Gynecologic Oncology (U.S.) has recommended this year that SLN mapping can be performed instead of routine pelvic LND for patients with apparent uterine-confined grade 1 and 2 endometrioid cancers. In patients with high-grade disease, completion LND with para-aortic assessment is reasonable until more data regarding the safety and efficacy of SLN biopsies alone become available (Holloway et al. 2017). The European Consensus Conference on endometrial cancer

in 2014 considered SLN mapping an experimental procedure and did not yet recommend it for a routine use at that time (Colombo et al. 2015).

2.5.2 Cervical cancer

Cervical cancer staging is still clinical, not surgical as in vulvar, ovarian and endometrial cancer (Pecorelli et al. 2009). Although it is more inaccurate when prognosis is considered, most new cases occur in less developed countries where the resources for surgical staging are insufficient. Currently, cervical cancer is the fourth most common female cancer worldwide with over half a million new cases per year. In 2012, 87 % of these cases occurred in the third world (Zigras et al. 2017). In Finland, cervical cancer is not very common with approximately 160 new cases per year in 2010–2014, but the incidence peaks among women in the fertile period of life (Figure 4). This cancer is usually diagnosed at an early stage, which allows surgical treatment and staging, in contrast to the situation in low-resource settings (Malila et al. 2017; Pecorelli et al. 2009).

LN involvement in cervical cancer is a known indicator of poor prognosis, especially if present in the early stage of the disease (Pecorelli et al. 2009). The information of LN metastasis is important for identifying patients who need adjuvant CRT. In an analysis by Cibula et al., FIGO stage predicted the prevalence of LN involvement: macrometastasis was present in 9.1% and micrometastasis in 3.6 % in patients of FIGO stage IA. In stage IB1, the rate of metastasis was 19.5 % and 6.2 % and in stage IB2 24.1 % and 12.1%, respectively ($p<0.001$). Ultrastaging was used in this evaluation. The presence of micrometastasis as well as macrometastasis was associated with significantly reduced OS (HR 6.86, 95 % CI 2.09–22.61, $p=0.002$ and 6.85, 95 % CI 2.59–18.05, $p<0.001$, respectively), and the predictive value of LN involvement was even greater than that of FIGO staging. The presence of ITC did not affect survival (Cibula et al. 2012). Since the size of micrometastasis and small macrometastasis is below the spatial resolution of PET, pretreatment imaging is not accurate enough in early stage disease to stratify patients into different prognostic categories. The SLN concept in cervical cancer offers a way of selecting LNs at greatest risk of metastatic involvement for ultrastaging but possibly also of reducing the risk of disturbing surgical side effects, e.g. lower leg lymphedema in this relatively young population (Bats et al. 2013; Zigras et al. 2017). Frozen sectioning does not seem to be of use in conjunction with SLN in cervical cancer. In a meta-analysis, the pooled sensitivity of frozen section analysis was only 60 %; this low figure is mainly

due to its inability to detect micrometastasis and small macrometastasis (Kadkhodayan et al. 2015).

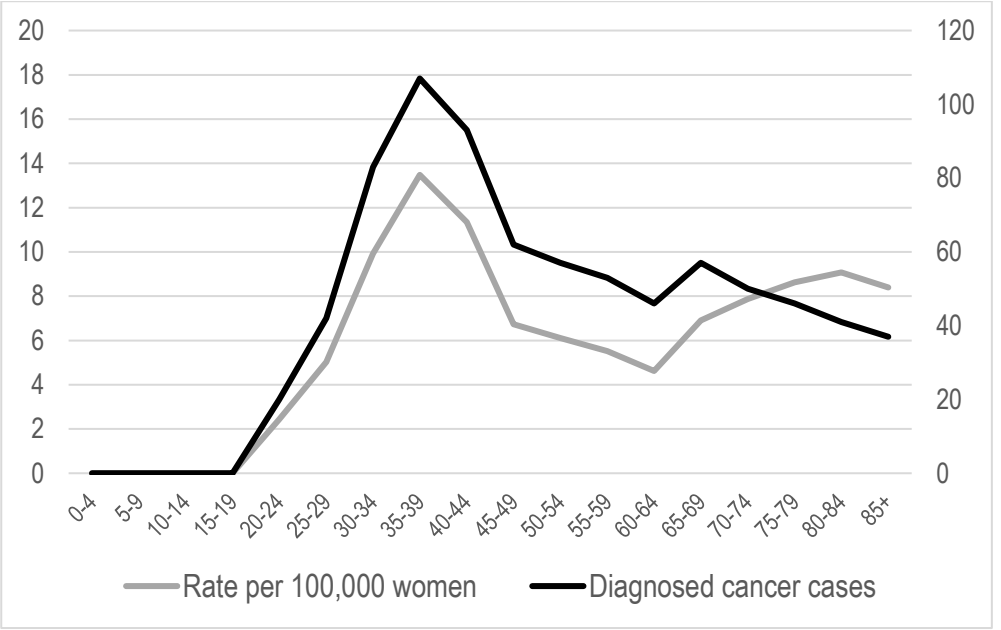

Figure 4. Incidence of cervical cancer and number of new cases in Finland during 2010–2014. Finnish Cancer Registry, interactive cancer statistics (Malila et al. 2017).

One valuable advantage of SLN mapping in cervical cancer is that it helps to locate possibly metastatic LNs outside the conventional LND fields ((i.e., external iliac, obturator, internal iliac or interiliac) (Kadkhodayan et al. 2015). The most common unexpected locations are the common iliac chain (6.6 %), parametria (4.3 %), lower para-aortic area (2 %), sacral chain (1.3 %) and inguinal chain (0.07 %) (Ouldamer et al. 2012). In the multicenter SENTICOL study, 19 % of intraoperatively detected SLNs were located in unexpected regions. 38 % of patients had at least one SLN in an unexpected region that was detected intraoperatively. 5 % of patients had SLNs only in unexpected locations (Bats et al. 2013).

According to an extensive meta-analysis, the pooled DR of SLN mapping in cervical cancer was 89 % and sensitivity 90 %. Performance was lowest if only blue dye was used as a tracer (DR 81 %, 95 % CI 75.7–85.2 % and sensitivity 86 %, 95 % CI 79.9–91.2 %). In relation to the injection and the injection technique, the success in SLN mapping was associated with a bigger volume or dilution of the blue dye and superficial cervical injections. With radiocolloid, a one-day protocol seemed more successful than a two-day protocol, although the difference was so little that it

probably lacks clinical relevance. In this meta-analysis, the pooled DR when using ICG was fairly low (77 %, 95 % CI 60.7–87.2 %) compared to traditional tracers (Kadkhodayan et al. 2015). Since this report was published, several new studies addressing this subject have been published, and equal or even better results have been reported with ICG (Diab 2017). Mini-invasive surgical techniques, such as traditional and robot-assisted laparoscopy, do not differ from open surgery in detecting the SLNs (Kadkhodayan et al. 2015).

The use of SPECT/CT in the SLN protocol helps the surgeon to locate SLNs more efficiently during surgery than does planar LSG, since it offers better anatomical localization. SPECT/CT reportedly decreases the retrieval time of SLNs during robot-assisted surgery for cervical cancer (Kadkhodayan et al. 2015).

In the aforementioned meta-analysis of Kadkhodayan et al., the DR in patients with a history of NACT was lower than the DR for the whole population, 74% (95 % CI 65.8–80.4), but sensitivity was comparable 95 % (95 % CI 74–99.9 %). Preoperative conization had no effect on DR (Kadkhodayan et al. 2015).

Since the uterine cervix is a midline structure, one would expect to find bilateral SLNs, which is considered optimal mapping. However, the bilateral DR varies and seems to be higher in younger patients and in those with small tumors (Kadkhodayan et al. 2015). Cormier et al. have suggested incorporating a surgical algorithm into the SLN mapping protocol for early cervical cancer, resembling the one for endometrial cancer (Chapter 2.5.1). The algorithm is presented in Figure 5. By applying the algorithm to the treatment of patients with FIGO stage IA1 + LVI to IIA disease, they identified all patients with metastatic LNs (sensitivity 100 % and FNR 0 %), thus potentially eradicating the need for complete bilateral LND in 75 % of patients (Cormier et al. 2011).

In conclusion, the SLN concept in cervical cancer is very sensitive for detecting LN metastasis, on condition that the mapping is bilateral and ultrastaging of the SLNs is used. The reports favoring abandoning systematic LND and replacing it with the SLN algorithm appear promising. One of the largest retrospective series to date demonstrated no difference in recurrence-free survival between patients with negative bilateral SLN biopsy alone vs. negative pelvic LND (Lennox & Covens 2017). The results from ongoing large prospective controlled trials hopefully establish the value of the SLN approach in early cervical cancer (Cibula et al. 2015).

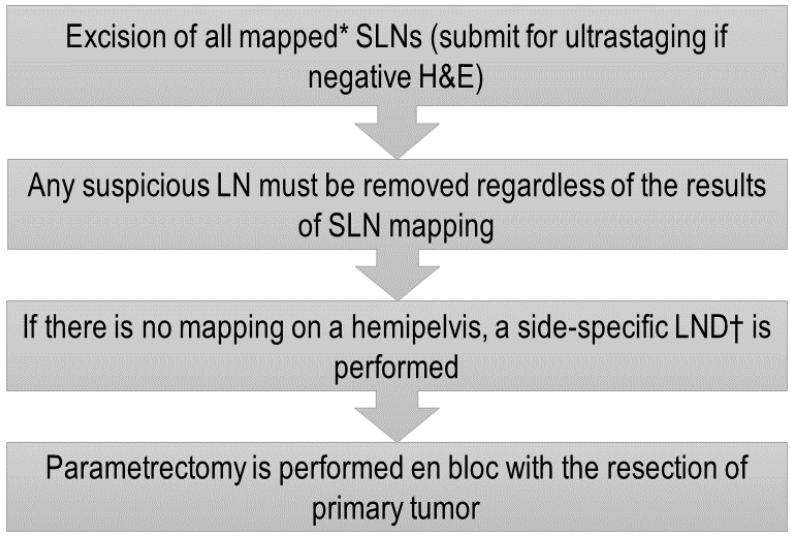

Figure 5. Surgical algorithm for SLN mapping in early cervical cancer. *Intracervical injection with blue dye, ^{99m}Tc-radiocolloid or both, †including interiliac/sub-aortic nodes. Reprinted from Gynecologic Oncology, Vol 122, Cormier et al., "Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer", pp. 275–280, Copyright (2011), with permission from Elsevier.

3 AIMS OF THE STUDY

This study was undertaken to assess the use of the sentinel lymph node method in vulvar and ovarian cancer. The specific aims of the sections were:

1. To evaluate the detection rate of sentinel lymph nodes and the reliability of the sentinel lymph node concept in different surgical stages of vulvar cancer (I).
2. To evaluate the expression of vascular endothelial growth factor C in malignant vulvar tumors and sentinel node metastases, the role of vascular endothelial growth factor C for lymphatic spread of vulvar cancer to the sentinel lymph nodes and beyond, and for the prognosis of the patients (II).
3. To test the feasibility of perioperative tracer injections (blue dye and radioisotope) to one ovary for locating sentinel lymph nodes during laparotomy, and to describe the differences – if any – in locations of blue and hot lymph nodes after injections to right and left ovary (III).
4. To examine the detection rate and locations of sentinel lymph nodes by blue dye and radioisotope in patients with possibly malignant ovarian tumors, and if possible, to evaluate the reliability of the method for detecting lymph node metastases (IV).

4 PATIENTS, MATERIALS AND METHODS

4.1 Patients and study design (I–IV)

4.1.1 Inclusion and exclusion criteria

For studies I and II, the study population was retrospectively collected among patients that presented with vulvar cancer at Tampere University Hospital from January 1st 2001 through June 30th 2005, and underwent vulvar surgery with mapping of SLNs. SLN mapping before IF LND was started in 2000, and in order to familiarize with the SLN method, it was performed for almost all patients that underwent groin surgery in our hospital. The reasons not to perform SLN mapping at the beginning of the operation included: 1) RT before surgery 2) no surgery at all 3) severe debilitation 4) primary vulvar procedure performed in some other hospital or at our hospital but before January 1st 2001 5) unknown origin of malignant disease in vulva. 47 patients who underwent the SLN mapping were identified from hospital records (I).

For study II, the availability of archived paraffin-embedded vulvar and lymphatic tissue specimens was crosschecked for all 47 women included in study I. The specimens were obtained from the Tissue Biobank and Research Services FinTiB, Fimlab Laboratories Inc., Tampere, Finland. Forty-six tumor samples from 44 patients with representative malignant growth in the specimen and 17 metastatic SLN samples were available for analysis. Three patients were excluded from the study due to the lack of suitable samples.

For study III, 16 patients with histologically proven high-risk EC (endometrioid adenocarcinoma grade 3, uterine papillary serous carcinoma, clear cell carcinoma or other risk factor(s) identified during preoperative workup) and normal-looking postmenopausal ovaries with no signs of extrauterine spread in preoperative imaging were recruited. All patients were scheduled for open surgery.

Twenty patients scheduled for open surgery for suspicious unilateral or bilateral ovarian masses were enrolled in study IV. The inclusion criteria were: 1) planned laparotomy to remove suspicious mass(es) and uterus, if intact 2) no signs of dissemination beyond the pelvis in preoperative imaging and 3) for premenopausal

women with the uterus was *in situ*, a negative pregnancy test within 24 hours before surgery. The exclusion criteria were: 1) a history of allergic reaction to blue dye or human albumin and 2) a suspicion of malignant spread to the abdominal cavity. Elevated serum CA-125 and/or ascites alone without any other signs of malignant spread were not exclusive.

4.1.2 Surgical procedures and lymphatic mapping

For study I, all patients had undergone radical vulvar surgery and unilateral or bilateral IF LND and in conjunction with the latter, SLN biopsy. Before groin surgery, ^{99m}Tc-labeled human albumin colloid (Nanocoll®; GE Healthcare, Saluggia, Italy) was injected at the Department of Nuclear Medicine, Tampere University Hospital, around the tumor site of 40 patients early in the morning preceding surgery or on the previous day. At the beginning of the operation, blue dye (Bleu Patenté V®; Guerbet, Paris, France) was injected peritumorally to the vulva of each of the 47 patients. During the groin surgery, all blue nodes or non-colored nodes following a blue lymphatic channel were considered to be a SLN. The radioactivity of the lymph nodes was checked with a handheld gamma probe. No preoperative lymphoscintigrams were taken.

In study III, at the beginning of each laparotomy, Nanocoll® and Bleu Patenté V® were injected near the hilum of the ovary, right and left alternately. The injection site depended on the order of entering the study: in patients with odd numbers, the injections were performed to the right ovary (8 patients), and in patients with even numbers, to the left ovary (8 patients). During a minimum of 10 minutes after tracer injections, the abdominal cavity was examined and the drainage of the blue dye noted, and then the TAH and BSO started. Omentectomy was also performed, if necessary according by endometrial histology. During pelvic and para-aortic lymphadenectomies, the blue nodes were located by inspection and hot lymph nodes with a handheld gamma probe. A count rate of at least 100 times background radiation was considered to signify a "hot" SLN. Each location was marked onto a map.

In study IV, at the beginning of the laparotomy after exposure of the adnexal mass(es), 1 mL of Nanocoll® was first slowly injected into one spot under the serosa next to the lateral junction of the ovarian tumor (mesovarium); a 27-gauge needle was used. To prevent tracer leakage from multiple needle holes and staining of the operation field, the needle was kept in place, the syringe changed, and 2 mL of Bleu Patenté V® was injected into the same spot. In the case of bilateral masses, the tracers

were injected in both sides. After injection, the abdominal cavity was examined for malignant disease after a minimum waiting period of 10 minutes, the cytological samples taken and the passage of the blue dye noted. Then the adnexal tumor(s) was/were removed, examined and a representative tissue sample was sent for frozen section analysis. After that, the uterus and the remaining normal adnex were removed, if intact. If the frozen sectioning proved to be malignant, pelvic and para-aortic LNDs were performed and at the same time, all blue and hot nodes (defined as above) were collected separately for histopathological examination and their number and locations marked onto a map. If the tumor proved to be benign or borderline and LND was not needed, the regional lymph node basins in the pelvis and para-aortic areas were examined transperitoneally for blue color and radioactivity, and the blue and hot spots marked onto a map.

4.1.3 Immunohistochemical analysis

VEGF-C protein expression of vulvar tumor and SLN samples were evaluated with IHC in study II. Representative samples from the invasive edges of the primary tumors and SLN metastases were selected for the study by one experienced pathologist. Four μm thick sections were cut from paraffin-embedded tissue blocks using a standard microtome. For IHC staining, the slides were then deparaffinized, rehydrated and subsequently pretreated with a PT-Module (Lab Vision, Fremont, CA, U.S.) at 98°C for 15 min in 0.05 M TrisHCl buffer, pH 9.0 containing 0.001 M EDTA. The primary VEGF-C antibody (Rabbit anti-VEGF-C; Invitrogen, Camarillo, CA, U.S.) was visualized with a PowerVision+ polymer kit (Leica Biosystems Newcastle Ltd., Newcastle, UK) and diaminobenzidine as chromogen (DABImmPact, Vectorlabs, Burlingame, CA, U.S.). The tissue sections were counterstained with hematoxylin (Mayer's hematoxylin, Oy FFChemicals Ab, Haukipudas, Finland). Human colon carcinoma samples, known to have a strong VEGF-C expression, were used as positive controls. Negative controls were made by omitting the primary VEGF-C antibody from the procedure.

Immunostained sections were scanned with an Aperio Scanscope XT (Aperio Technologies, Vista, CA, U.S.) and subjected to visual analysis on a computer screen. Two observers, blinded to the clinicopathological information of the patients, assessed the scans. The assessments were first performed independently and then pooled. If the assessments of two observers were discordant, the scans were reassessed and consensus was reached. The IHC staining intensity was scored semiquantitatively as negative (no staining at all), weak (some scattered stained cells

or faint more widespread staining), moderate (more abundant widespread staining or focal intensive staining) or strong (almost all cells intensively stained). For statistical analysis, negative and weak staining were combined as "negative" and moderate and strong staining as "positive" staining.

4.2 Methods

For study I, the following data was collected for analysis from the patient charts: age at the time of the surgery, whether it was possible to identify a SLN, technique for identification of the SLN (radioisotope, dye or both), site of the primary tumor, type of surgery, surgical stage of the disease (FIGO 1988), histopathology, status of the SLN (positive or negative for malignant cells) and of other regional LNs and the presence or absence of other metastases.

This data was used also for study II, although, as described in 9.1.1, three patients were excluded due to the lack of suitable specimens. Follow-up data was also collected from the hospital charts. It included the date of surgery, data on adjuvant treatment and its duration, if any, duration of the follow-up, date of the observation and location(s) of recurrence(s), if any, final date of follow-up and the date and cause of death, if it took place during the follow-up period. The results of the VEGF-C expression analysis of vulvar tumors and SLNs were combined with the clinical data for risk and survival analysis.

For study III, the following data was recorded: time from the injection to the beginning of the surgical procedure and to detection of the first sentinel lymph node, technique for SLN identification, count rate of a hot SLN, calculated net radioactivity of the Nanocoll® injection and any intraoperative complications. The following demographic data was collected from the records: age at the time of surgery, BMI, previous abdominal procedures, postoperative complications, final histopathological results and stage of the uterine cancer.

For study IV, the following data was recorded: exact locations and method(s) of detecting the SLNs, time of injections, start of removal of the tumor(s) and of identification of the SLNs, and intraoperative complications, together with the demographic data including age, BMI, previous abdominal surgery, postoperative complications, and stage and results of the final pathological examination including the metastatic status of harvested SLNs and regional LNs.

For studies III and IV, a map of the essential regions of lymphatic drainage was drawn, and it was used to mark the exact locations of the detected SLNs in both studies. The regions were: upper para-aortic, lower para-aortic, upper pelvic (common iliac) and lower pelvic (external iliac and obturator) (Figure 6).

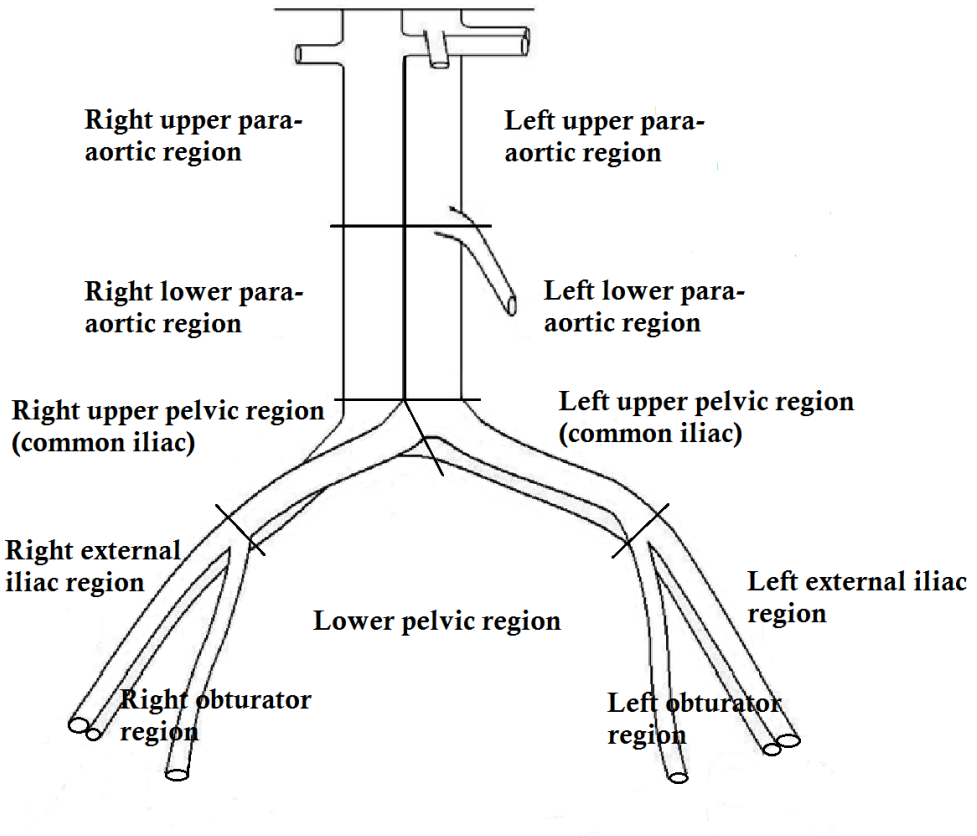

Figure 6. Map of essential regions of ovarian lymphatic drainage.

4.3 Statistical analysis

For study I, the SLN DRs for all used mapping techniques (dye, radioisotope and both), FIGO stages and primary tumor sites were calculated per patient by dividing the number of successfully mapped cases with the total number of patients. The false-negative rate and negative predictive value were calculated for all procedures and FIGO stages I–II and III–IV, separately (calculation, see Chapter 2.2.3).

For study II, the concordance between the primary assessments by two observers of the VEGF-C expression was evaluated with Cohen's unweighted kappa test. Associations between VEGF-C staining and clinicopathological parameters were analyzed using Fisher's exact test, odds ratio and relative risk. Disease-specific and progression-free survival curves were calculated using Kaplan-Meier's survival analysis and compared with the log rank test.

For study III and IV, the overall SLN DR was calculated per patient. The 2-sided Fisher's exact test was used to compare the differences between the locations of the right and left ovarian SLNs. For study IV, Pearson's correlation coefficient was used to estimate the association between tumor size and SLN numbers. The effect of BMI on visibility of the blue dye was analyzed using odds ratio.

In studies II, III and IV, a p-value less than 0.05 was considered statistically significant. SPSS Statistics for Windows (version 19.0 released 2010, IBM Corp., Armonk, NY, USA) was used for the statistical calculations.

4.4 Ethical considerations

The patients in study I underwent SLN mapping during operative treatment of vulvar cancer during a familiarizing period (2001–2005) of the SLN method. Training of the SLN method had begun one year earlier in our hospital, but to exclude a learning curve effect, the data of the first year was excluded from the analysis. Informed consent from the patients for SLN mapping was not obtained in study I. Written informed consent was obtained from all patients participating in the studies on mapping of the SLNs of ovary or ovarian tumors (III, IV).

For study II, the use of archived tissue specimens for IHC was approved by the National Supervisory Authority for Welfare and Health (Valvira). Retrospective collection of patient data from the hospital records for studies I and II and the protocols for studies III and IV were approved by the Ethics Committee of the Pirkanmaa Hospital District.

5 SUMMARY OF RESULTS

Study population, patient demographics and FIGO stages are shown in Table 8.

Table 8. Study population, patient demographics and FIGO stages. Age (years) and BMI (kg/m^2) are presented as medians (range). In Study IV, stages of borderline tumors are marked with *. †FIGO stage not given to metastatic tumor. BOT = borderline ovarian tumor, N.A. = not available

	Study I	Study II	Study III	Study IV
Number of patients	47	44	16	20
Study population	Vulvar cancer	Vulvar cancer	High-risk endometrial cancer	Ovarian tumors: benign 11 (55 %) BOT 4 (20 %) malignant 4 (20 %) metastatic 1 (5 %)†
Age	76 (43-93)	76 (44-93)	69 (58-77)	63 (41-81)
BMI	N.A.	N.A.	27 (20-42)	26 (22-36)
FIGO stage (1988)				
I	11 (23 %)			
II	14 (30 %)			
III	21 (45 %)			
IV	1 (2 %)			
FIGO stage (2009)				
I		19 (43 %)	11 (69 %)	
II		4 (9 %)	0 (0 %)	
III		20 (46 %)	5 (31 %)	
IV		1 (2 %)	0 (0 %)	
FIGO stage (2014)				
I				4*+1 (20 %*+5 %)
II				1 (5 %)
III				2 (10 %)
IV				0 (0 %)

5.1 Sentinel lymph node method in vulvar cancer (I)

5.1.1 Sentinel lymph node detection rates

SLNs were detected during groin surgery in 46 patients, giving an overall DR of 98 % (46/47). In 40 of 47 patients, both tracers were used for SLN mapping, and in 36 patients (90 %), the SLN was located with both. In this population, 3 additional SLNs were located by inspection only (7.5 %, 3/40) and one by gamma detector only (2.5%, 1/40), giving a success rate of 100 % for the combined method. In all three cases in which the detection of radiation failed, the reason was gamma probe malfunction. In 7 patients, only blue dye was injected at the beginning of the surgery, and the SLN was not visually identifiable in only one of them, giving a success rate of 85 % for the use of blue dye. The exact DRs as related to the FIGO stage and the technique are presented in Table 9.

Table 9. Detection rates by FIGO staging and identification method. *Handheld gamma probe failed to operate. Reprinted from Acta Obstetricia Gynecologica Scandinavica, Vol. 86, Nyberg et al., "Sentinel node and vulvar cancer: a series of 47 patients", pp. 615–619, Copyright (2007), with permission from John Wiley and Sons.

FIGO stage 1988	Success of dye technique, number (percentage)		Success of isotope technique, number (percentage)		Success of combined technique, number (percentage)		Total success rates
	Yes	No	Yes	No	Yes	No	
I	11/11 (100 %)	0/11 (0 %)	10/10 (100 %)	0/10 (0 %)	10/10 (100 %)	0/10 (0 %)	11/11 (100 %)
II	13/14 (93 %)	1/14 (7 %)	9/10 (90 %)	1*/10 (10 %)	10/10 (100 %)	0/10 (0 %)	14/14 (100 %)
III	21/21 (100 %)	0/21 (0 %)	18/20 (90 %)	2*/20 (10 %)	20/20 (100 %)	0/20 (0 %)	21/21 (100 %)
IV	0/1 (0 %)	1/1 (100 %)	0/0 (0 %)	0/0 (0 %)	0/0 (0 %)	0/0 (0 %)	0/1 (0 %)
Total	45/47 (96 %)	2/47 (4 %)	37/40 (93 %)	3*/40 (7 %)	40/40 (100 %)	0/40 (100 %)	46/47 (98 %)

The only patient, in whom SLN detection did not succeed with either technique, had a FIGO stage IV lateral tumor and she had nodal metastasis in both the groin

and the pelvis. The overall DR of SLNs for FIGO stages I–II was 100 % (25/25) and for FIGO Stages III–IV 95 % (21/22).

5.1.2 Reliability of the sentinel lymph node method

In 18 patients (39 %, 18/46), metastatic SLNs were found, and five of them (28 %, 5/18) had other regional lymph node metastases. Among 28 patients with negative SLNs, one (4 %, 1/28) had regional lymph node metastasis, i.e., a false negative. The status of SLNs compared to that of regional lymph nodes by different FIGO Stages is presented in Table 10.

The FNR of the SLN method for all FIGO stages together was 5.3 %. For stages I–II, it was 0 % and for stages III–IV it was 6.7 %. The NPV of a disease-free SLN for FIGO stages I–II was 1.00, while for FIGO stages III–IV it was 0.86.

Table 10. Sentinel and regional lymph node status by FIGO staging. *False-negative SLN. Reprinted from Acta Obstetricia Gynecologica Scandinavica, Vol. 86, Nyberg et al., "Sentinel node and vulvar cancer: a series of 47 patients", pp. 615-619, Copyright (2007), with permission from John Wiley and Sons.

FIGO stage 1988	Patients with SLN –		Patients with SLN +		Total
	Regional nodes –	Regional nodes +	Regional nodes –	Regional nodes +	
I	11	0	0	0	11
II	10	0	3	1	14
III	6	1*	10	4	21
IV	-	-	-	-	SLN not identifiable
Total	27	1*	13	5	46

5.2 VEGF-C in predicting lymph node metastasis and clinical course of vulvar cancer (II)

5.2.1 Patient data and follow-up

The median follow-up time was 39 months (range 0.6-109 months). The stage of the disease of all patients was revised to comply with FIGO 2009, see Table 8. During the assessment of the IHC, one previously missed SLN metastasis was found and

the patient was restaged accordingly. Tumor characteristics and data on the treatment and follow-up are presented in Table 11.

Table 11. Tumor characteristics, treatment and follow-up.

Variables		Number of patients
Histology and grade	Squamous cell carcinoma	43 (98 %)
	grade 1	22 (50 %)
	grade 2	14 (32 %)
	grade 3	7 (16 %)
	Anaplastic carcinoma	1 (2 %)
	grade 3	1 (2 %)
SLN metastasis at time of the surgery	No	23 (52 %)
	Yes	20 (46 %)
	SLN not detected	1 (2 %)
Postoperative adjuvant therapy	No adjuvant therapy	22 (50 %)
	Radiation therapy	21 (48 %)
	Concurrent chemoradiation	1 (2 %)
Alive at end of follow-up	Yes	22 (50 %)
	No	22 (50 %)
	Died of vulvar cancer or related cause	15 (34 %)
	Died of other cause	7 (16 %)

During the follow-up, one patient died of cancer less than three weeks after surgery and two patients during adjuvant radiotherapy before completion of the treatment (7 %, 3/44). Thirteen patients (30 %) had a recurrence after the completion of the treatment, 7 in the vulva and 6 outside the vulva. Three out of 7 patients (43 %) with vulvar recurrence were salvaged by reoperation and were alive at the end of the follow-up, while all six patients with a recurrence outside vulva died. At the end of follow-up, half of the patients were alive.

5.2.2 Immunohistochemistry and interobserver agreement

The interobserver agreement regarding VEGF-C expression in tumor tissue and SLN samples was substantial; Cohen's unweighted kappa for concordance in tumor samples was 0.69 (95 % CI 0.48–0.90) and in SLN samples 0.72 (95 % CI 0.50–1.06).

5.2.3 Tissue samples and VEGF-C expression

Of 46 tumor samples, only 7 % (3/46) of the invasive edges of the vulvar tumors did not express any VEGF-C. The expression was weak in 26 % (12/46). Thus, altogether 15 tumors (33 %) were classified as VEGF-C negative. The staining was moderate or strong in 53 % (25/46) and 13 % (6/46) of the tumor edges, respectively, and thus 31 (67 %) tumors were classified as VEGF-C positive. There was no difference in the median age of patients with VEGF-C negative and VEGF-C positive tumors (75.5 vs. 76 years, p=1.00). There was no statistically significant difference in VEGF-C expression between low-grade and high-grade tumors (p=0.36) (Figure 7).

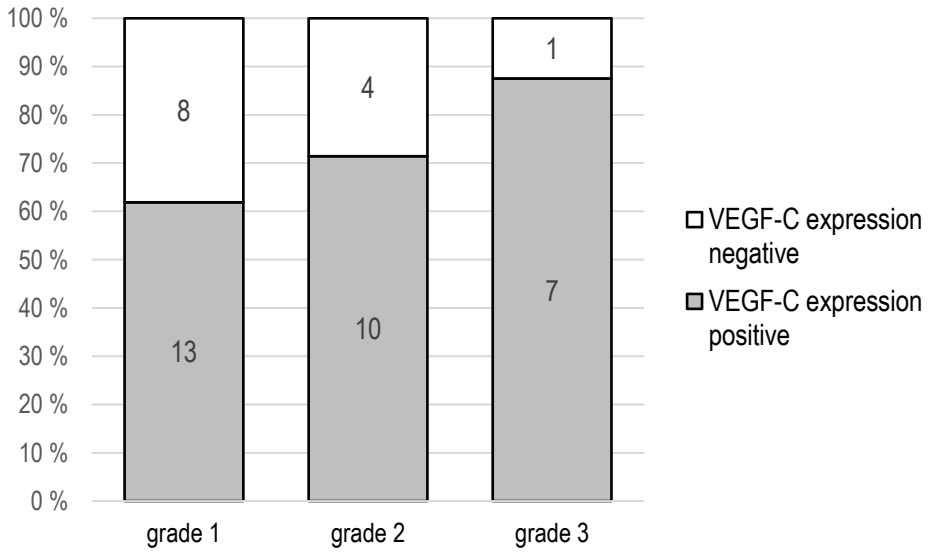

Figure 7. VEGF-C expression in invasive edges of vulvar cancer according to histological grade. The grade of one tumor was not available. Reprinted from Journal of Cancer Research & Therapy, Vol. 5, Nyberg et al., "Can vascular endothelial growth factor C expression be of use in predicting surgical stage or prognosis in vulvar cancer?", pp. 50-55, Copyright (2017), with permission from NobleResearch

5.2.4 VEGF-C expression and surgical stage

Seventeen out of 30 (57 %) VEGF-C positive and 8 out of 14 (57 %) VEGF-C negative tumors were advanced (> Stage I) at the time of surgery. The risk of more advanced surgical stage was the same with VEGF-C positive and negative tumor groups (OR 0.98, 95 % CI 0.27–3.53, p=0.98). Nor did the risk of having SLN metastasis at the time of surgery differ between VEGF-C positive and negative

tumors (47 %, 14/30 and 46 %, 6/13, respectively, OR 1.02, 95 % CI 0.28–3.77, p=0.98).

5.2.5 Sentinel lymph node metastasis and VEGF-C expression

The SLN metastases were VEGF-C negative in 24 % (4/17) and VEGF-C positive in 76 % (13/17) of the cases. The SLN metastases of VEGF-C positive primary tumors expressed VEGF-C in 91% (10/11) of the cases as compared to 50% (3/6) of the SLN metastases of VEGF-C negative vulvar tumors (p=0.099).

In 38 % (5/13) of patients whose SLN metastases expressed VEGF-C, metastatic non-SLNs were also found. However, when the SLN metastasis was VEGF-C negative, there were no other LN metastases (0/4; OR 5.82, 95 % CI 0.26–130.89, p=0.267). The positive predictive value of VEGF-C expression in the SLN metastasis in relation to non-SLN metastases was 38 % and the negative predictive value was 100 %.

5.2.6 VEGF-C expression and clinical course of vulvar cancer

Excluding three patients who died during the primary treatment, the patients with VEGF-C positive primary tumors relapsed more often (39 %, 11/28) during the follow-up than the patients with VEGF-C negative tumors (15 %, 2/13), but the risk was not statistically significant (RR 2.55, 95 % CI 0.66–9.90, p=0.18). The VEGF-C positive tumors recurred mostly in the vulvar area (64 %, 7/11) while the VEGF-C negative tumors recurred in the inguinal area (100 %, 2/2). The risk of groin recurrence was significantly lower, when the tumor was VEGF-C positive (RR 0.36, 95 % CI 0.16–0.79, p=0.01).

The DSS as a function of the VEGF-C expression of the primary tumors is shown in Figure 8 and there was no association between these variables (log rank test, p=0.83). There was also no statistically significant difference in PFS between patients with VEGF-C negative and positive tumors (log rank test, p=0.19) (Figure 9).

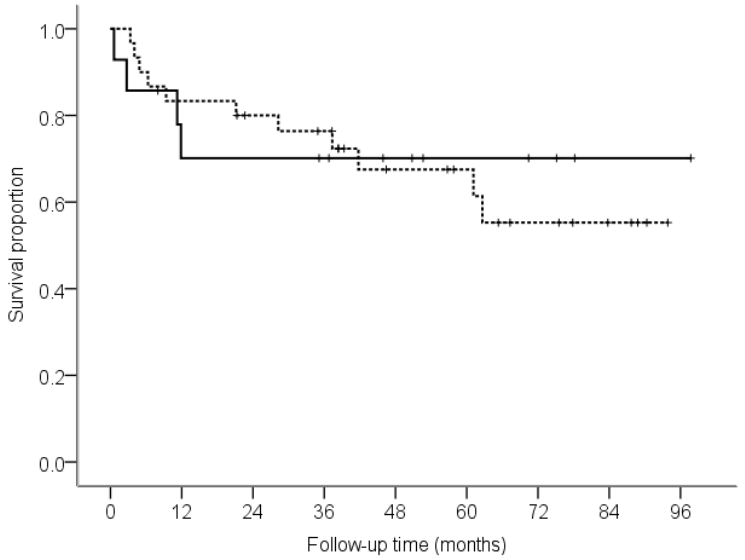

Figure 8. Disease-specific survival (Kaplan-Meier) by VEGF-C expression of vulvar tumors (log rank test p=0.83). Dotted line: positive VEGF-C expression, black line: negative VEFG-C expression, +: censored. Reprinted from Journal of Cancer Research & Therapy, Vol. 5, Nyberg et al., "Can vascular endothelial growth factor C expression be of use in predicting surgical stage or prognosis in vulvar cancer?", pp. 50-55, Copyright (2017), with permission from NobleResearch.

There was no difference in the risk of recurrence between patients with VEGF-C positive and negative SLN metastases (5/12 and 1/3, respectively, RR 1.25, 95 % CI 0.22–7.08, p=0.80), respectively. DSS and PFS by VEGF-C expression of SLN metastasis are shown in Figure 10 and Figure 11. No statistically significant differences were observed by the log rank test (p=0.45 and p=0.78, respectively). The only recurrence in the group with VEGF-C negative SLN metastasis appeared in vulvar area whereas three out of five recurrences (60 %) in the group with VEGF-C positive SLN metastasis appeared in inguinal area and two recurrences (40 %) in vulvar area. These figures are too small for statistical analysis.

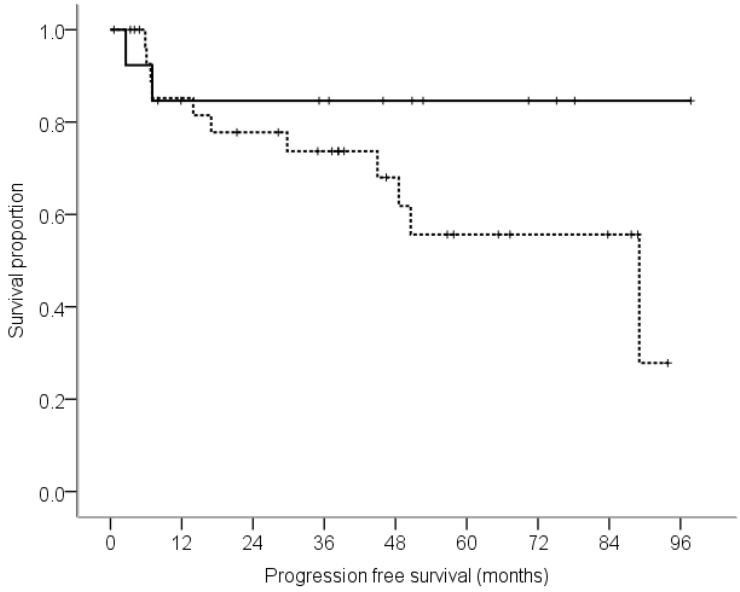

Figure 9. Progression-free survival (Kaplan-Meier) by VEGF-C expression of vulvar tumors (log rank test p=0.19). Dotted line: positive VEGF-C expression, black line: negative VEFG-C expression, +: censored. Reprinted from Journal of Cancer Research & Therapy, Vol. 5, Nyberg et al., "Can vascular endothelial growth factor C expression be of use in predicting surgical stage or prognosis in vulvar cancer?", pp. 50-55, Copyright (2017), with permission from NobleResearch.

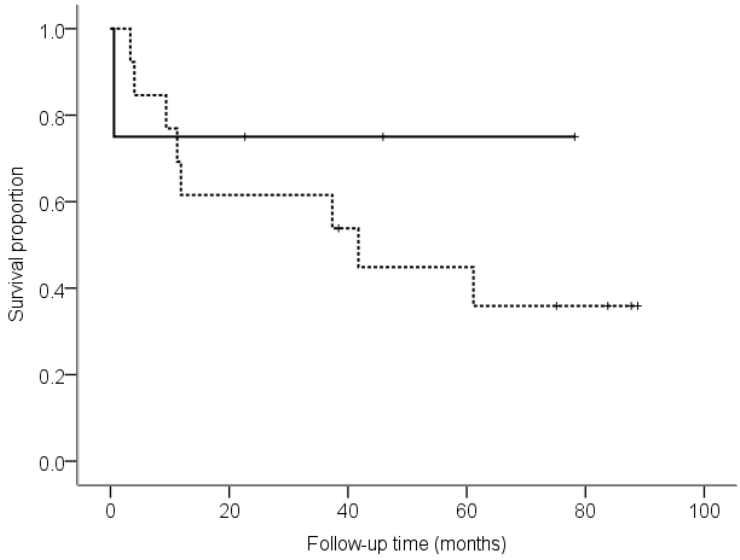

Figure 10. Disease-specific survival (Kaplan-Meier) by VEGF-C expression of SLN metastases (log rank test p=0.45). Dotted line: positive VEGF-C expression, black line: negative VEFG-C expression, +: censored.

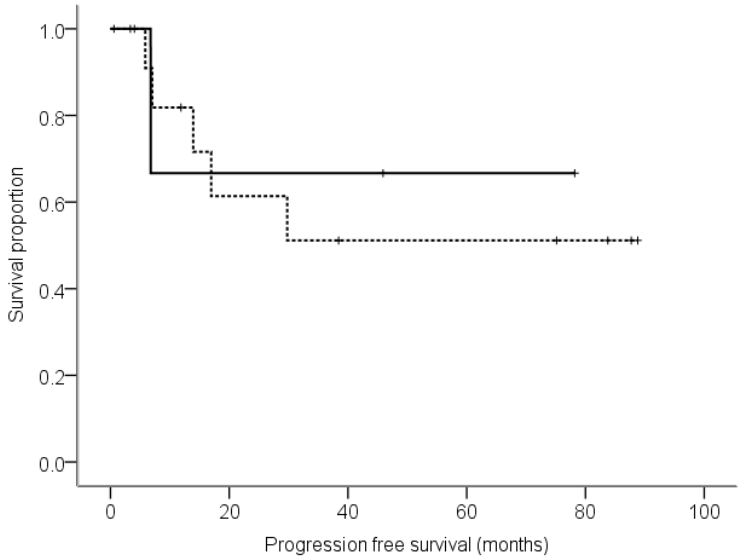

Figure 11. Progression-free survival (Kaplan-Meier) by VEGF-C expression of SLN metastases (log rank test p=0.78). Dotted line: positive VEGF-C expression, black line: negative VEFG-C expression, +: censored.

5.3 Intraoperative detection of ovarian sentinel lymph nodes (III)

The median volume of injected Nanocoll® was 0.8 (0.2–1.0) mL during the procedure, and the median measured and calculated net activity was 19 MBq (4.3–25.8 MBq). The interval between the injections and the beginning of hysterectomy ranged from 10 to 21 minutes (median 15 minutes). The median interval from injection to the detection and removal of the first SLN was 138 minutes (105–215 minutes).

5.3.1 Sentinel lymph node detection rate and numbers

At least one SLN was detected in all but one patient (15 of 16), resulting in a DR of 94 %. The total number of collected SLNs was 30, 24 of which (80 %) were both blue-stained and radioactive. One SLN (3 % of all) was detected only visually and 5 SLNs (17 % of all) only with a gamma detector. Sixteen SLNs were detected after a right-sided injection and 14 after a left-sided injection. The median number of detected SLNs per patient was two (range, 1-3), and there was no difference between the median number of SLNs after right- or left-sided injections (2 [range, 1–3] vs. 2 [range, 1–3], respectively).

There was no correlation between the time from tracer injections to the detection of the SLN and the number of detected SLNs, indicating that the tracers did not move along to the second echelon lymph nodes. The median interval from the tracer injection to the retroperitoneal detection of all SLN(s) was 138 minutes (range, 105–215 minutes). Even then, the blue dye was visible in most of the SLNs (25/30, 75 %).

5.3.2 Locations of sentinel lymph nodes

The locations of all detected SLNs are presented in Figure 12. All SLNs were located on the para-aortic area ipsilateral to the injection site. Twenty of them (67 %) were located under the level of inferior mesenteric artery (IMA) and ten (33 %) above the IMA level. When comparing this distribution between right and left ovary, the difference was significant. After the tracer injections to the right ovary, 94 % (15 of 16) were located under the IMA level, whereas 64 % (9 of 14) were located above IMA level after left-sided injections (p=0.001).

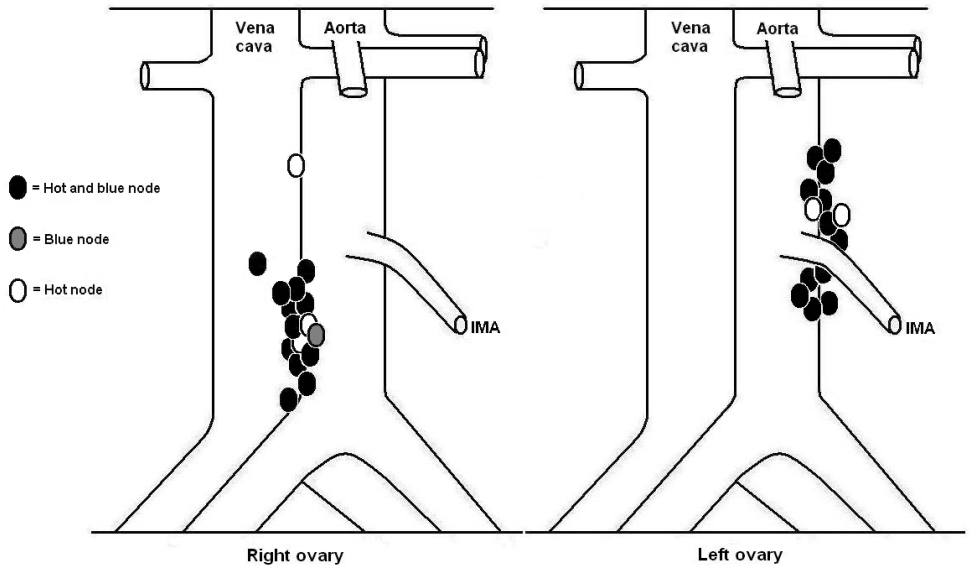

Figure 12. Locations of sentinel lymph nodes after tracer injections to the right and left ovary. IMA = inferior mesenteric artery. Reprinted from the International Journal of Gynecological Cancer, Vol. 21, Nyberg et al., "Ovarian sentinel node: is it feasible?", pp. 568-572, Copyright (2011), with permission from Wolters Kluwer.

5.3.3 Adverse effects

Due to the use of radioisotope, all patients were exposed to a small amount of radiation. The calculated median net activity was 19 MBq per patient, giving an effective radiation dose of less than 0.1 mSv to the patient. This effective dose equates with a chest X-ray from 2 directions (Suutari 2017).

There was one complication caused by the SLN mapping. One patient experienced an allergic reaction in the form of resilient hypotension and urticaria, observed 15 to 20 minutes after tracer injections. It was treated with etilefrine, hydrocortisone and norepinephrine intravenously and did not prevent the completion of the operation. Two months later, the patients underwent skin testing and was diagnosed with the type I hypersensitivity towards blue dye.

5.4 Sentinel lymph node method in ovarian cancer (IV)

There were 11 right-sided (55 %), 7 left-sided (35 %) and 2 bilateral ovarian tumors (10 %) in the study group. Patient data is presented in Table 12. All 20 patients underwent successful injection of both tracers to the mesovarium of the ovarian tumor(s). For 3 patients, careful liberation of adhesions after previous abdominal hysterectomy or supravaginal amputation was needed. The median injected activity of Nanocoll® to one spot was 20.0 MBq (17.0–26.6 MBq). The interval between the tracer injections and the beginning of the removal of the tumor(s) was 10 to 33 minutes, median 12 minutes.

All tumors were opened and a frozen-section sample taken and sent for pathological examination. In eleven patients, the result was benign, which was confirmed by the final histopathologic examination. In three patients, the examination resulted in BOTs and LND was not needed. One patient had a history of breast cancer, and the tumor turned out to be metastatic with unexpected carcinosis. No LND was performed in this case. For one severely obese patient (BMI 36) LND was omitted due to difficult surgical conditions in spite of a malignant result in the frozen-section analysis. LND was also passed for another patient with a right-sided malignant tumor, unexpected spread to the abdominal cavity and a suboptimal surgical result. For 17 women, the SLN mapping was therefore performed transperitoneally. In one patient, the pathologist suspected BOT but could not exclude invasion. Since she also had palpable lymph nodes in the para-aortic area, LND was performed. However, the final histopathology did show BOT. Altogether, the retroperitoneum of 3 patients was opened, SLNs mapped and removed for reliability analysis.

Table 12. Patient demographics. In all cases, cytology was taken. Reprinted from the International Journal of Gynecological Cancer, Vol. 27, Nyberg et al., "Sentinel Node and Ovarian Tumors: a Series of 20 Patients", pp. 684-689, Copyright (2017), with permission from Wolters Kluwer.

Patient number	Age (years)	BMI	Tumor Side	Previous surgery	Performed operations	Final diagnosis and FIGO Stage
#1	80.5	32.0	bilateral		TAH, BSO	Cystadenofibroma
#2	57.7	28.3	left	SVA, BS	BO, ADH	Serous cystadenofibroma
#3	64.6	24.2	right		TAH, BSO, OM, APP, BP	Mucinous BOT stage 1C2
#4	70.2	27.5	right		TAH, BSO	Serous cystadenofibroma
#5	56.4	26.0	right		TAH, BSO, OM, APP, BP	Serous BOT stage 1A
#6	63.0	25.6	left		TAH, BSO	Serous cystadenoma
#7	41.1	24.3	right	APP	TAH, BSO, LND, OM	Endometrioid adenocarcinoma stage 1C2
#8	80.7	28.7	right		TAH, BSO, OM, APP	Serous adenocarcinoma grade 2 stage 3A2
#9	62.5	22.9	left	VH	BSO, OM, APP, BP	Mucinous BOT stage 1A
#10	75.1	27.4	right	TAH, BS, APP	BO, ADH	Mucinous cystadenoma
#11	43.0	23.0	right		TAH, BSO	Mucinous cystadenoma
#12	63.8	24.9	left		TAH, BSO	Fibroma
#13	63.6	27.7	left		TAH, BSO	Brenner's tumor / mucinous cystadenoma
#14	65.5	25.3	left		TAH, BSO	Mucinous cystadenoma
#15	43.6	23.7	right	APP	TAH, BSO, LND, OM	Serous cystadenocarcinoma grade 3 stage 3A1
#16	55.6	27.6	right	TAH	BSO, OM, APP, ADH	Metastatic breast cancer
#17	62.1	21.5	right		TAH, BSO, LND, OM, APP	Serous BOT stage 1C3
#18	58.1	25.1	bilateral		TAH, BSO	Adenofibroma
#19	68.9	25.9	left		TAH, BSO	Serous cystadenoma
#20	68.5	36.1	right		TAH, BSO, OM, APP	Serous adenocarcinoma grade 3 stage 2A

ADH = liberation of adhesions; APP = appendicectomy; BO = bilateral oophorectomy; BOT = borderline ovarian tumor; BP = peritoneal biopsies; BS = bilateral salpingectomy; BSO = bilateral salpingo-oophorectomy; FIGO = International Federation of Gynecology and Obstetrics; LND = pelvic&para-aortic lymph node dissections; OM = omentectomy; SVA = supravaginal uterine amputation; TAH = total abdominal hysterectomy; VH = vaginal hysterectomy.

5.4.1 Sentinel lymph node detection rate and numbers

A total of 36 SLN sites were detected; the DR was 100 % and the median number of SLNs 2 (1–3) per unilateral tumor. There were no differences in the median SLN count between right and left ovarian tumors (2 [1–3] vs. 2 [1–2]). In case of bilateral tumors, the median number was 2 (2–3).

Eighteen of all SLNs (50 %) were detected both visually and with a gamma detector, 17 (47 %) by means of radiation only, and one (3%) only visually. With transperitoneal mapping (28 SLN locations), 43 % (12/28) were found by combining dye and radiotracer, 54 % (15/28) with radiation only and 4 % (1/28) with dye only. Eight SLNs were collected from lymphadenectomized patients, 75% (6/8) with the combined method and 25 % (2/8) with the use of a gamma detector. The median interval between injections and identification of SLNs was 56 minutes (28–126 minutes) in the transperitoneal group and 129 minutes (49–180 minutes) in the retroperitoneal group.

In the group of patients with a BMI less than 27, 92 % (11/12) had at least one blue-stained SLN, whereas the figure was only 25 % (2/8) in patients with BMI 27 and over. The odds for blue dye being visible in SLNs with BMI $\geq$ 27 compared to BMI $<$ 27 was 0.03 (95 % CI 0.02–0.41, p=0.008).

5.4.2 Locations of sentinel lymph nodes

In 18 patients with unilateral tumors, the SLNs were mostly located ipsilaterally (83 %, 15/18), and contralaterally in only one patient (6 %). Bilateral SLN sites were found in 11 % (2 patients). In both patients with bilateral ovarian masses, all the SLNs were detected bilaterally. Of all SLNs, 78 % (28/36) were located in the para-aortic regions, and in 60 % of all patients (12/20), SLNs were found exclusively in the para-aortic region. In 30 % of the patients (6/20), the SLNs were located both in the para-aortic and pelvic regions, and only one patient (10 %) had the solitary SLN in the pelvis. The distribution and laterality of the SLN locations are described more closely in Table 13.

Eleven patients with a right-sided ovarian mass had 20 SLN sites located after the tracer injections. The distribution of the SLNs after right-sided injections is shown in Figure 13. Most of the SLNs were located ipsilaterally (85 %) and in the para-aortic area (70 %), and drift of the tracers to the contralateral side occurred both to the para-aortic (10 %) and high pelvic areas (5%). No SLNs were found in the contralateral lower pelvic area.

Table 13. Distribution and laterality of SLN locations in 20 patients by side of the ovarian tumor, which was also the tracer injection site. Reprinted from the International Journal of Gynecological Cancer, Vol. 27, Nyberg et al., "Sentinel Node and Ovarian Tumors: a Series of 20 Patients", pp. 684-689, Copyright (2017), with permission from Wolters Kluwer.

Tumor location	Number of patients	The region of SLN site			Unilateral SLNs		Bilateral SLNs
		Para-aortic only	Both para-aortic and pelvic	Pelvic only	Ipsilateral	Contra-lateral	
Right-sided	11 (55 %)	7 (64 %)	2 (18 %)	2 (18 %)	9 (82 %)	1 (9 %)	1 (9 %)
Left-sided	7 (35 %)	5 (71 %)	2 (29 %)	0 (0 %)	6 (86 %)	0 (0 %)	1 (14 %)
Bilateral	2 (10 %)	0 (0 %)	2 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	2 (100 %)
Total	**20 (100 %)**	**12 (60 %)**	**6 (30 %)**	**2 (10 %)**	**15 (75 %)**	**1 (5 %)**	**4 (20 %)**
					16 (80 %)		

Seven patients with a left-sided ovarian mass had 11 SLN sites located after a left-sided injection. The distribution of SLNs after a left-sided injection is shown in Figure 14. After the tracer injections, the SLNs were located mainly in the para-aortic area (82 %), and 91 % of the SLNs were ipsilateral. Tracers drifted to the contralateral side only in the high para-aortic area (9 %).

The SLN sites of the right and left ovarian tumors were distributed asymmetrically. Although the difference was not statistically significant, the SLNs after a left-sided injection seemed to be located higher than the SLNs after a right-sided injection. After a left-sided injection, the only area the tracers drifted to contralaterally was the upper para-aortic region; after a right-sided injection, tracer drifted to the upper pelvic, lower para-aortic and upper para-aortic region.

After bilateral injections in two patients, 5 SLN sites were found; two in the right lower para-aortic region (40 %), one in the left higher para-aortic region (20 %), one in the right external iliac region (lower pelvic; 20 %) and one in the left common iliac region (upper pelvic; 20 %). Here, too, the SLNs on the left were located higher than on the right side.

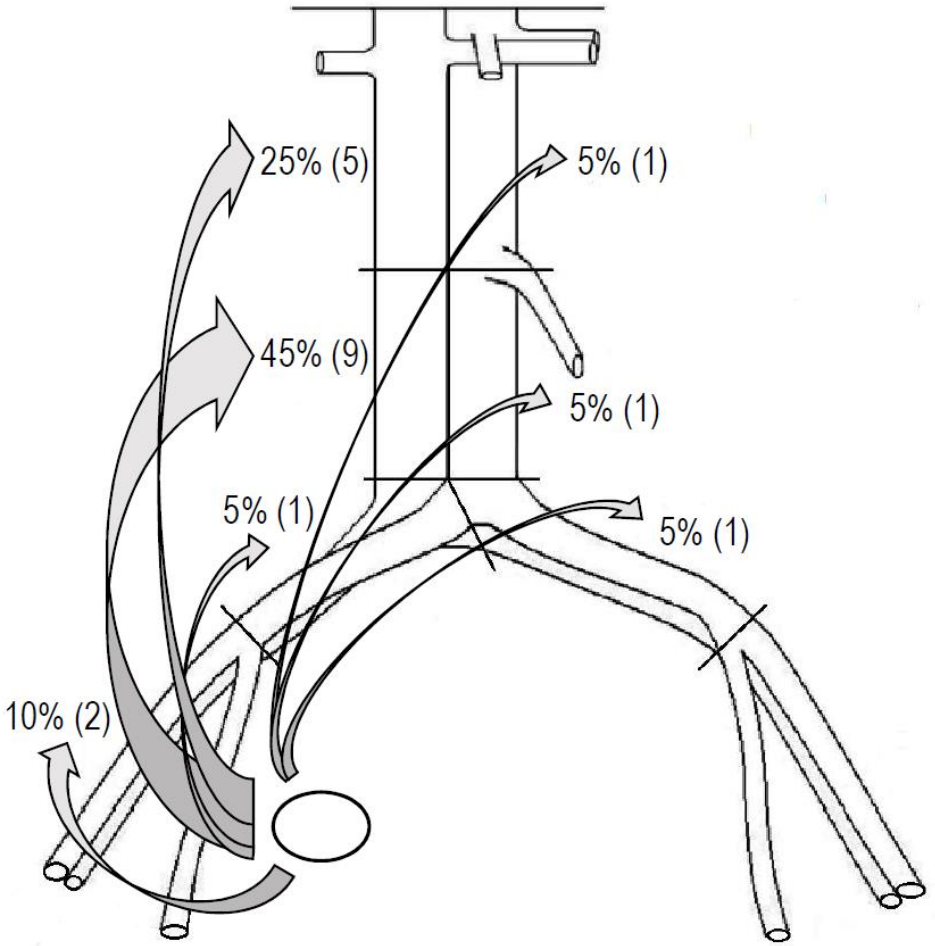

Figure 13. Distribution of SLNs after tracer injections next to the right ovarian tumors (11 in all). Twenty SLNs were located; percentages (numbers).

5.4.3 Reliability of sentinel lymph node method

Three patients underwent systematic pelvic and para-aortic LND and the detected SLNs were collected separately for histopathologic examination (Table 14). In one of these patients (#17), the final diagnosis was not malignant but BOT. However, endosalpingiosis was present in two of the three SLNs of this patients. Patient #15 was the only one who had a metastasis also in other LNs, and in this case, a metastatic para-aortic SLN correctly predicted the final FIGO stage of her cancer.

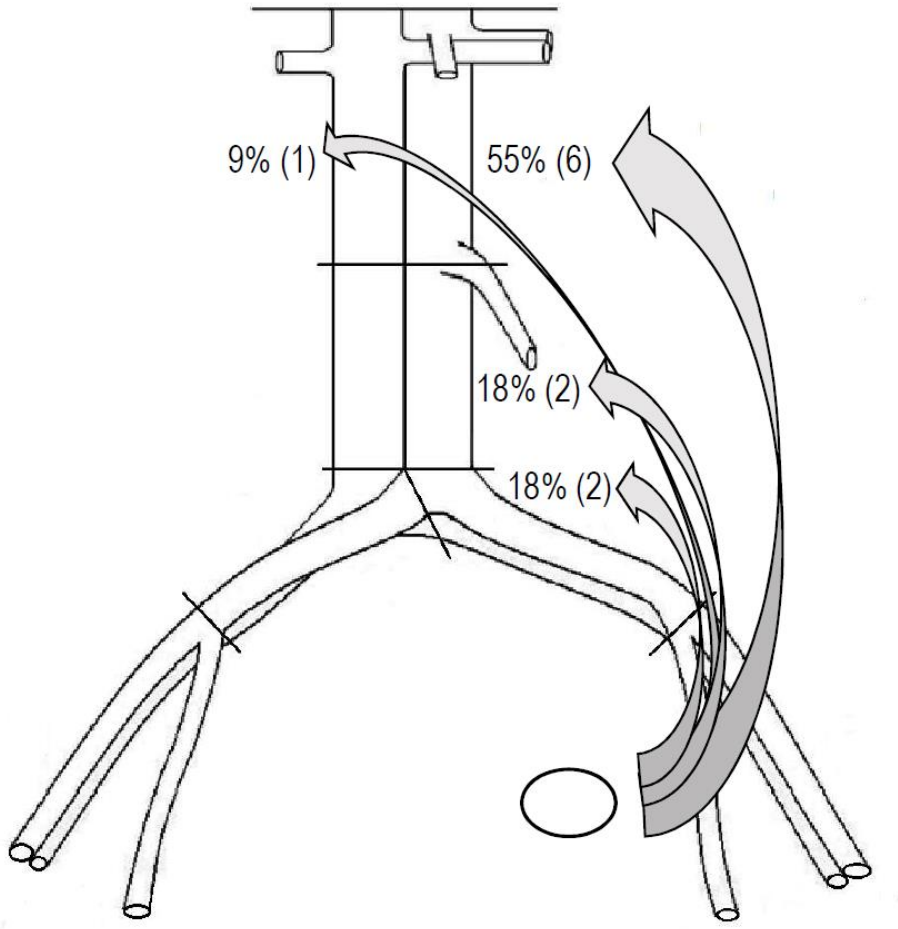

Figure 14. Distribution of SLNs after tracer injections next to the left ovarian tumors (7 in all). Eleven SLNs were located; percentages (numbers).

The number of patients with metastatic LNs in this study was not large enough to allow evaluation of the reliability of the SLN method for patients with ovarian cancer.

5.4.4 Adverse effects

The calculated median net radioactivity emitted by the radioisotope was 20 MBq per patient (range, 17.0-40.8 MBq), as in study III. The patients were exposed to an effective radiation dose of less than 0.1 mSv.

No adverse effects due to the SLN mapping or allergic reactions to tracers occurred.

Table 14. Histopathologic results of retroperitoneal SLN sampling compared with other LNs. PA = para-aortic. *Endosalpingiosis. Reprinted from the International Journal of Gynecological Cancer, Vol. 27, Nyberg et al., "Sentinel Node and Ovarian Tumors: a Series of 20 Patients", pp. 684-689, Copyright (2017), with permission from Wolters Kluwer.

Patient number	Tumor Side	Systematic lymphadenectomy and SLN results according to anatomic region							
		Right PA SLNs (metastatic)	Right PA LNs (metastatic)	Left PA SLNs (metastatic)	Left PA LNs (metastatic)	Right pelvic SLNs (metastatic)	Right pelvic LNs (metastatic)	Left pelvic SLNs (metastatic)	Left pelvic LNs (metastatic)
#7	right	3 (0)	10 (0)	0 (0)	19 (0)	0 (0)	24 (0)	0 (0)	31 (0)
#15	right	**2 (1)**	**6 (2)**	0 (0)	9 (0)	0 (0)	10 (2)	0 (0)	12 (0)
#17	right	3 (0*)	7 (0)	0 (0)	7 (0)	0 (0)	8 (0)	0 (0)	7 (0)

6 DISCUSSION

6.1 Sentinel lymph node method in vulvar cancer (I)

In our series of 47 patients, we showed that SLN mapping with blue dye and ^{99m}Tc-nanocolloid is an accurate and feasible method of diagnosing lymphatic metastasis in early VC (FIGO 1988 stage I–II), a true target of the SLN concept. In this population, the DR of the combined technique was 100 % and the FNR 0 %. Our overall DR of 98 % in an unselected population was also high; the only SLN detection failure concerned a patient with stage IV disease, and the only false-negative SLN result was in a patient with stage III disease. These stages, however, are not included in the current guidelines of the SLN method in vulvar cancer (Table 6), since the risk of false-negative SLN increases as stage advances (Levenback et al. 2012). In our study, the FNR for stages III and IV was 6.7 %.

The performance of blue dye (DR of 96 %) in our series was surprisingly high compared to the literature (pooled DR for blue dye alone 69 %, 95 % CI 63–74 %) and even higher than that of the radioisotope in our study (DR of 93 %), although the latter is in line with the literature (pooled DR for radioisotope alone 94 %, 95 % CI 91–96 %) (Meads et al. 2014). One explanation for this success is that the first year of use of the new method at our department was not included in this retrospective analysis in order to exclude the effect of a learning curve, which could be longer with the blue dye (Chapter 2.2.7.1).

We did not use a preoperative LSG. On three occasions, the handheld gamma detector failed to operate, which was the main cause of failure to detect SLNs with the radioisotope. With LSG, this kind of problem could have been avoided, as suggested by de Hullu et al. (De Hullu et al. 2004) and described in Chapter 2.2.5.1. Klapdor et al. have concluded that, compared to LSG, SPECT/CT provides the surgeon with important additional information, facilitates intraoperative SLN detection and predicts aberrant lymphatic drainage (Klapdor et al. 2015). At present, the LSG is taken preoperatively at our department and the location of a hot SLN is marked on the skin of the patient.

Since the publication of our study in 2007, a large prospective study has conclusively proven the safety of the SLN concept in early vulvar cancer (van der Zee et al. 2008; Te Grootenhuis et al. 2016), and it has been established as an

important part of the surgical treatment. Our work, which was an accuracy study, has been cited in several reviews and meta-analyses, which have concluded that the combined technique of identification of SLNs is an accurate way of diagnosing LN metastasis in VC (eg. Meads et al. 2014; Oonk et al. 2015; Tu et al. 2015). In the future, blue dye might be replaced with ICG, which offers many benefits. In a randomized comparison of different lymphatic tracers, optical guidance with NIR fluorescence had a higher DR (100% compared to 77% with blue dye). Since ICG is invisible under normal light, it does not – in contrast to blue dyes – smudge the surgical field. Also, ICG migrates faster than blue dyes to the SLNs and the tissue penetration of NIR light is better than that of normal light, which allows earlier and deeper visualization of the lymphatic channels and SLNs (Schaafsma et al. 2013).

6.2 VEGF-C in predicting lymph node metastasis and clinical course of vulvar cancer (II)

We showed that most of the vulvar malignant tumors express VEGF-C on their invasive edges. High grade tumors expressed VEGF-C more often than low grade tumors, but the association between histological grade and VEGF-C expression was not statistically significant. VEGF-C was also expressed in three quarters of the SLN metastases, more often when the primary tumor expressed it, although the association was not significant.

Our findings of the frequency of VEGF-C expression in vulvar cancer differs from the only other published report of Jach et al. In their much smaller population, VEGF-C expression was observed only in 10 % (1 out of 10 tissue samples) of vulvar SCC cases. The carcinoma specimens they used for the IHC analysis were individually selected. However, the authors neither specified which part of the tumor their specimens represented nor the histological grades of the tumors – a feature that in our study had a small, albeit non-significant, effect on the frequency of VEGF-C expression, (Jach et al. 2011). The expression of VEGF-C in 111 cervical SCC samples was heterogeneous within tumors and significantly higher in the marginal portions of the carcinomas compared with the central regions (Gombos et al. 2005). In our study, we also focused on the invasive edge of vulvar tumors and recorded the same phenomenon. Furthermore, the semiquantitative scoring system of Jach et al. to assess VEGF-C expression took into account the percentage of VEGF-C positive cells. Using the central parts of the vulvar tumors for the immunostaining analysis might have diminished their scores even when the staining was strong.

It is known that SCCs originating from many different organs express VEGF-C, e.g. tumors of oral cavity (Karatzanis et al. 2012), esophageal cancer (Peng et al. 2013), and cervical cancer (Zhang et al. 2017). The positive expression is associated with poorer prognosis and higher risk of lymphatic metastasis. In our study, tumoral VEGF-C expression did not associate with the surgical stage or the frequency of SLN metastases.

The risk of relapse did not significantly differ between VEGF-C positive and negative tumors. In our study population, 43 % of patients with local recurrence were successfully salvaged, while all groin recurrences were fatal. The risk of groin recurrence was significantly lower in the VEGF-C positive tumor group than in VEGF-C negative tumor group. Fewer groin recurrences and more favorable prognosis of vulvar recurrences might explain why the tumoral VEGF-C expression had no impact on DSS, although VEGF-C positive tumors recurred slightly more often. VEGF-C expression in SLN metastasis did not affect the recurrence rate nor the survival of the patients.

Currently, the size of SLN metastasis is the only well-established prognostic factor for non-SLN metastases in relation to characteristics of a SLN metastasis. GROINSS-V study showed that the risk of non-SLN metastasis increases with the size of the SLN metastasis. There was no size cutoff below which the likelihood of non-SLN metastasis was close to zero. Therefore additional treatment should be offered to all SLN positive patients, which always increases side effects and lowers quality of life (Oonk et al. 2010). The absence of VEGF-C expression in the SLN metastasis seemed to act as a predictor of cancer-free non-SLNs, but the small number of metastatic SLN samples limits interpretation of this finding. This observation would be worthwhile to test with a larger sample size. If true, it could serve as a prognostic factor when considering additional treatment for VC.

Otherwise, VEGF-C did not seem to be a useful indicator of the prognosis in VC. This is probably due to the different courses of disease with local or regional recurrence.

6.3 Intraoperative detection of ovarian sentinel lymph node (III)

We showed that by injecting common tracers, Patent Blue and ^{99m}Tc-radiocolloid, to one normal ovary it was possible to locate ovarian SLNs intraoperatively with a high DR of 94 %. One to three SLNs (median of 2) were located per patient, and they were consistently located in certain para-aortic areas that were in concordance

with the previous literature about LN metastasis in ovarian cancer (Panici & Angioli 2002; Fournier et al. 2009; Kleppe et al. 2011). All SLNs were ipsilateral.

The SLNs after left-sided injection were often located above the IMA level (63 %), while almost all SLNs of the right ovary (94 %) were located below it. The difference was statistically significant. Likewise, Negishi et al. reported a similar difference during their experiment with activated charcoal solution. They found that the SLNs of the left ovary were restricted to the para-aortic area, especially above the IMA, but the SLNs of the right ovary were also detected in the pelvis (Negishi et al. 2004).

Nomura et al. reported that bilateral LN metastasis were present in 33 % and contralateral LN metastasis in 13 % of their patients (Nomura et al. 2010). We did not detect any bilateral, contralateral or pelvic SLNs. The relatively small sample size and the learning curve related to the introduction of a new procedure might have affected that. Since the stumps of the suspensory ligaments in the pelvis contain remnants of both blue dye and radioactivity, it can distract a surgeon and hamper the detection of ipsilateral pelvic SLNs. In our next study (IV), both bilateral and contralateral SLNs were detected (Table 13).

One allergic reaction to blue dye was encountered during this section. It was manageable without discontinuation of the surgery. Altogether 83 patients were included in our whole study and no other allergic reactions emerged, yielding an incidence of allergic reactions caused by blue dye of 1.2 %. This is in line with previous reports (Chapter 2.2.4.1) It is still good to remember that despite of their apparent harmlessness, blue dyes can cause severe side effects to the patients.

We discovered that a 10-minute interval between the injection and the removal of the adnex was enough to allow the tracers to migrate to the lymphatic system. Although it is not common to excise the primary tumor before mapping of the SLNs, doing so did not seem to disrupt the mapping. This would be of essence when surgically treating suspicious ovarian masses by laparotomy; the surgeon would be able to first remove the tumor and send it to the frozen section, and only then perform the SLN mapping, if necessary. Conventional tracers allowed a longer time for retroperitoneal SLN mapping than expected. All this lead to the conclusion that it would be possible to investigate the SLN method also in the surgical treatment of early ovarian cancer.

6.4 Sentinel lymph node method in ovarian cancer (IV)

In this section, we showed that when injected into one spot of the mesovarium, conventional tracers (Patent Blue and ^{99m}Tc-radiocolloid) swiftly migrate to SLNs allowing removal of the ovarian tumor for frozen sample and SLN mapping after receiving the result. The DR was 100 %, and 1–3 SLNs (median 2) were detected from each patient. Again, the SLNs of the left ovary were located higher than those of the right ovary: para-aortic alone (71 vs. 64 %, and above IMA 64 vs. 30 %, respectively), para-aortic and pelvic (29 vs. 18 %) and pelvis alone (0 vs. 18 %). There were no SLNs related to a left ovarian tumor in the lower pelvis (external iliac and obturator areas), but pelvic SLNs were located in the common iliac region. This result is clinically remarkable although it was not statistically significant. After unilateral injection (18 patients), 83 % of the SLNs were ipsilateral, 11 % bilateral and 6 % contralateral. There were two patients who underwent bilateral injection, and both had only bilateral SLNs.

The SLN locations we reported are in line with the other published studies of SLN in relation to ovarian cancer (Kleppe et al. 2014; Hassanzadeh et al. 2016; Angelucci et al. 2016; Buda et al. 2017) (Table 15). Although the proposed tracer injection sites have varied from the ovarian cortex or parenchyma to the adnexal ligaments and mesovarium, all have led to the same conclusion: the SLNs are most often found in the para-aortic area with or without pelvic SLNs. Solitary pelvic SLNs are rare. Table 16 shows a detailed summary of the literature.

Table 15. SLN locations per patient in all published SLN mapping studies. *Five patients with ovarian cancer were already included in Kleppe et al 2014.

Publication	Number of patients with successful SLN mapping	Para-aortic SLNs alone	Para-aortic and pelvic SLNs	Pelvic SLNs alone
Vanneuville et al. 1991	12	4	8	0
Negishi et al. 2004	11	7	4	0
Nyberg et al. 2011 (III)	15	15	0	0
Kleppe et al. 2014	21	14	5	2
Hassanzadeh et al. 2016	25	21	2	2
Angelucci et al. 2016	5	2	2	1
Buda et al. 2017	9	6	2	1
Speth et al. 2017	3*	2	0	1
Nyberg et al. 2017 (IV)	20	12	6	2
Total (percentage)	**121 (100 %)**	**83 (69 %)**	**29 (24 %)**	**9 (7 %)**

Table 16. Publications on ovarian SLN mapping, 1991–2017. DR = detection rate, LT = laparotomy, LSC = laparoscopy, IMA = inferior mesenteric artery, ICG = indocyanine green.

Publication	Patients	Tracers	Injection sites	Means of detection	DR	Main results
Vanneuville et al. 1991	14 pts; LSC for benign cause	^{99m}Tc-colloid	Mesovarium of a normal ovary	Postoperative LSG (4-6 h)	86 %	Isolated para-aortic SLN in 33 %, combined pelvic/para-aortic in 67 %. Laterality not available.
Negishi et al. 2004	11 pts; LT for endometrial or fallopian tumors	Activated charcoal solution (CH40)	Unilateral ovarian cortex	Visual during LND	100 %	Isolated para-aortic SLN in 64 %, combined pelvic/para-aortic in 36 %. SLNs of left ovary were located solely on para-aortic region, while SLNs of right ovary were also found on pelvis. 45 % of SLNs were bilateral.
Nyberg et al. 2011	16 pts; LT for high risk EC	^{99m}Tc-albumin colloid + blue dye	Hilum of one normal ovary	γ-detector + visual during LND	94 %	Isolated para-aortic SLNs in 100 %. SLNs of left ovary located higher in relation to IMA than those of right ovary (p=0.001). All SLNs were ipsilateral.
Kleppe et al. 2014	21 pts; LT for ovarian tumor	^{99m}Tc-albumin colloid + blue dye	Suspensory and proper ovarian ligaments	γ-detector + visual during LND/LT	100 %	Isolated para-aortic SLNs in 67 %, combined pelvic/para-aortic in 24 %, isolated pelvic SLNs in 9 %. Resected SLNs of left ovary located solely above IMA. 6 % of SLNs were on contralateral side. LN metastasis in 1/6 pts, FNR 0 %
Hassan-zadeh et al. 2016	35 pts; LT for ovarian tumor	^{99m}Tc-Phytate (+ blue dye in 4 pts in group 2)	1) ovarian cortex (10 pts) 2) proper and suspensory ovarian ligaments (25 pts)	γ-detector during LND /LT + postop LSG	1) 40 % 2) 84 %	Isolated para-aortic SLN in 84 %, combined pelvic/para-aortic in 8 %, isolated pelvic in 8 %. Laterality was not mentioned. LN metastasis in 3/10 pts, FNR 0 %

Angelucci et al. 2016	5 pts; LSC for early ovarian cancer	ICG	Ovarian pedicle (3 pts), broad ligament (1 pt), ovarian paren-chyma (1 pt)	Visual (SPIES camera)	100 %	Isolated para-aortic SLNs in 40 %, combined pelvic/para-aortic in 40 %, isolated pelvic in 20 %. All SLNs were ipsilateral. LN metastasis in 0/5 pts. FNR not conceivable.
Buda et al. 2017	10 pts; LSC for early ovarian (7) or cervical cancer (3)	ICG	Suspensory and proper ovarian ligaments	Visual (SPIES camera)	90 %	Isolated para-aortic SLNs in 67 %, combined pelvic/para-aortic in 22 %, isolated pelvic in 11 %. No SLNs of right ovary above IMA. All SLNs were ipsilateral. LN metastasis in 0/7 pts, FNR not conceivable.
Speth et al. 2017	8 pts; LT for high risk EC (3) and early ovarian cancer (5)†	^{99m}Tc-albumin colloid + blue dye	Suspensory and proper ovarian ligaments	γ-detector + visual during LND, postoperative SPECT/CT	100 %	Isolated para-aortic SLNs in 67 %, isolated pelvic in 33 %. Bilateral SLNs in 33 %. Discrepancies between intraoperative γ-detector imaging and postoperative SPECT/CT, but no residual SLNs reliably detected in postoperative SPECT/CT.
Nyberg et al. 2017	20 pts; LT for ovarian tumor(s)	^{99m}Tc-albumin colloid + blue dye	Mesovarium (18 unilateral, 2 bilateral)	γ-detector + visual during LND/LT	100 %	Isolated para-aortic SLNs in 60 %, combined pelvic/para-aortic in 30 %, isolated pelvic in 10 %. 75 % of SLN were ipsilateral, 5 % contralateral and 20 % bilateral. LN metastasis in 1/3 pts; FNR 0 %
† The results of five ovarian cancer patients were included in Kleppe et al. 2014.						

Our observation of SLNs related to tumors of the left ovary locating on average higher than SLNs related to right ovarian tumors is consistent with previous results. Kleppe et al. reported that in six patients who underwent LND, all SLNs related to the left ovary were located just below the renal vein, while the SLNs related to the right ovary were mainly located at the level of IMA (Kleppe et al. 2014). Similar results were reported by Negishi et al. as discussed in Chapter 6.3 (Negishi et al. 2004). Buda et al. also reported that they located almost half of the left-ovary-related SLNs above the IMA (Buda et al. 2017). This is consistent with human anatomy; ovarian lymphatic vessels run along the suspensory ligaments which drain into the para-aortic area and the left-sided ovarian vessels branch from the aorta and great veins more cephalad than the right-sided vessels (Ushijima 2007; Kleppe et al. 2015; Paño et al. 2015).

The site of tracer injection has varied, partly because some of the studies have just concentrated on exploring the lymphatic network of a healthy ovary. When treating a suspicious ovarian mass, tracers cannot be injected into the tumor. Ovarian ligaments have been suggested to be suitable for this purpose; in several studies, the tracers have been injected into four different spots on the dorsal and ventral sides of the suspensory and proper ovarian ligaments (Kleppe et al. 2014; Hassanzadeh et al. 2016; Buda et al. 2017; Speth et al. 2017). No specific differences in distribution of SLN locations by different injection sites have been observed in Table 15. According to our experience, when using conventional tracers, the operation field can be smudged with the tracers that leak from the needle holes, impeding identification of pelvic SLNs. The same has been noted by others (Buda et al. 2017; Speth et al. 2017). The solution is either to minimize the number of injection holes or to use ICG, which is invisible to the surgeon's eyes under regular light. We suggest that one injection spot to the mesovarium that contains plenty of lymphatic vessels (Kleppe et al. 2015) is enough to ensure successful SLN mapping and comparable results.

Kimmig et al. have just published two educational videos on ICG-guided targeted compartmental LND in early EOC by robotic-assisted surgery. They suggest to that the ovarian tumor is removed en bloc together with its draining lymphatic vessels and at least the first 2 SLNs along each channel. They injected ICG into the uterine fundus on the side of the ovarian tumor (Kimmig, Buderath, et al. 2017; Kimmig, Rusch, et al. 2017). This idea is based on an ontogenetic approach to the logoregional spread of gynecologic cancers. According to it, adult tissues are mapped with respect to their developmental origin, so called ontogenetic compartments. The boundaries of the ontogenic compartments suppress tumor spread in the early stages of tumor

growth and – according to this theory – their surgical treatment would improve both local and regional control. As the upper uterine fundus is referred to the same developmental upper Mullerian compartment as the fallopian tubes (except the fimbriae), the mesosalpinges and the utero-ovarian vascular anastomoses, an injection to the uterine fundus next to the origin of the fallopian tubes and proper ovarian ligament would be logical (Santiago et al. 2016). However, since the ovaries and ovarian ligaments are not part of the paramesonephric-mesonephric-Mullerian tubercle complex, some open questions remain about the true equivalence of this injection site and ovarian lymphatics. The authors have not published by far any patient series on their results in SLN detection or the reliability of compartmental ICG-guided surgery for detecting metastatic LNs.

In our study, blue dye performed better than for Kleppe et al who reported retroperitoneal SLN staining in only 25 % of patients. In our study, 46% of the SLN locations showed coloring by transperitoneal search, 75% by retroperitoneal search. Hassanzadeh et al., on the other hand, reported blue staining of SLNs in 100 % of the patients. Whether blue dye is useful at all depends probably on the interval between injection and SLN mapping as well as on the BMI of the patient. In our study, the risk of blue dye not being visible was higher when the patient's BMI was 27 or more. Replacing blue dye with ICG would probably lead to visual identification of SLNs also in overweight patients, although obesity *per se* has been reported to be a contributing factor for reduced DR and false-negative SLNs also with ICG (Polom et al. 2011; Darin et al. 2016). When using blue dye and/or radiocolloids, we waited for an interval of 10 to 21 minutes before removal of the ovarian tumor / injection site to allow for migration of the tracers. Angelucci et al. reported that the transport of ICG to the SLNs took place in only 1–3 minutes (median 2 minutes), which allowed swift progression of the operation (Angelucci et al. 2016). ICG certainly merits further investigation in this setting, merely due to its rapid transportation from the injection site to SLNs which allows swift removal of the tumor for frozen analysis.

At present, over 80 patients have undergone an experimental SLN mapping related to ovarian tumors, but only 5 have had LN involvement (Table 16). Not all enrolled patients had ovarian cancer, or then there was an unexpected spread of the cancer in the abdominal cavity. In all aforementioned 5 patients, SLN correctly identified metastatic disease giving a pooled FNR of 0 %. One patient in our series whose final pathological diagnosis was BOT, had endosalpigiosis in a resected para-aortic SLN. Although her disease was not malignant, this finding could be related to the lymphatic flow from the adnex. The present findings do not help in deciding

how to proceed when a positive SLN is encountered. Should it only upstage the patient and be the reference to adjuvant chemotherapy? Or should the patient undergo complete LND for debulking? That remains to be evaluated in further studies.

The ratio of the total number of patients enrolled to the number of patients with LN involvement tells how difficult it is for one department to recruit series large enough to draw conclusions about the reliability of the SLN concept in EOC; to gather 50 patients with LN involvement, one should recruit over 800 patients in a reliability study. A multicenter prospective trial is warranted. Also, long-term follow-up of the operated patients would be required to reliably ensure the safety of the injection techniques.

6.5 Strengths and limitations

Our first study (sentinel lymph node method in vulvar cancer) was a retrospective analysis of the experiences of one clinic. It included patients of advanced stages (47 % of study population), which nowadays are not deemed suitable for the SLN method. One could question the relevance of including this group of patients to the study, but the purpose was to ensure that the team becomes familiar with the SLN technique. To our surprise, the technique worked surprisingly well also in this group. In addition to the retrospective nature of the study, another limitation was the use of conventional histopathological methods for the analysis of the SLNs. No ultrastaging was performed, possibly missing some micrometastasis. Later during study II, one patient with a low-volume metastasis in a SLN was identified and restaged. The strength of study I was that a limited group of experienced surgeons performed all the operations. The analysis was not started from the absolute beginning of the learning curve. In this way, the results represent an established procedure more accurately.

In study II (VEGF-C in predicting lymph node metastasis and the clinical course of vulvar cancer), our sample size of 44 tumors was representative considering the rarity of VC but apparently not large enough to show statistical significance between subgroups, although some trends were observed. The strengths of study II were a long follow-up time and its clinical orientation. The median follow-up of 39 months was sufficient for observing recurrences. We chose a simple IHC scoring system, bearing in mind its applicability to patient care.

Study III (intraoperative detection of ovarian sentinel lymph node) was a prospective feasibility study that recruited patients who underwent similar LND

under laparotomy as patients with ovarian cancer. Due to logistical reasons in our clinic, patient recruitment was slower than expected. Therefore, we decided to analyze the results after 16 recruited patients. Although all operations, except one, were performed by the same surgeon – who actually also assisted in the remaining operation – the effect of a learning curve must be taken into account. The strength of study III was the meticulous recording all findings related to timing and location of SLNs. The concept itself was pioneering and generated an international interest in the SLN method in EOC.

Study IV (sentinel lymph node method in ovarian cancer) was also a prospective feasibility study undertaken in an authentic population with ovarian tumors. As in study III, the locations of the SLNs were carefully mapped in relation to surrounding anatomical structures, which was a notable strength of this study. The recruitment of suitable patients was also slow also for this study, mainly because only one surgeon performed the SLN procedures. That, however, ensured an identical performance of all procedures and minimized the effect of a learning curve. The limitation was a small number of patients who finally underwent LND and could be included in the evaluation of the reliability of the SLN method in EOC.

7 SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the use of the SLN approach for nodal staging in vulvar and ovarian cancers. The accuracy of combined injections of blue dye and ^{99m}Tc-colloid in detecting SLNs and the reliability of the method in the treatment of vulvar cancer was investigated in a retrospective series (I). The expression of VEGF-C in malignant vulvar tumors and SLN metastases was then assessed and the results were combined with information on surgical stage, nodal involvement and clinical course of the disease (II). A pilot study of ovarian SLN mapping with conventional tracers (blue dye and ^{99m}Tc-colloid) was conducted in patients undergoing pelvic and para-aortic lymphadenectomies for high-risk endometrial cancer (III). After that, the same method was tested in a series of patients with ovarian tumors (IV).

The main findings and conclusions are:

1. The use of blue dye and ^{99m}Tc-colloid in patients with early stage vulvar cancer yielded a high SLN detection rate of 100 %, and there were no false-negative sentinel nodes. The combined method seemed safe to use in early vulvar cancer.
2. VEGF-C was expressed moderately or strongly by 67% of the malignant vulvar tumors in their invasive edges. Expression was also present in 76 % of the SLN metastases. Positive VEGF-C expression was not significantly associated with higher surgical stage, presence of SLN metastasis, higher recurrence rate or poorer prognosis. Negative VEGF-C expression in SLN metastases might serve as an indicator of metastasis-free non-SLN, but this assumption needs to be verified in a larger study.
3. The use of conventional tracers was feasible for ovarian SLN mapping during laparotomy; the detection rate was 94 %. The SLNs were located in certain para-aortic regions, which was in line with previous studies. SLNs related to the left ovary were mostly detected above the IMA level, whereas almost all SLNs related to the right ovary were located below that level. The difference was statistically significant ($p=0.001$).
4. Study IV showed that a one-spot mesovarian tracer injection is enough to allow the detection of SLNs in all patients (detection rate 100 %). Most of the SLNs were located in the para-aortic region; 30 % of them with pelvic

SLNs and 60 % without pelvic SLNs. Isolated pelvic SLNs were rare (10 %). One patient had a nodal metastasis, and a positive SLN predicted the involvement correctly (FNR 0 %). The SLN concept deserves to be investigated further in relation to the surgical treatment of early ovarian cancer.

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9 REFERENCES

Alazraki, N.P. et al., 1997. Lymphoscintigraphy, the sentinel node concept, and the intraoperative gamma probe in melanoma, breast cancer, and other potential cancers. *Seminars in Nuclear Medicine*, 27(1), pp.55–67.

Albrecht, I. & Christofori, G., 2011. Molecular mechanisms of lymphangiogenesis in development and cancer. *International Journal of Developmental Biology*, 55(4–5), pp.483–494.

Alex, J.C. et al., 1993. Gamma-probe-guided lymph node localization in malignant melanoma. *Surgical Oncology*, 2(5), pp.303–308.

Alex, J.C. & Krag, D.N., 1993. Gamma-probe guided localization of lymph nodes. *Surgical Oncology*, 2(3), pp.137–43.

Alkatout, I. et al., 2015. Vulvar cancer: epidemiology, clinical presentation, and management options. *International journal of women's health*, 7, pp.305–13.

Amchova, P., Kotolova, H. & Ruda-Kucerova, J., 2015. Health safety issues of synthetic food colorants. *Regulatory Toxicology and Pharmacology*, 73(3), pp.914–922.

Angelucci, M. et al., 2016. Laparoscopic indocyanine green sentinel lymph node mapping in early ovarian cancer. A pilot study and review of the literature. *Italian Journal of Gynaecology and Obstetrics*, 28(5), pp.23–28.

Angioli, R. et al., 2008. Update on lymphadenectomy in early and advanced ovarian cancer. *Current Opinion in Obstetrics and Gynecology*, 20(1), pp.34–39.

Aragona, A.M. et al., 2012. Tailoring the Treatment of Locally Advanced Squamous Cell Carcinoma of the Vulva: Neoadjuvant Chemotherapy Followed by Radical Surgery. *International Journal of Gynecological Cancer*, 22(7), pp.1258–1263.

Ayhan, A. et al., 2008. Prognostic factors for recurrence and survival in primary vulvar squamous cell cancer. *Acta Obstetricia et Gynecologica Scandinavica*, 87(11), pp.1143–1149.

Bachmann, C. et al., 2012. Nodal status-its impact on prognosis in advanced ovarian cancer. *Journal of Cancer Research and Clinical Oncology*, 138(2), pp.261–267.

Baiocchi, G. et al., 2013. Prognostic value of the number and laterality of metastatic inguinal lymph nodes in vulvar cancer: Revisiting the FIGO staging system. *European Journal of Surgical Oncology*, 39(7), pp.780–785.

Bakkar, R. et al., 2014. Stage IIIC Ovarian/Peritoneal Serous Carcinoma: A Heterogeneous Group of Patients With Different Prognoses. *International Journal of Gynecological Pathology*, 33(3), pp.302–308.

Barlin, J.N. et al., 2012. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: Beyond removal of blue nodes. *Gynecologic Oncology*, 125(3), pp.531–535.

Barton, D.P.J., Naik, R. & Herod, J., 2009. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC Trial) A Randomized Study. *International Journal of Gynecological Cancer*, 19(8), p.1465.

Bats, A.-S. et al., 2013. The sentinel node technique detects unexpected drainage pathways and allows nodal ultrastaging in early cervical cancer: insights from the multicenter prospective SENTICOL study. *Annals of surgical oncology*, 20(2), pp.413–22.

Van Beekhuizen, H.J. et al., 2014. Lymph Node Count at Inguinofemoral Lymphadenectomy and Groin Recurrences in Vulvar Cancer. *International Journal of Gynecological Cancer*, 24, pp.773–778.

Bentivegna, E. et al., 2016. Fertility-sparing surgery in epithelial ovarian cancer : a systematic review of oncological issues. *Ann Oncol*, Nov(27(11)), pp.1994–2004.

van den Berg, N.S. et al., 2014. Hybrid tracers for sentinel node biopsy. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 58(2), pp.193–206.

van den Berg, N.S., van Leeuwen, F.W.B. & van der Poel, H.G., 2012. Fluorescence guidance in urologic surgery. *Current Opinion in Urology*, 22, pp.109–120.

Betterman, K.L. & Harvey, N.L., 2016. The lymphatic vasculature: Development and role in shaping immunity. *Immunological Reviews*, 271(1), pp.276–292.

Bézu, C. et al., 2011. Anaphylactic response to blue dye during sentinel lymph node biopsy. *Surgical Oncology*, 20(1), pp.e55–e59.

Bockisch, A. et al., 2009. Hybrid Imaging by SPECT/CT and PET/CT: Proven Outcomes in Cancer Imaging. *Seminars in Nuclear Medicine*, 39(4), pp.276–289.

Bogani, G. et al., 2017. Minimally Invasive Surgical Staging in Early-stage Ovarian Carcinoma: A Systematic Review and Meta-analysis. *Journal of minimally invasive gynecology*, 24(4), pp.552–562.

Boone, B. et al., 2008. The role of VEGF-C staining in predicting regional metastasis in melanoma. *Virchows Archiv*, 453(3), pp.257–265.

Borgstein, P.J. et al., 1998. Sentinel Lymph Node Biopsy in Breast Cancer: Guidelines and Pitfalls of Lymphoscintigraphy and Gamma Probe Detection. *Journal of the American College of Surgeons*, 186(3), pp.275–283.

Bromham, N. et al., 2017. Axillary treatment for operable primary breast cancer. *The Cochrane database of systematic reviews*, 1, p.CD004561.

Buda, A. et al., 2016. Impact of Indocyanine Green for Sentinel Lymph Node Mapping in Early Stage Endometrial and Cervical Cancer: Comparison with Conventional Radiotracer (99m)Tc and/or Blue Dye. *Annals of surgical oncology*, 23(7), pp.2183–2191.

Buda, A. et al., 2017. Near-infrared Fluorescence-guided Sentinel Node Mapping of the Ovary With Indocyanine Green in a Minimally Invasive Setting: A Feasible Study. *Journal of Minimally Invasive Gynecology*, 24(1), pp.165–170.

Burke, T.W. et al., 1995. Surgical therapy of T1 and T2 vulvar carcinoma: further experience with radical wide excision and selective inguinal lymphadenectomy. *Gynecologic oncology*, 57(2), pp.215–220.

Cabanas, R.M., 1977. An approach for the treatment of penile carcinoma. *Cancer*, 39(2), pp.456–

466.

Cao, W. et al., 2014. VEGF-C expression is associated with the poor survival in gastric cancer tissue. *Tumour Biol*, 35(4), pp.3377–3383.

Chagpar, A.B. et al., 2005. Factors predicting failure to identify a sentinel lymph node in breast cancer. *Surgery*, 138(1), pp.56–63.

Chokoeva, A.A. et al., 2015. Vulvar cancer: a review for dermatologists. *Wiener Medizinische Wochenschrift*, 165(7–8), pp.164–177.

Chung, M.K. et al., 2012. Lymphatic vessels and high endothelial venules are increased in the sentinel lymph nodes of patients with oral squamous cell carcinoma before the arrival of tumor cells. *Annals of Surgical Oncology*, 19(5), pp.1595–1601.

Cianfarani, F. et al., 2012. Expression of vascular endothelial growth factor-C in primary cutaneous melanoma predicts sentinel lymph node positivity. *Journal of Cutaneous Pathology*, 39(9), pp.826–834.

Cibula, D. et al., 2012. Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. *Gynecologic Oncology*, 124(3), pp.496–501.

Cibula, D., Oonk, M.H.M. & Abu-Rustum, N.R., 2015. Sentinel lymph node biopsy in the management of gynecologic cancer. *Current opinion in obstetrics & gynecology*, 27, pp.66–72.

Cochran, A.J. et al., 2015. Is Sentinel Node Susceptibility to Metastases Related to Nodal Immune Modulation ? *Cancer Journal*, 21(1), pp.39–46.

Cohn, D.E. et al., 2002. Prospective evaluation of positron emission tomography for the detection of groin node metastases from vulvar cancer. *Gynecologic oncology*, 85(1), pp.179–84.

Coleman, R.L. et al., 2013. Is bilateral lymphadenectomy for midline squamous carcinoma of the vulva always necessary? An analysis from Gynecologic Oncology Group (GOG) 173. *Gynecologic Oncology*, 128, pp.155–159.

Colombo, N. et al., 2016. Bevacizumab in ovarian cancer: Focus on clinical data and future perspectives. *Critical Reviews in Oncology/Hematology*, 97, pp.335–348.

Colombo, N. et al., 2015. ESMO–ESGO–ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. *Radiotherapy and Oncology*, 117(3), pp.559–581.

Cormier, B. et al., 2011. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. *Gynecologic Oncology*, 122(2), pp.275–280.

Coso, S., Bovay, E. & Petrova, T. V., 2014. Pressing the right buttons: Signaling in lymphangiogenesis. *Blood*, 123(17), pp.2614–2624.

Courtney-Brooks, M. et al., 2010. Does the number of nodes removed impact survival in vulvar cancer patients with node-negative disease? *Gynecologic Oncology*, 117, pp.308–311.

Cousins, A. et al., 2014. Clinical relevance of novel imaging technologies for sentinel lymph node identification and staging. *Biotechnology Advances*, 32(2), pp.269–279.

Creager, A.J. & Geisinger, K.R., 2002. Intraoperative evaluation of sentinel lymph nodes for breast carcinoma: current methodologies. *Advances in anatomic pathology*, 9(4), pp.233–43.

Crosbie, E.J. et al., 2010. The accuracy of the sentinel node procedure after excision biopsy in

squamous cell carcinoma of the vulva. *Surgical Oncology*, 19(4), pp.e150–e154.

Cserni, G., 2012. Intraoperative analysis of sentinel lymph nodes in breast cancer by one-step nucleic acid amplification. *Journal of clinical pathology*, 65(3), pp.193–9.

Czerniecki, B.J. et al., 2001. Revolutionary impact of lymphoscintigraphy and intraoperative sentinel node mapping in the clinical practice of oncology. *Seminars in Nuclear Medicine*, 31(2), pp.158–64.

Dadras, S.S. et al., 2005. Tumor lymphangiogenesis predicts melanoma metastasis to sentinel lymph nodes. *Modern Pathology*, 18(9), pp.1232–1242.

Darin, M.C. et al., 2016. Role of Indocyanine Green in Sentinel Node Mapping in Gynecologic Cancer: Is Fluorescence Imaging the New Standard? *Journal of Minimally Invasive Gynecology*, 23(2), pp.186–193.

Dauphine, C.E. et al., 2006. Intraoperative injection of technetium-99m sulfur colloid is effective in the detection of sentinel lymph nodes in breast cancer. *American Journal of Surgery*, 192(4), pp.423–426.

Davidson, B. & Tropé, C.G., 2014. Ovarian cancer: diagnostic, biological and prognostic aspects. *Women's health (London, England)*, 10(5), pp.519–33.

Deppe, G. et al., 2013. Chemotherapy of vulvar cancer: A review. *Wiener Klinische Wochenschrift*, 125(5–6), pp.119–128.

Deppe, G., Mert, I. & Winer, I.S., 2014. Management of squamous cell vulvar cancer: a review. *The journal of obstetrics and gynaecology research*, 40(5), pp.1217–25.

Diab, Y., 2017. Sentinel Lymph Nodes Mapping in Cervical Cancer: a Comprehensive Review. *International Journal of Gynecological Cancer*, 27(1), pp.154–158.

van Diest, P.J. et al., 1999. Pathological investigation of sentinel lymph nodes. *European Journal of Nuclear Medicine*, 26(4 Suppl), pp.S43–S49.

Dittmer, C. et al., 2011. Epidemiology of vulvar and vaginal cancer in Germany. *Archives of Gynecology and Obstetrics*, 284(1), pp.169–174.

Van Doorn, H.C. et al., 2016. Repeat sentinel lymph node procedure in patients with recurrent vulvar squamous cell carcinoma is feasible. *Gynecologic Oncology*, 140(3), pp.415–419.

Ebert, B. et al., 2011. Cyanine dyes as contrast agents for near-infrared imaging in vivo: acute tolerance, pharmacokinetics, and fluorescence imaging. *Journal of biomedical optics*, 16(6), pp.66003-1-66003–9.

Ehrisman, J. et al., 2016. Performance of sentinel lymph node biopsy in high-risk endometrial cancer. *Gynecologic Oncology Reports*, 17, pp.69–71.

El-Ghobashy, A.E. & Saidi, S.A., 2009. Sentinel lymph node sampling in gynaecological cancers: Techniques and clinical applications. *European Journal of Surgical Oncology*, 35, pp.675–685.

Ennik, T.A. et al., 2011. Effects of Previous Surgery on the Detection of Sentinel Nodes in Women With Vulvar Cancer. *International Journal of Gynecological Cancer*, 21(9), pp.1679–1683.

Estourgie, S.H. et al., 2003. What is a false-negative result for sentinel node procedures in breast cancer? *Journal of Surgical Oncology*, 82(3), pp.141–142.

Even-Sapir, E. et al., 2003. Lymphoscintigraphy for sentinel node mapping using a hybrid

SPECT/CT system. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, 44(9), pp.1413–20.

Fagotti, A. et al., 2008. Prospective validation of a laparoscopic predictive model for optimal cytoreduction in advanced ovarian carcinoma. *American Journal of Obstetrics and Gynecology*, 199(6).

Falcetta, F.S. et al., 2016. Laparoscopy versus laparotomy for FIGO stage I ovarian cancer. *The Cochrane database of systematic reviews*, 10, p.CD005344.

Faul, C.M. et al., 1997. Adjuvant radiation for vulvar carcinoma: Improved local control. *International Journal of Radiation Oncology Biology Physics*, 38(2), pp.381–389.

Fons, G. et al., 2009. Adjuvant radiotherapy in patients with vulvar cancer and one intra capsular lymph node metastasis is not beneficial. *Gynecologic Oncology*, 114(2), pp.343–345.

Forstner, R., Meissnitzer, M. & Cunha, T.M., 2016. Update on Imaging of Ovarian Cancer. *Current Radiology Reports*, 4(6), p.31.

Fournier, M. et al., 2009. Lymph node involvement in epithelial ovarian cancer sites and risk factors in a series of 355 patients. *International Journal of Gynecological Cancer*, 19(8), pp.1307–1313.

Frangioni, J. V., 2003. In vivo near-infrared fluorescence imaging. *Current Opinion in Chemical Biology*, 7(5), pp.626–634.

Gadducci, A. et al., 2012. Clinico-pathological and biological prognostic variables in squamous cell carcinoma of the vulva. *Critical Reviews in Oncology/Hematology*, 83, pp.71–83.

Gallego, E. et al., 2011. Stromal expression of vascular endothelial growth factor C is relevant to predict sentinel lymph node status in melanomas. *Virchows Archiv*, 458(5), pp.621–630.

Gasimli, K. et al., 2016. Lymph Node Involvement Pattern and Survival Differences of FIGO IIIC and FIGO IIIA1 Ovarian Cancer Patients After Primary Complete Tumor Debulking Surgery: A 10-Year Retrospective Analysis of the Tumor Bank Ovarian Cancer Network. *Annals of Surgical Oncology*, 23(4), pp.1279–1286.

Gasparri, M.L. et al., 2016. Circulating tumor cells as trigger to hematogenous spreads and potential biomarkers to predict the prognosis in ovarian cancer. *Tumor Biology*, 37(1), pp.71–75.

Giammarile, F. et al., 2014. The EANM clinical and technical guidelines for lymphoscintigraphy and sentinel node localization in gynaecological cancers. *European Journal of Nuclear Medicine and Molecular Imaging*, 41(7), pp.1463–1477.

Gill, B.S. et al., 2015. Impact of adjuvant chemotherapy with radiation for node-positive vulvar cancer: A National Cancer Data Base (NCDB) analysis. *Gynecologic Oncology*, 137, pp.365–372.

Gioux, S., Choi, H.S. & Frangioni, J. V., 2010. Image-guided surgery using invisible near-infrared light: Fundamentals of clinical translation. *Molecular Imaging*, 9(5), pp.237–255.

Gombos, Z. et al., 2005. Peritumoral lymphatic vessel density and vascular endothelial growth factor C expression in early-stage squamous cell carcinoma of the uterine cervix. *Clinical cancer research*, 11(23), pp.8364–8371.

Gordinier, M.E. et al., 2003. Groin recurrence in patients with vulvar cancer with negative nodes

on superficial inguinal lymphadenectomy. *Gynecologic Oncology*, 90(3), pp.625–628.

Te Grootenhuis, N.C. et al., 2016. Sentinel nodes in vulvar cancer: Long-term follow-up of the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V) i. *Gynecologic Oncology*, 140, pp.8–14.

Hacker, N.F., 2017. Quality control in ovarian cancer surgery. *Best Practice & Research Clinical Obstetrics and Gynaecology*, 41, pp.96–107.

Hacker, N.F., Eifel, P.J. & van der Velden, J., 2015. Cancer of the vulva. *International journal of gynaecology and obstetrics*, 131 Suppl, pp.S76-83.

Hacker, N.F. & Rao, A., 2016. Surgery for advanced epithelial ovarian cancer. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 41, pp.71–87.

Hagen, B. et al., 2016. Indocyanine green fluorescence imaging of lymph nodes during robotic-assisted laparoscopic operation for endometrial cancer. A prospective validation study using a sentinel lymph node surgical algorithm ☆. *Gynecologic Oncology*, 143(3), pp.479–483.

Handgraaf, H.J.M. et al., 2014. Real-time near-infrared fluorescence guided surgery in gynecologic oncology: A review of the current state of the art. *Gynecologic Oncology*, 135(3), pp.606–613.

Hassanzade, M. et al., 2013. Lymphatic mapping and sentinel node biopsy in squamous cell carcinoma of the vulva: Systematic review and meta-analysis of the literature. *Gynecologic Oncology*, 130(1), pp.237–245.

Hassanzadeh, M. et al., 2016. Lymphatic mapping and sentinel node biopsy in ovarian tumors: a study using intra-operative Tc-99m-Phytate and lymphoscintigraphy imaging. *Journal of Ovarian Research*, 9(1), p.55.

Hauspy, J. et al., 2007. Sentinel lymph node in vulvar cancer. *Cancer*, 110(5), pp.1015–1023.

Heaps, J.M. et al., 1990. Surgical-pathologic variables predictive of local recurrence in squamous cell carcinoma of the vulva. *Gynecologic oncology*, 38(3), pp.309–314.

Hinten, F. et al., 2015. Clitoral involvement of squamous cell carcinoma of the vulva: localization with the worst prognosis. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology*, 41(4), pp.592–598.

Hirakawa, S., 2009. From tumor lymphangiogenesis to lymphvascular niche. *Cancer Science*, 100(6), pp.983–989.

Hirakawa, S. et al., 2007. VEGF-C-induced lymphangiogenesis in sentinel lymph nodes promotes tumor metastasis to distant sites. *Blood*, 1009, pp.1010–1017.

Holloway, R.W. et al., 2017. Sentinel lymph node mapping and staging in endometrial cancer: A Society of Gynecologic Oncology literature review with consensus recommendations. *Gynecologic Oncology*, p.In press.

Holloway, R.W. et al., 2016. Sentinel lymph node mapping with staging lymphadenectomy for patients with endometrial cancer increases the detection of metastasis. *Gynecologic Oncology*, 141(2), pp.206–210.

Hoon, D.S. et al., 2014. Molecular analysis of sentinel lymph nodes and search for molecular signatures of the metastatic potential of breast cancer. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 58, pp.180–92.

Hoon, D.S.B. et al., 2006. Molecular mechanisms of metastasis. *Cancer and Metastasis Reviews*, 25(2),

pp.203–220.

Hoon Suh, D. et al., 2013. Improvements to the FIGO staging for ovarian cancer: Reconsideration of lymphatic spread and intraoperative tumor rupture. *Journal of Gynecologic Oncology*, 24(4), pp.352–358.

Horowitz, N.S. et al., 2012. Phase II trial of erlotinib in women with squamous cell carcinoma of the vulva. *Gynecologic Oncology*, 127(1), pp.141–146.

Hosseinian, L. et al., 2016. Methylene Blue: Magic Bullet for Vasoplegia? *Anesthesia and Analgesia*, 122(1), pp.194–201.

How, J. et al., 2017. Impact of sentinel lymph node mapping on recurrence patterns in endometrial cancer. *Gynecologic Oncology*, 144(3), pp.503–509.

de Hullu, J.A. et al., 2001. Sentinel lymph node detection in locally recurrent carcinoma of the vulva. *British Journal of Obstetrics and Gynaecology*, 108, pp.766–768.

De Hullu, J.A. et al., 2004. Pitfalls in the sentinel lymph node procedure in vulvar cancer. *Gynecologic Oncology*, 94(1), pp.10–15.

De Hullu, J.A. et al., 2002. Vulvar carcinoma: The price of less radical surgery. *Cancer*, 95(11), pp.2331–2338.

Hutteman, M., van der Vorst, J.R. & Vahrmeijer, A.L., 2012. Optimization of Near-Infrared Fluorescent Sentinel Lymph Node Mapping for Vulvar Cancer. *American Journal of Obstetrics & Gynecology*, 206(1), pp.1–11.

Huxley, N. et al., 2015. A systematic review and economic evaluation of intraoperative tests [RD-100i one-step nucleic acid amplification (OSNA) system and metasin test] for detecting sentinel lymph node metastases in breast cancer. *Health Technology Assessment*, 19(2), pp.1–246.

Hyde, S.E. et al., 2007. Squamous cell carcinoma of the vulva with bulky positive groin nodes - Nodal debulking versus full groin dissection prior to radiation therapy. *International Journal of Gynecological Cancer*, 17(1), pp.154–158.

Ignatov, T. et al., 2016. Adjuvant radiotherapy for vulvar cancer with close or positive surgical margins. *Journal of Cancer Research and Clinical Oncology*, 142(2), pp.489–495.

Jach, R. et al., 2011. Expression of vascular endothelial growth factors VEGF-C and -D, VEGFR-3, and comparison of lymphatic vessels density labeled with D2-40 antibodies as a prognostic factors in vulvar epithelial neoplasia (VIN) and invasiva vulvar cancer. *Neuroendocrinology Letters*, 32(4), pp.530–539.

Jain, R., Dandekar, P. & Patravale, V., 2009. Diagnostic nanocarriers for sentinel lymph node imaging. *Journal of Controlled Release*, 138(2), pp.90–102.

Jayson, G.C. et al., 2014. Ovarian cancer. *The Lancet*, 384(9951), pp.1376–1388.

Jelovac, D. & Armstrong, D.K.D., 2011. Recent progress in the diagnosis and treatment of ovarian cancer. *CA: a cancer journal for clinicians*, 61(3), pp.183–203.

Kadkhodayan, S. et al., 2015. Sentinel node biopsy for lymph nodal staging of uterine cervix cancer: a systematic review and meta-analysis of the pertinent literature. *European journal of surgical oncology*, 41(1), pp.1–20.

Kamran, M.W. et al., 2014. Whole-body [18F]fluoro-2-deoxyglucose positron emission

tomography scan as combined PET-CT staging prior to planned radical vulvectomy and inguinofemoral lymphadenectomy for squamous vulvar cancer: a correlation with groin node metastasis. *European Journal of Gynaecological Oncology*, 35(3), pp.230–235.

Kang, S. et al., 2011. Sentinel lymph node biopsy in endometrial cancer: Meta-analysis of 26 studies. *Gynecologic Oncology*, 123, pp.522–527.

Karatzanis, A.D. et al., 2012. Molecular pathways of lymphangiogenesis and lymph node metastasis in head and neck cancer. *European Archives of Oto-Rhino-Laryngology*, 269, pp.731–737.

Karnezis, A.N. et al., 2016. The disparate origins of ovarian cancers: pathogenesis and prevention strategies. *Nature Reviews Cancer*, 17(1), pp.65–74.

Kataria, K., Srivastava, A. & Qaiser, D., 2016. What Is a False Negative Sentinel Node Biopsy: Definition, Reasons and Ways to Minimize It? *Indian Journal of Surgery*, 78(5), pp.396–401.

Kelley, L.M. & Holmes, D.R., 2011. Tracer agents for the detection of sentinel lymph nodes in breast cancer: Current concerns and directions for the future. *Journal of Surgical Oncology*, 104(1), pp.91–96.

Khoury-Collado, F. et al., 2009. Improving sentinel lymph node detection rates in endometrial cancer: How many cases are needed? *Gynecologic Oncology*, 115(3), pp.453–455.

Khoury-Collado, F., St Clair, C. & Abu-Rustum, N.R., 2016. Sentinel Lymph Node Mapping in Endometrial Cancer: An Update. *The oncologist*, 21(4), pp.461–466.

Kilvaer, T.K. et al., 2015. Lymphangiogenic markers and their impact on nodal metastasis and survival in non-small cell lung cancer - A structured review with meta-analysis. *PLoS ONE*, 10(8), pp.1–17.

Kim, C.H. et al., 2013. Pathologic Ultrastaging Improves Micrometastasis Detection in Sentinel Lymph Nodes During Endometrial Cancer Staging. *Int J Gynecol Cancer*, 23(5), pp.964–970.

Kim, H.S. et al., 2013. Impact of intraoperative rupture of the ovarian capsule on prognosis in patients with early-stage epithelial ovarian cancer: A meta-analysis. *European Journal of Surgical Oncology*, 39(3), pp.279–289.

Kimmig, R., Rusch, P., et al., 2017. Aortic utero-ovarian sentinel nodes and left infrarenal aortic lymph node dissection by ICG supported navigation. *Gynecologic Oncology Reports*, 20, pp.22–23.

Kimmig, R., Buderath, P., et al., 2017. Surgical treatment of early ovarian cancer with compartmental resection of regional lymphatic network and indocyanine-green-guided targeted compartmental lymphadenectomy (TCL , paraaortic part). *Journal of Gynecologic Oncology*, 28(3), p.e41.

Klapdor, R. et al., 2017. Groin Recurrences in Node Negative Vulvar Cancer Patients After Sole Sentinel Lymph Node Dissection. *International Journal of Gynecological Cancer*, 27, pp.166–170.

Klapdor, R. et al., 2016. Outcome After Sentinel Lymph Node Dissection in Vulvar Cancer: A Subgroup Analysis of the AGO-CaRE-1 Study. *Annals of Surgical Oncology*, 487.

Klapdor, R. et al., 2015. SPECT/CT for SLN dissection in vulvar cancer: Improved SLN detection and dissection by preoperative three-dimensional anatomical localisation. *Gynecologic Oncology*, 138(3), pp.590–596.

Kleppe, M. et al., 2011. Lymph node metastasis in stages i and II ovarian cancer: A review. *Gynecologic Oncology*, 123, pp.610–614.

Kleppe, M. et al., 2014. The Detection of Sentinel Nodes in Ovarian Cancer: A Feasibility Study. *Journal of Nuclear Medicine*, 55, pp.1799–1804.

Kleppe, M. et al., 2015. Understanding Lymphatic Drainage Pathways of the Ovaries to Predict Sites for Sentinel Nodes in Ovarian Cancer. *International Journal of Gynecological Cancer*, 25(8), pp.1405–1414.

Kojima, H. et al., 2005. Clinical significance of vascular endothelial growth factor-C and vascular endothelial growth factor receptor 3 in patients with T1 lung adenocarcinoma. *Cancer*, 104(8), pp.1668–1677.

Kole, M. & Robison, K., 2016. Sentinel Lymph Node Evaluation in Vulvar Cancer: The New Standard of Care. *Journal of Cancer Clinical Trials*, 1(3), p.1000115.

Koshiyama, M., Matsumura, N. & Konishi, I., 2017. Subtypes of Ovarian Cancer and Ovarian Cancer Screening. *Diagnostics*, 7(1), p.12.

Krag, D.N., 1998. Minimal Access Surgery for Staging Regional Lymph Nodes: The Sentinel-node Concept. *Current Problems in Surgery*, 35(11), pp.952–1016.

Krag, D.N. et al., 1993. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surgical Oncology*, 2(6), p.335–9; discussion 340.

Kuol, N. et al., 2017. The mechanisms tumor cells utilize to evade the host's immune system. *Maturitas*, (April), p.In Press.

Lai, H.C. et al., 2011. Interference of patent blue dye with pulse oximetry readings, methemoglobin measurements, and blue urine in sentinel lymph node mapping: A case report and review of the literature. *Acta Anaesthesiologica Taiwanica*, 49(4), pp.162–164.

Landkroon, A.P., De Hullu, J.A. & Ansink, A.C., 2006. Repeat sentinel lymph node procedure in vulval carcinoma. *British Journal of Obstetrics and Gynaecology*, 113(11), pp.1333–1336.

Layfield, D.M. et al., 2011. Intraoperative assessment of sentinel lymph nodes in breast cancer. *British Journal of Surgery*, 98(1), pp.4–17.

Le, T. et al., 2007. The definition of optimal inguinal femoral nodal dissection in the management of vulva squamous cell carcinoma. *Annals of Surgical Oncology*, 14(7), pp.2128–2132.

Lennox, G.K. & Covens, A.L., 2017. Can sentinel lymph node biopsy replace pelvic lymphadenectomy for early cervical cancer? *Gynecologic Oncology*, 144(1), pp.16–20.

Levenback, C. et al., 2001. Intraoperative lymphatic mapping and sentinel node identification with blue dye in patients with vulvar cancer. *Gynecologic oncology*, 83(2), pp.276–81.

Levenback, C. et al., 1994. Intraoperative lymphatic mapping for vulvar cancer. *Obstetrics and Gynecology*, 84(2), pp.163–167.

Levenback, C.F. et al., 2012. Lymphatic mapping and sentinel lymph node biopsy in women with squamous cell carcinoma of the vulva: a gynecologic oncology group study. *Journal of Clinical Oncology*, 30(31), pp.3786–3791.

Levenback, C.F. et al., 2009. Sentinel lymph node biopsy in patients with gynecologic cancers Expert panel statement from the International Sentinel Node Society Meeting, February 21, 2008. In *Gynecologic oncology*. pp. 151–156.

Liersch, R. et al., 2012. Induced lymphatic sinus hyperplasia in sentinel lymph nodes by VEGF-C as the earliest premetastatic indicator. *International Journal of Oncology*, 41(6), pp.2073–2078.

Lohela, M. et al., 2009. VEGFs and receptors invelved in angiogenesis versus lymphangiogenesis. *Current Opinion in Cell Biology*, 21, pp.154–165.

López-Ruiz, M.E. et al., 2016. One-step nucleic acid amplification (OSNA) for the detection of sentinel lymph node metastasis in endometrial cancer. *Gynecologic Oncology*, 143(1), pp.54–59.

Louis-Sylvestre, C. et al., 2005. Sentinel node localization should be interpreted with caution in midline vulvar cancer. *Gynecologic Oncology*, 97(1), pp.151–154.

Luchini, C. et al., 2016. Prognostic implications of extranodal extension in node-positive squamous cell carcinoma of the vulva: A systematic review and meta-analysis. *Surgical oncology*, 25(1), pp.60–65.

Madeddu, G. & Spanu, A., 2004. Use of tomographic nuclear medicine procedures, SPECT and pinhole SPECT, with cationic lipophilic radiotracers for the evaluation of axillary lymph node status in breast cancer patients. *European Journal of Nuclear Medicine and Molecular Imaging*, 31(1), pp.S23–S34.

Madu, M.F., Wouters, M.W.J.M. & van Akkooi, A.C.J., 2017. Sentinel node biopsy in melanoma: Current controversies addressed. *European Journal of Surgical Oncology*, 43(3), pp.517–533.

Maggioni, a et al., 2006. Randomised study of systematic lymphadenectomy in patients with epithelial ovarian cancer macroscopically confined to the pelvis. *British journal of cancer*, 95(6), pp.699–704.

Mahner, S. et al., 2015. Adjuvant therapy in lymph node-positive vulvar cancer: The AGO-CaRE-1 study. *Journal of the National Cancer Institute*, 107(3), p.dju426.

Malila, N. et al., 2017. Interactive cancer statistics. *Finnish Cancer Registry statistics*, p.1. Available at: http://tilastot.syoparekisteri.fi/syovat/?_inputs_&language=%22en%22 [Accessed June 29, 2017].

Mansfield, A.S. et al., 2012. The presence of sinusoidal CD163(+) macrophages in lymph nodes is associated with favorable nodal status in patients with breast cancer. *Virchows Archiv : an international journal of pathology*, 461(6), pp.639–646.

Mariani, A. et al., 2008. Prospective assessment of lymphatic dissemination in endometrial cancer: A paradigm shift in surgical staging. *Gynecologic Oncology*, 109(1), pp.11–18.

Masannat, Y. et al., 2006. Properties and characteristics of the dyes injected to assist axillary sentinel node localization in breast surgery. *European Journal of Surgical Oncology*, 32(4), pp.381–384.

Matsuo, K. et al., 2014. Effect of Lymphovascular Space Invasion on Survival of Stage I Epithelial Ovarian Cancer. *Obstetrics & Gynecology*, 123(5), pp.957–965.

Matsuo, K. et al., 2012. Significance of lymphovascular space invasion in epithelial ovarian cancer. *Cancer Medicine*, 1(2), pp.156–164.

Meads, C. et al., 2014. Sentinel lymph node biopsy in vulval cancer: systematic review and meta-analysis. *British journal of cancer*, 110(12), pp.2837–2846.

Messina, J.L. & Rosa, M., 2015. Pathologic evaluation of sentinel nodes. *Cancer Journal*, 21(1),

pp.33–38.

Micheletti, L., Bogliatto, F. & Massobrio, M., 2005. Groin lymphadenectomy with preservation of femoral fascia: Node dissection for treatment of vulvar cancer. *World Journal of Surgery*, 29, pp.1268–1276.

Micheletti, L. & Preti, M., 2014. Surgery of the vulva in vulvar cancer. *Best Practice and Research: Clinical Obstetrics and Gynaecology*, 28(7), pp.1074–1087.

Mieog, J.S.D. et al., 2011. Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer. *Annals of surgical oncology*, 18(9), pp.2483–91.

Morice, P. et al., 2003. Lymph node involvement in epithelial ovarian cancer: Analysis of 276 pelvic and paraaortic lymphadenectomies and surgical implications. *Journal of the American College of Surgeons*, 197(2), pp.198–205.

Morice, P. et al., 2011. Recommendations of the Fertility Task Force of the European Society of Gynecologic Oncology about the conservative management of ovarian malignant tumors. *International Journal of Gynecological Cancer*, 21(5), pp.951–963.

Morrison, J. et al., 2012. Chemotherapy versus surgery for initial treatment in advanced ovarian epithelial cancer. *Cochrane Database Systematic Reviews*, 15(8), p.CD005343.

Morrow, M. et al., 1999. Learning sentinel node biopsy: results of a prospective randomized trial of two techniques. *Surgery*, 126(4), pp.714–720.

Morton, D.L. et al., 1992. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Archives of Surgery*, 127(4), pp.392–399.

Motomura, K., 2015. Sentinel node biopsy for breast cancer: past, present, and future. *Breast cancer (Tokyo, Japan)*, 22(3), pp.212–220.

Mutch, D.G., 2009. The new FIGO staging system for cancers of the vulva, cervix, endometrium and sarcomas. *Gynecologic Oncology*, 115(3), pp.325–328.

Nathanson, S.D., Shah, R. & Rosso, K., 2015. Sentinel lymph node metastases in cancer: causes, detection and their role in disease progression. *Seminars in Cell & Developmental Biology*, 38, pp.106–16.

Navalkissoor, S. et al., 2015. SPECT/CT in imaging sentinel nodes. *Clinical and Translational Imaging*, 3(3), pp.203–215.

NCCN, 2016. Vulvar cancer (Squamous Cell Carcinoma) Version 1.2017. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)*, pp.1–24.

Negishi, H. et al., 2004. Lymphatic mapping and sentinel node identification as related to the primary sites of lymph node metastasis in early stage ovarian cancer. *Gynecologic Oncology*, 94(1), pp.161–166.

Nick, A.M. et al., 2016. A framework for personalized surgical approach to ovarian cancer. *Nature reviews. Clinical oncology.*, 1848(4), pp.3047–3054.

Nomura, H. et al., 2010. Lymph node metastasis in grossly apparent stages I and II epithelial ovarian cancer. *International Journal of Gynecological Cancer*, 20(3), pp.341–345.

Nooij, L.S. et al., 2015. Groin surgery and risk of recurrence in lymph node positive patients with vulvar squamous cell carcinoma. *Gynecologic Oncology*, 139(3), pp.458–464.

Nooij, L.S. et al., 2016. Tumour-free margins in vulvar squamous cell carcinoma: Does distance really matter? *European Journal of Cancer*, 65, pp.139–149.

Nowak, M. et al., 2015. Current clinical application of serum biomarkers to detect ovarian cancer. *Przeglad Menopauzalny*, 14(4), pp.254–259.

Nyberg, R.H., Korkola, P. & Mäenpää, J.U., 2017. Sentinel Node and Ovarian Tumors: a Series of 20 Patients. *International Journal of Gynecologic Cancer*, 27(4), pp.684–689.

Näyhä, V. V & Stenbäck, F.G., 2007. Increased angiogenesis is associated with poor prognosis of squamous cell carcinoma of the vulva. *Acta Obstetricia et Gynecologica Scandinavica*, 86, pp.1392–1397.

Oldan, J.D. & Patel, P.S., 2014. Positron Emission Tomography/Computed Tomography for Gynecologic Malignancies. *Obstetrical & Gynecological Survey*, 69(4), pp.545–556.

Oonk, M.H. et al., 2010. Size of sentinel-node metastasis and chances of non-sentinel-node involvement and survival in early stage vulvar cancer: Results from GROINSS-V, a multicentre observational study. *The Lancet Oncology*, 11(7), pp.646–652.

Oonk, M.H.M. et al., 2017. European Society of Gynaecological Oncology Guidelines for the Management of Patients With Vulvar Cancer. *International Journal of Gynecological Cancer*, 27(4), pp.832–837.

Oonk, M.H.M., Hollema, H. & van der Zee, A.G.J., 2015. Sentinel node biopsy in vulvar cancer: Implications for staging. *Best practice & research. Clinical obstetrics & gynaecology*, 29(6), pp.812–821.

Origoni, M. et al., 1992. Prognostic value of pathological patterns of lymph node positivity in squamous cell carcinoma of the vulva stage III and IVA FIGO. *Gynecologic oncology*, 45(3), pp.313–6.

Ouldamer, L. et al., 2012. Unusual localizations of sentinel lymph nodes in early stage cervical cancer: A review. *Surgical Oncology*, 21, pp.e153–e157.

Paik, E.S. et al., 2015. Survival analysis of revised 2013 FIGO staging classification of epithelial ovarian cancer and comparison with previous FIGO staging classification. *Obstetrics & gynecology science*, 58(2), pp.124–34.

Paladini, D. et al., 1994. Prognostic significance of lymph node variables in squamous cell carcinoma of the vulva. *Cancer*, 74(9), pp.2491–2496.

Panici, P.B. et al., 2008. Systematic pelvic lymphadenectomy vs no lymphadenectomy in early-stage endometrial carcinoma: Randomized clinical trial. *Journal of the National Cancer Institute*, 100(23), pp.1707–1716.

Panici, P.B. & Angioli, R., 2002. Role of lymphadenectomy in ovarian cancer. *Best Practice and Research: Clinical Obstetrics and Gynaecology*, 16(4), pp.529–551.

Paño, B. et al., 2015. Pathways of Lymphatic Spread in Gynecologic Malignancies. *RadioGraphics*, 35(3), pp.916–945.

Parthasarathy, A. et al., 2006. The benefit of adjuvant radiation therapy in single-node-positive squamous cell vulvar carcinoma. *Gynecologic Oncology*, 103(3), pp.1095–1099.

Pastushenko, I. et al., 2016. Increased Angiogenesis and Lymphangiogenesis in Metastatic Sentinel Lymph Nodes Is Associated With Nonsentinel Lymph Node Involvement and

Distant Metastasis in Patients With Melanoma. *The American Journal of Dermatopathology*, 38(5), pp.338–46.

Pecorelli, S., Zigliani, L. & Odicino, F., 2009. Revised FIGO staging for carcinoma of the cervix. *International Journal of Gynecology and Obstetrics*, 105, pp.107–108.

Peng, J. et al., 2013. Prognostic significance of vascular endothelial growth factor expression in esophageal carcinoma: a meta-analysis. *Journal of Balkan Union of Oncology*, 18(2), pp.398–406.

Penick, E. et al., 2017. Vulva. In D. S. Cho et al., eds. *Principles and practice of gynecologic cancer*. Philadelphia: Wolters Kluwer, p. 1024.

Pepa, C. Della et al., 2015. Ovarian cancer standard of care: Are there real alternatives? *Chinese Journal of Cancer*, 34(1), pp.17–27.

Pereira, E.R. et al., 2015. The lymph node microenvironment and its role in the progression of metastatic cancer. *Seminars in Cell and Developmental Biology*, 38, pp.98–105.

Del Pino, M., Rodriguez-Carunchio, L. & Ordi, J., 2013. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology*, 62(1), pp.161–75.

Van Der Ploeg, I.M.C. et al., 2008. The hybrid SPECT/CT as an additional lymphatic mapping tool in patients with breast cancer. *World Journal of Surgery*, 32(9), pp.1930–1934.

Podgrabinska, S. & Skobe, M., 2014. Role of lymphatic vasculature in regional and distant metastases. *Microvascular Research*, 95, pp.46–52.

Podratz, K.C., Symmonds, R.E. & Taylor, W.F., 1982. Carcinoma of the vulva: analysis of treatment failures. *American journal of obstetrics and gynecology*, 143(3), pp.340–351.

Polom, K. et al., 2011. Current trends and emerging future of indocyanine green usage in surgery and oncology: A literature review. *Cancer*, 117(21), pp.4812–4822.

Powless, C.A. et al., 2011. Risk factors for lymph node metastasis in apparent early-stage epithelial ovarian cancer: Implications for surgical staging. *Gynecologic Oncology*, 122, pp.536–540.

Prat, J., 2015. Ovarian, fallopian tube and peritoneal cancer staging: Rationale and explanation of new FIGO staging 2013. *Best Practice and Research: Clinical Obstetrics and Gynaecology*, 29, pp.858–869.

Prat, J., 2014. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. *International Journal of Gynecology and Obstetrics2*, 124, pp.1–5.

Preti, M. et al., 2014. Vulvar intraepithelial neoplasia. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 28(7), pp.1051–1062.

Raspagliesi, F. et al., 2006. Clinical and pathological prognostic factors in squamous cell carcinoma of the vulva. *Gynecologic Oncology*, 102(2), pp.333–337.

Raspagliesi, F. et al., 2014. Role of paclitaxel and cisplatin as the neoadjuvant treatment for locally advanced squamous cell carcinoma of the vulva. *Journal of Gynecologic Oncology*, 25(1), pp.22–29.

Reade, C.J., Eiriksson, L.R. & Mackay, H., 2014. Systemic therapy in squamous cell carcinoma of the vulva: Current status and future directions. *Gynecologic Oncology*, 132(3), pp.780–789.

Robison, K. et al., 2014. Long-term follow-up of vulvar cancer patients evaluated with sentinel

lymph node biopsy alone. *Gynecologic Oncology*, 133(3), pp.416–420.

Rossi, E.C. et al., 2017. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. *The Lancet Oncology*, 18(3), pp.384–392.

Saad, Z.Z. & Buscombe, J.R., 2015. Sentinel lymph node: established and new areas of use. *Clinical and Translational Imaging*, 3(3), pp.225–236.

Saha, S. et al., 2016. Safety of radioactive sentinel node biopsy for breast cancer and the pregnant surgeon – A review. *International Journal of Surgery*, 36, pp.298–304.

Sahdev, A., 2016. CT in ovarian cancer staging: How to review and report with emphasis on abdominal and pelvic disease for surgical planning. *Cancer Imaging*, 16(1), pp.1–9.

Santiago, I.A., Gomes, A.P. & Heald, R.J., 2016. An ontogenetic approach to gynecologic malignancies. *Insights into Imaging*, 7, pp.329–339.

Sappey, M.P.C., 1874. *Anatomie, physiologie, pathologie des vaisseaux lymphatiques considérés chez l'homme et les vertébrés* Première p. A. Delahaye, ed., Paris, France: Libraire-Editeur.

Schaafsma, B.E. et al., 2013. Near-infrared fluorescence sentinel lymph node biopsy in vulvar cancer: A randomised comparison of lymphatic tracers. *BJOG*, 120, pp.758–764.

Schaafsma, B.E. et al., 2011. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *Journal of Surgical Oncology*, 104(3), pp.323–332.

Schauer, A.J. et al., 2005a. General Techniques in Surgical Investigations. In U. Heilmann, ed. *The Sentinel Lymph Node Concept*. Berlin Heidelberg: Springer-Verlag Berlin Heidelberg, pp. 89–99.

Schauer, A.J. et al., 2005b. Lymphatic Drainage to the SLN. In U. Heilmann, ed. *The Sentinel Lymph Node Concept*. Berlin Heidelberg: Springer-Verlag Berlin Heidelberg, pp. 51–57.

Schauer, A.J. et al., 2005c. Main Techniques of Sentinel Lymph Node Labeling. In U. Heilmann, ed. *The Sentinel Lymph Node Concept*. Berlin Heidelberg: Springer-Verlag Berlin Heidelberg, pp. 5–9.

Schuurman, M.S. et al., 2013. Trends in incidence and survival of Dutch women with vulvar squamous cell carcinoma. *European Journal of Cancer*, 49(18), pp.3872–80.

Sharma, D.N., 2012. Radiation in vulvar cancer. *Current opinion in Obstetrics & Gynecology*, 24, pp.24–30.

Shylasree, T.S., Bryant, A. & Howells, R.E.J., 2011. Chemoradiation for advanced primary vulvar cancer. *The Cochrane Library*, (4), pp.1–3.

Sinno, A.K. et al., 2014. A comparison of colorimetric versus fluorometric sentinel lymph node mapping during robotic surgery for endometrial cancer. *Gynecologic Oncology*, 134(2), pp.351–361.

Sleeman, J.P., 2015. Pre-metastatic conditioning of organ microenvironments by tumors: beyond preparing the soil. *Journal of Molecular Medicine*, 93(11), pp.1171–1172.

Sobin, L. et al., 2015. Principles of cancer staging. In B. O'Sullivan et al., eds. *UICC Manual of Clinical Oncology*. Chichester, England: Wiley-Blackwell, pp. 34–39.

Speth, S.C.J.M. et al., 2017. Comparison of intra-operative gamma probe detection with postoperative SPECT/CT of sentinel nodes related to the ovary. *Journal of Nuclear Medicine*, 58, pp.243–245.

Stacker, S.A. et al., 2014. Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nature Reviews Cancer*, 14(3), pp.159–172.

van der Steen, S. et al., 2010. New FIGO staging system of vulvar cancer indeed provides a better reflection of prognosis. *Gynecologic Oncology*, 119(3), pp.520–525.

Stehman, F.B., Ali, S. & DiSaia, P.J., 2009. Node count and groin recurrence in early vulvar cancer: A Gynecologic Oncology Group study. *Gynecologic Oncology*, 113(1), pp.52–56.

Stoffels, I. et al., 2014. Cost-effectiveness of preoperative SPECT/CT combined with lymphoscintigraphy vs. lymphoscintigraphy for sentinel lymph node excision in patients with cutaneous malignant melanoma. *European Journal of Nuclear Medicine and Molecular Imaging*, 41(9), pp.1723–1731.

Strien, L., Leidenius, M. & Heikkilä, P., 2012. False-positive and false-negative sentinel node findings in 473 breast cancers. *Human Pathology*, 43(11), pp.1940–1947.

Suh, D.H. et al., 2016. Major clinical research advances in gynecologic cancer in 2015. *J Gynecol Oncol*, 27(6), p.e53.

Sundar, S., Neal, R.D. & Kehoe, S., 2015. Diagnosis of ovarian cancer. *BMJ*, 351(September), p.h4443.

Suster, S. & Wong, T.Y., 1994. On the discriminatory value of anti-HPCA-1 (CD-34) in the differential diagnosis of benign and malignant cutaneous vascular proliferations. *The American Journal of Dermatopathology*, 16(4), pp.355–363.

Suutari, J. (Säteilyturvakeskus), 2017. Röntgentutkimusten säteilyannoksia. *Säteily terveydenhuollossa*. Available at: http://www.stuk.fi/aiheet/sateily-terveydenhuollossa/rontgentutkimukset/rontgentutkimusten-sateilyannoksia [Accessed October 8, 2017].

Sznurkowski, J.J., 2016. Vulvar cancer: initial management and systematic review of literature on currently applied treatment approaches. *European Journal of Cancer Care*, 25(4), pp.638–646.

Sznurkowski, J.J., Milczek, T. & Emerich, J., 2013. Prognostic factors and a value of 2009 FIGO staging system in vulvar cancer. *Arch Gynecol Obstet*, 287, pp.1211–1218.

Tabbaa, Z.M. et al., 2012. Impact of the new FIGO 2009 staging classification for vulvar cancer on prognosis and stage distribution. *Gynecologic Oncology*, 127(1), pp.147–152.

Tamaki, Y., 2016. One-step nucleic acid amplification (OSNA): where do we go with it? *International Journal of Clinical Oncology*, 22(1), pp.1–8.

Tamaki, Y., 2015. One-step nucleic acid amplification assay (OSNA) for sentinel lymph node biopsy. *Breast Cancer*, 22(3), pp.230–4.

Tammela, T. et al., 2005. The biology of vascular endothelial growth factors. *Cardiovascular Research*, 65(3), pp.550–563.

Tanaka, E. et al., 2006. Image-Guided Oncologic Surgery Using Invisible Light: Completed Pre-Clinical Development for Sentinel Lymph Node Mapping. *Annual Surgical Oncology*, 13(12), pp.1671–1681.

Tardelli, E. et al., 2016. Sentinel Lymph Node Biopsy in Cutaneous Melanoma: Standard and New Technical Procedures and Clinical Advances. A Systematic Review of the Literature. *Clinical Nuclear Medicine*, 41(12), pp.e498–e507.

Terada, K.Y., Shimizu, D.M. & Wong, J.H., 2000. Sentinel Node Dissection and Ultrastaging in Squamous Cell Cancer of the Vulva. *Gynecologic Oncology*, 76, pp.40–44.

Tew, K. et al., 2005. Meta-analysis of sentinel node imprint cytology in breast cancer. *British Journal of Surgery*, 92(9), pp.1068–1080.

Thevarajah, S., Huston, T.L. & Simmons, R.M., 2005. A comparison of the adverse reactions associated with isosulfan blue versus methylene blue dye in sentinel lymph node biopsy for breast cancer. *American Journal of Surgery*, 189(2), pp.236–239.

Thompson, J.F. & Uren, R.F., 2000. What is a "sentinel" lymph node? *European Journal of Surgical Oncology*, 26(2), pp.103–104.

Treseler, P., 2006. Pathologic examination of the sentinel lymph node: What is the best method? *Breast Journal*, 12(Suppl. 2), pp.s143–s151.

Trietsch, M.D. et al., 2015. Genetic and epigenetic changes in vulvar squamous cell carcinoma and its precursor lesions: A review of the current literature. *Gynecologic Oncology*, 136(1), pp.143–157.

Trimbos, J.B., 2011. Lymphadenectomy in ovarian cancer: standard of care or unnecessary risk. *Current Opinion in Oncology*, 23, pp.507–511.

Trimbos, J.B., 2017. Surgical treatment of ovarian cancer. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 41, pp.60–70.

Tu, H., Huang, H. & Liu, J., 2015. [Meta-analysis of application value of sentinel lymph node biopsy in early-stage vulval squamous cell carcinoma]. *Zhonghua yi xue za zhi*, 95(33), pp.2715–2719.

Uren, R. et al., 1993. Lymphoscintigraphy in high-risk melanoma of the trunk: predicting drainging node groups, defining lymphatic channels and locating the sentinel node. *Journal of Nuclear Medicine*, 34(9), pp.1435–40.

Uren, R.F., Nieweg, O.E. & Thompson, J.F., 2016. Sentinel Lymph Node Biopsy: Evolution of the Technique Since the Original Description by Morton et al. in 1992. *Critical Reviews in Oncogenesis*, 21(1–2), pp.7–17.

Ushijima, K., 2007. Management of retroperitoneal lymph nodes in the treatment of ovarian cancer. *International Journal of Clinical Oncology*, 12(3), pp.181–186.

Valdés Olmos, R.A., Rietbergen, D.D., et al., 2014. Contribution of SPECT/CT imaging to radioguided sentinel lymph node biopsy in breast cancer, melanoma, and other solid cancers: from "open and see" to "see and open". *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 58(2), pp.127–139.

Valdés Olmos, R.A. et al., 1999. Lymphoscintigraphy in oncology: A rediscovered challenge. *European Journal of Nuclear Medicine*, 26(Suppl), pp.S2–S10.

Valdés Olmos, R.A., Vidal-Sicart, S., et al., 2014. The GOSTT concept and hybrid mixed / virtual / augmented reality environment radioguided surgery. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 58(2), pp.207–15.

Valdés Olmos, R.A. & Vidal-Sicart, S., 2016. Introducing new perspectives in radioguided intervention. *Clinical and Translational Imaging*, 4(5), pp.307–311.

Vargas, R. et al., 2014. Tumor size, depth of invasion, and histologic grade as prognostic factors of lymph node involvement in endometrial cancer: A SEER analysis. *Gynecologic Oncology*, 133(2), pp.216–220.

Verbeek, F.P.R. et al., 2015. Sentinel Lymph Node Biopsy in Vulvar Cancer using Combined Radioactive and Fluorescence Guidance. *International Journal of Gynecologic Cancer*, 33(4), pp.395–401.

Vidal-Sicart, S. et al., 2014. Contribution of perioperative imaging to radioguided surgery. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, 58(2), pp.140–160.

Vidal-Sicart, S. & Valdés Olmos, R.A., 2016. Synergism of SPECT/CT and portable gamma cameras for intraoperative sentinel lymph node biopsy in melanoma, breast cancer, and other malignancies. *Clinical and Translational Imaging*, 4(5), pp.313–327.

Vidal, F. et al., 2013. Evaluation of the Sentinel Lymph Node Algorithm With Blue Dye Labeling for Early-Stage Endometrial Cancer in a Multicentric Setting. *International Journal of Gynecological Cancer*, 23(7), pp.1237–1243.

Viswanathan, A.N. et al., 2013. Relationship of margin status and radiation dose to recurrence in post-operative vulvar carcinoma. *Gynecologic Oncology*, 130(3), pp.545–549.

van der Vorst, J.R. et al., 2013. Dose optimization for near-infrared fluorescence sentinel lymph node mapping in patients with melanoma. *The British journal of dermatology*, 168(1), pp.93–8.

Wakisaka, N. et al., 2015. Primary tumor-secreted lymphangiogenic factors induce pre-metastatic lymphvascular niche formation at sentinel lymph nodes in oral squamous cell carcinoma. *PLoS ONE*, 10(12), pp.1–14.

Wang, Z. et al., 2015. Expression of CDK1 Tyr15 , pCDK1 Thr161 , Cyclin B1 (Total) and pCyclin B1 Ser126 in Vulvar Squamous Cell Carcinoma and Their Relations with Clinicopatological Features and Prognosis. *PLOS ONE*, 10(4), p.e0121398.

Wasnik, A.P., 2013. Multimodality imaging of ovarian cystic lesions: Review with an imaging based algorithmic approach. *World Journal of Radiology*, 5(3), p.113.

Webber, K. & Friedlander, M., 2016. Chemotherapy for epithelial ovarian, fallopian tube and primary peritoneal cancer. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 41, pp.126–138.

Weidle, U.H. et al., 2016. Mechanisms and targets involved in dissemination of ovarian cancer. *Cancer Genomics and Proteomics*, 13(6), pp.407–424.

WNA, 2017. Supply of Radioisotopes. *World Nuclear Association: Radioisotopes in Medicine*, p.1. Available at: http://www.world-nuclear.org/information-library/non-power-nuclear-applications/radioisotopes-research/radioisotopes-in-medicine.aspx [Accessed June 27, 2017].

Woelber, L. et al., 2012. Prognostic role of lymph node metastases in vulvar cancer and implications for adjuvant treatment. *Int J Gynecol Cancer*, 22(3), pp.503–508.

Woelber, L. et al., 2013. Secondary sentinel node biopsy after previous excision of the primary tumor in squamous cell carcinoma of the vulva. *Annals of Surgical Oncology*, 20(5), pp.1701–1706.

Wright, A.A. et al., 2016. Neoadjuvant chemotherapy for newly diagnosed, advanced ovarian cancer: Society of Gynecologic Oncology and American Society of Clinical Oncology Clinical Practice Guideline. *Gynecologic Oncology*, 34(28), pp.3460–3473.

Zanzonico, P. & Heller, S., 2000. The intraoperative gamma probe: basic principles and choices available. *Seminars in nuclear medicine*, 30(1), pp.33–48.

van der Zee, A. et al., 2016. Vulvar Cancer Guidelines - complete report. *ESGO Guidelines & Quality Indicators*, pp.1–86.

van der Zee, A.G.J. et al., 2008. Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. *Journal of Clinical Oncology*, 26(6), pp.884–889.

Zhang, J. et al., 2017. Prognostic role of vascular endothelial growth factor in cervical cancer: a meta-analysis. *Oncotarget*, 8(15), pp.24797–24803.

Zhang, Z. et al., 2016. Prognostic significance of high VEGF-C expression for patients with breast cancer: An update meta analysis. *PLoS ONE*, 11(11), pp.1–15.

Zheng, W., Aspelund, A. & Alitalo, K., 2014. Lymphangiogenic factors, mechanisms, and applications. *The Journal of Clinical Investigation*, 124(3), pp.878–887.

Zhou, J.H., Shan, G.P. & Chen, Y.W., 2016. The effect of lymphadenectomy on survival and recurrence in patients with ovarian cancer: a systematic review and meta-analysis. *Japanese Journal of Clinical Oncology*, 46(8), pp.718–726.

Zigras, T. et al., 2017. Early Cervical Cancer: Current Dilemmas of Staging and Surgery. *Current oncology reports*, 19(8), p.51.

Zong, S. et al., 2016. Prognostic significance of VEGF-C immunohistochemical expression in colorectal cancer: A meta-analysis. *Clinica Chimica Acta*, 458, pp.106–114.

Zweizig, S., Korets, S. & Cain, J.M., 2014. Key concepts in management of vulvar cancer. *Best Practice & Research Clinical Obstetrics and Gynaecology*, 28, pp.959–966.

10 ORIGINAL COMMUNICATIONS

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ORIGINAL ARTICLE

Sentinel node and vulvar cancer: a series of 47 patients

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Abstract

Background. There is growing interest to apply the sentinel node technique in the treatment of vulvar cancer. *Methods.* All charts of the patients operated on for vulvar cancer at Tampere University Hospital from January 1, 2001 through June 30, 2005 were retrospectively reviewed. Demographic, clinical, and histopathological information was collected from each patient. The sentinel lymph node mapping was done intraoperatively either with a combination of the radioisotope and dye techniques (40 patients) or with the dye technique alone (7 patients). The sentinel lymph node was dissected separately for histopathological evaluation, and then a routine inguinal lymphadenectomy was performed. *Results*. The final FIGO surgical Stage distribution was: Stage I, 11 (23%); Stage II, 14 (30%); Stage III, 21 (45%); and Stage IV, 1 (2%). Sentinel lymph node was identified in 46 (98%) women with either one or both of the methods. In Stage I–II, the sentinel lymph node identification rate was 25/25 (100%) with the combined method. The only patient with unidentified sentinel lymph node had lymphatic spread beyond inguinal area or Stage IV disease. Eighteen of the sentinel lymph nodes (39%) were positive for tumor cells, and in 5 cases additional metastatic nodes were found. One patient with macroscopically enlarged metastatic inguinal nodes and Stage III disease had a negative sentinel lymph node. In the 25 patients with Stage I–II disease, the false-negative rate of the sentinel lymph node method was 0/4, giving a negative predictive value of 1.00. *Conclusions*. A sentinel node identification rate of 98% with a false-negative rate of 0% in the patients with Stage I–II disease is an encouraging finding.

Key words: *Vulvar carcinoma, operative treatment, sentinel node*

The overall survival of patients with vulvar cancer strongly depends on the spread of the disease to the inguinofemoral lymph nodes. Therefore, patients with early vulvar cancer currently undergo a total dissection of inguinal and femoral nodes both for staging and therapeutical purposes (1).

Patients with vulvar cancer are mostly elderly, with a mean age of 70 at presentation. These women often have significant co-morbidity, like venous insufficiency of the lower extremities, which makes them especially vulnerable to the complications of inguinofemoral lymphadenectomy. The short-term complications include wound healing problems and infections, and the long-term complications lymph edema and chronic cellulitis of the legs, which is present in 24–70% of patients (1,2).

The sentinel lymph node (SLN) procedure can offer an alternative approach to total inguinofemoral lymphadenectomy. The method has already been accepted in the surgical management of breast cancer and cutaneous melanoma (3,4). The concept of the SLN is based on the assumption that the first node to receive the lymphatic drainage from the malignant tumor should be the first site of the metastatic spread. If this node – the SLN – is free of the disease, so should the entire lymphatic basin

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of the groin area in the case of vulvar cancer. Therefore, a tumor-free sentinel node allows the surgeon to omit the inguinofemoral lymphadenectomy, sparing the patient from the many complications of the procedure (1,2).

There is still obscurity relating to the SLN method in the management of vulvar cancer. The site of the primary tumor impacts the usefulness of the SLN procedure. Lateral tumors are more likely to spread to the ipsilateral nodes, but concerning midline (both clitoral and posterior) tumors, the malignant spread can occur bilaterally or directly to the deep nodes. The lymphatic channels can be obstructed by the gross metastatic disease, causing the SLN not to be detectable even when there are positive groin nodes. Prior to vulvar surgery, even an excisional biopsy can alter the course of lymphatic drainage, leaving the SLN method uncertain. The most reliable technique in identification of the SLN (dye, radioisotope or combined) still needs to be validated. The learning curve in detecting the sentinel node has been established also in vulvar cancer (1,2,5,6).

The purpose of this study was to retrospectively evaluate: 1. how often the SLN was identified intraoperatively at Tampere University Hospital, and 2. how reliably a negative or positive SLN reflects the actual nodal status in the inguinofemoral area of patients with vulvar cancer.

Patients and methods

We retrospectively collected data from all patients who presented with vulvar cancer at Tampere University Hospital over a period of 4.5 years, from January 1, 2001 through June 30, 2005. The rather systematic SLN mapping at our hospital was started in the year 2000. To familiarize with the method, the procedure was done on most of the patients who underwent lymphadenectomy regardless of the clinical staging. The reasons not to perform the sentinel node included: radiation therapy prior to surgery, no surgery at all (palliative radiation therapy, patient deceased prior to surgery), incomplete surgery (patient too ill for lymphadenectomy), the primary operation performed at some other hospital or at our hospital but before January 1, 2001, or unknown origin of the malignant disease. Of a total of 74 patients with vulvar cancer treated at our hospital during the 4.5 years, 27 were excluded from the study for the reasons listed above, which left 47 women for this analysis.

A combined method of two different techniques was mostly used for the SLN mapping. For every patient, Patent Blue® dye was injected perilesionally during the surgery for the lymphatic mapping. The SLN was the first node following the blue-colored lymph duct, whether or not the node itself was blue. For 40/47 patients, technetium-99-colloid radioisotope was injected around the site of the tumor early in the morning preceding surgery or on the previous day. It is to be noted that any lymphoscintigram was not taken preoperatively, but the SLN was rather identified with a hand-held gamma probe intraoperatively (Navigator GPS, Tyco HealthCare, Norwalk, CT, USA). The SLN was dissected separately for histopathological evaluation, followed by a complete inguinal lymphadenectomy. Moreover, ipsilateral pelvic nodes were biopsied if any inguinal spread was found or suspected. All the SLN procedures were performed by one of two gynecologic oncologists (T.K. or J.M.).

With the approval of the local ethics committee, the following information was retrospectively collected from each patient: age at the time of the surgery, the technique for the identification of the SLN (radioisotope, dye or both), the site of the primary tumor, the type of surgery, the surgical stage of the disease, the histopathology report, whether it was possible to identify a SLN, and whether other metastases were present. One pathologist (J.P.) reviewed the original histological slides for this study.

Results

The age and stage distribution, and the histopathological findings

The median age of the patients was 76 years (range 43–93 years) at the time of operation. Forty-six tumors were squamocellular carcinomas, while one was anaplastic. The FIGO surgical stage and the histological grade appear in Table I. Seven of the 21 Stage III tumors had spread to urethra and/or vagina (with simultaneous inguinal nodal involvement in three cases), while the rest represented lymphatic spread only. Of the 47 primary tumors, 16 (34%) were lateral and 22 (47%) were central (four of them clitoral), respectively. Nine (19%) of the patients had bilateral tumors.

The identification of the SLN

For 40 women, the combined dye and radioisotope method for SLN mapping was used. In 36 cases (90%) the SLN was identifiable with both techniques. In no instance was the SLN missed, which makes the total identification rate of the combined method 100%. In three cases the SLN was identified

Table I. The stage and grade distribution in patients with vulvar cancer

	Stage I	Stage II	Stage III	Stage IV	Total
Grade 1	8	7	8	1	24 (51%)
Grade 2	3	5	8	0	16 (34%)
Grade 3	0	2	5	0	7 (15%)
Total	11 (23%)	14 (30%)	21 (45%)	1 (2%)	47 (100%)

by the dye only, because the hand-held detector failed to operate. In one case of FIGO stage II disease with a bilateral primary tumor the SLN was identifiable with the radioisotope only.

In seven women, only the dye technique was used for the SLN mapping. In one case the SLN could not be identified. She had a FIGO Stage IV Grade 1 disease, her primary tumor was positioned laterally, and there were positive lymph nodes in the inguinal and obturator areas.

The success rate of the SLN identification as related to the FIGO stage and the technique used is presented in Table II. In Stage I–II disease, the SLN was identifiable with the dye technique in 24 of 25 patients (96%) and with the radioisotope in 19 of 20 patients (95%), respectively, giving a total success rate of 25/25 or 100%.

The identification rate according to the primary site of the tumor and the method of identification is shown in Table III. In all, of the total 47 dye mappings performed, the SLN was identified in 45 cases (96%) and of the 40 radioisotope mappings performed, the SLN was identified in 37 cases (93%). The total SLN identification rate for all patients together was 98% (46 out of 47).

The reliability of SLN method

Of the 46 SLNs identified, 18 (39%) harbored cancer cells (Tables IV and V). Five patients (1 with Stage II and 4 with Stage III disease) had other metastatic inguinal nodes also. There was only one patient with Stage III disease, who had inguinal metastases in spite of a negative SLN. Thus, the false-negative rate of the SLN method was in FIGO Stage I–II 0/4 or 0%, and in FIGO Stage III–IV 1/14 or 7.1%, respectively. The negative predictive value of a disease-free SLN in FIGO Stage I–II was 1.00, while it was in FIGO Stage III–IV 0.86.

Discussion

SLN mapping offers a promising minimally invasive alternative for surgical staging of vulvar cancer. The usefulness of the SLN method depends especially on the intraoperative identification rate of the SLN and the number of false-negative SLNs.

In two studies of 51 and 52 patients by Ansink et al. (7) and Levenback et al. (6), the use of blue dye alone led to an identification rate of 56% and 88%, respectively. In subsequent studies, a combined method of blue dye and radioisotope has given identification rates in the order of 96–100% (2). In our material, the total intraoperative SLN identification rate was 100% with the combination of blue dye and radioisotope. With the dye alone the results were surprisingly good; 96% of the SLNs were identified. With the radioisotope and hand-held gamma detector, 93% of the SLNs were identified.

In our study, three perioperative radioisotope mappings failed because the hand-held tracer did not operate. This problem could have been avoided with a preoperative lymphoscintigraphy, presented by de Hullu et al. (8). According to de Hullu et al., lymphoscintigraphy offers a reliable preoperative estimate of the number and location of the SLNs, which can be problematic using intraoperative mapping only. Especially in the case of medially situated

Table II. The success rate of the sentinel node (SLN) identification by the FIGO stage and the method used

	Dye mapping: success		Radioisotope mapping: success		Combination: success		
FIGO stage	Yes	No	Yes	No	Yes	No	Total success rate
Stage I	11/11 (100%)	0/11 (0%)	10/10 (100%)	0/10 (0%)	10/10 (100%)	0/10 (0%)	11/11 (100%)
Stage II	13/14 (93%)	1/14 (7%)	9/10 (90%)	1*/10 (10%)	10/10 (100%)	0/10 (0%)	14/14 (100%)
Stage III	21/21 (100%)	0/21 (0%)	18/20 (90%)	2*/20 (10%)	20/20 (100%)	0/20 (0%)	21/21 (100%)
Stage IV	0/1 (0%)	1/1 (100%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/1 (0%)
Total	45/47 (96%)	2/47 (4%)	37/40 (92.5%)	3*/40 (7.5%)	40/40 (100%)	0/40 (0%)	46/47 (98%)

*Hand-held gamma detector failed to operate.

Table III. The SLN identification rate according to the primary tumor site and the technique used

	SLN identification rate by the technique used		
Primary tumor site	Dye mapping	Radioisotope mapping	Total
Unilateral	94% (15/16)	93% (14/15)	94% (15/16)
Central	100% (22/22)	94% (16/17)	100% (22/22)
Bilateral	89% (8/9)	88% (7/8)	100% (9/9)
Total	96% (45/47)	93% (37/40)	98% (46/47)

tumors, lymphoscintigraphy also shows whether the lymphatic drainage is uni- or bilateral (9).

The primary site of the tumor affects the SLN identification rate. Levenback et al. (6) reported a higher identification rate for unilateral than for midline tumors (90 versus 69%), respectively. Although in the present study the only failure to identify a SLN was associated with a lateral tumor, the fact that the patient had a Stage IV disease means that there is no true discrepancy between our results and those of Levenback et al.

Our false-negative rate of 0% in early vulvar cancer or the true target for the SLN procedure (9) compares well with previous reports (1,2). One may question the relevance of also including patients with more advanced disease. We did so only because we wanted to familiarize with the SLN technique. Hence, we performed the SLN procedure systematically to almost all surgically treated patients in addition to an ipsi- or bilateral lymphadenectomy, regardless of clinical stage. Our only false-negative case had advanced (FIGO Stage III) vulvar cancer with preoperatively palpable inguinal nodes, and the patient for whom the SLN could not be detected had Stage IV disease. The SLN procedure would not have been implemented for either of them if the method had been in routine clinical use.

Two recent publications have focused on the prognostic value of the SLN procedure in early vulvar cancer. Terada et al. followed 21 patients with a T1 vulvar squamocellular cancer for a median of 4.6 years after radical local excision and sentinel node dissection (10). A combined method with isosulfan blue dye and radioisotope was used to localize SLN. In their material, none of the patients with negative sentinel node developed groin or distant metastases. Three-year disease-free survival for patients with a negative sentinel node was 100% (95% CI 81.5–100%). Patients with positive sentinel node (3/21) subsequently underwent complete inguinofemoral lymphadenectomy. Of these three women, two have died of cancer, one with local recurrence and one with a groin recurrence. Martinez-Palones et al. (11) compared two series of patients with T1–T2 vulvar cancer. The first group was a prospective series of 28 patients (between years 2000 and 2005) who underwent vulvectomy and lymphadenectomy with SLN identification, and the second one was a retrospective series of 27 patients (between 1995 and 2000) who underwent the same operations without SLN identification. A combined technique with isosulfan blue dye and radioisotope was used in SLN mapping. In their work, the identification rate was 96% (27/28) and the false negative rate 1/7 or 14.3%. During a median follow-up of 60 months (range 6–110) in the non-SLN group and 22.5 months (range 0–64) in the SLN group, they found 6 recurrences in the non-SLN group and 8 recurrences (5 in vulva and 3 in groin) in the SLN group (26.9% versus 28.6%, $p = 0.893$). In the SLN group, four of the recurrences were found in patients with positive SLN and one in a patient with a failure of the SLN identification. Two of the recurrences were associated with a large tumor size and one with a persistent vulvar human papilloma virus infection. Consequently, both studies indicate that in early vulvar cancer a negative sentinel node is a favorable prognostic factor. On the other hand, a positive sentinel node appears to carry a significant risk for a recurrence.

Table IV. The rate of metastatic SLN according to the FIGO stage

FIGO stage	Negative SLN	Metastatic SLN	Not identifiable	Total
Stage I	11	0	0	11
Stage II	10	4	0	14
Stage III	7	14	0	21
Stage IV	0	0	1	1
Total	28	18	1	47

Table V. Sentinel and inguinal node findings as compared to FIGO stage

	Negative SLN		Metastatic SLN		
FIGO stage	Inguinal nodes −	Inguinal nodes +	Inguinal nodes −	Inguinal nodes +	Total
Stage I	11	0	0	0	11
Stage II	10	0	3	1	14
Stage III	6	1*	10	4	21
Stage IV	–	–	–	–	SLN not found
Total	27	1	13	5	46

*False-negative sentinel node.

In conclusion, an identification rate of 100% and a false-negative rate of 0% in the patients with early vulvar cancer further imply that the SLN procedure is feasible and reliable in vulvar cancer. In accordance with previous studies, the present results are in favor of using the combined method to identify the sentinel node(s). Unfortunately the present knowledge on the significance of the SLN procedure in vulvar cancer relies on rather small Phase II studies only. Due to the rarity of vulvar cancer and the lack of prospective randomized trials, the clinicians are encouraged to join in multicenter studies like the Groningen International Study on Sentinel Nodes in Vulvar Cancer (GROINS-V-II).

References

1. Dhar KK, Woolas RP. Lymphatic mapping and sentinel node biopsy in early vulvar cancer. BJOG. 2005;112:696–702.
2. De Hullu JA, Oonk MHM, van der Zee AGJ. Modern management of vulvar cancer. Curr Opin Obstet Gynecol. 2004;16:65–72.
3. Von Smitten K. Surgical management of breast cancer in the future. Acta Oncol. 2000;39:437–9.
4. Ra JH, McMasters KM, Spitz FR. Should all melanoma patients undergo sentinel lymph node biopsy? Curr Opin Oncol. 2006;18:185–8.
5. Levenback C, Burke TW, Morris M, Malpica A, Lucas KR, Gershenson DM. Potential applications of intraoperative lymphatic mapping in vulvar cancer. Gynecol Oncol. 1995; 59:216–20.
6. Levenback C, Coleman RL, Burke TW, Bodurka-Bevers D, Wolf JK, Gershenson DM. Intraoperative lymphatic mapping and sentinel node identification with blue dye in patients with vulvar cancer. Gynecol Oncol. 2001;83:276–81.
7. Ansink AC, Sie-Go DM, van der Velden J, Sijmons EA, de Barros Lopez A, Monaghan JM, et al. Identification of sentinel lymph node in vulvar carcinoma patients with the aid of a patent blue V injection: a multicenter study. Cancer. 1999;86:652–6.
8. De Hullu JA, Doting E, Piers DA, Hollema H, Aalders JG, Koops HS, et al. Sentinel lymph node identification with technetium-99m-labeled nanocolloid in squamous cell cancer of the vulva. J Nucl Med. 1998;39:1381–5.
9. De Hullu JA, Oonk MHM, Ansink AC, Hollema H, Jager PL, van der Zee AGJ. Pitfalls in the sentinel lymph node procedure in vulvar cancer. Gynecol Oncol. 2004;94:10–5.
10. Terada KY, Shimizu DM, Jiang CS, Wong JH. Outcomes for patients with T1 squamous cell cancer of the vulva undergoing sentinel node biopsy. Gynecol Oncol. 2006;102: 200–3.
11. Martinez-Palones JM, Péres-Benaventi MA, Gil-Moreno A, Diaz-Feijoo B, Roca I, Garcia-Jiménez A, et al. Comparison of recurrence after vulvectomy and lymphadenectomy with and without sentinel node biopsy in early stage vulvar cancer. Gynecol Oncol. 2006;103:865–70.

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Original research

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Can vascular endothelial growth factor C expression be of use in predicting surgical stage or prognosis in vulvar cancer?

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Abstract

Introduction: Nodal metastasis is a main prognostic factor in vulvar cancer. Increased vascular endothelial growth factor C (VEGF-C) expression has been associated with lymph node metastasis and poor prognosis in many cancers. The aim of this retrospective study was to investigate VEGF-C expression pattern in the invasive edge of vulvar cancer and in sentinel lymph node metastasis, and its association with the stage and prognosis. *Methods:* Tumor and sentinel lymph node samples from 44 patients were evaluated with immunohistochemistry, and the results were linked with the clinicopathological data. *Results:* Sixty-seven percent of primary tumors and 76% of sentinel lymph node metastases expressed VEFG-C. Positive VEGF-C expression of the primary tumor did not predict surgical Stage or sentinel lymph node involvement. The risk of relapse was not significantly higher with VEGF-C expressing tumors than with VEGF-C negative tumors (RR 2.55, 95% CI 0.66-9.90, p = 0.18). The risk of groin recurrence was significantly lower with VEGF-C positive than negative tumors (RR 0.36, 95% CI 0.16-0.79, p = 0.01). Survival was similar in both groups. No non-sentinel lymph node metastases were found in case of negative VEGF-C expression in the sentinel lymph node metastasis, whereas with positive VEGF-C expression they were found in 5/13 (38%) of cases. *Conclusions:* Tumoral VEGF-C expression was not associated with higher surgical Stage or poorer prognosis in vulvar cancer. However, absence of its expression in sentinel lymph node metastasis might indicate a low risk for non-sentinel lymph node metastases.

Keywords: vulvar cancer; VEGF-C; sentinel lymph node; nodal metastasis; lymphatic spread; prognosis; recurrence

Introduction

Nodal metastatic involvement is the most important prognostic factor in vulvar cancer. Node-negative patients have a 5-year survival rate of 70 - 98% but those with positive nodes only 12 - 41% [1]. Size of the primary tumor, presence of lymphovascular invasion and the depth of invasion are known to increase the risk for nodal metastasis [2–4], as well as the central location of the primary tumor [5]. Biologic prognostic variables are not as well known. Increased tumor angiogenesis and altered vessel characteristics are suggested to lead into a shorter disease-free survival [6]. Expression levels of matrix metalloproteinase-2 expression higher than 50% are also an indicator of a lower five-year survival rate [7]. Over-expression of tissue matrix metalloproteinases, transmembrane protein CD44 and its isoforms, thrombospondin-1, vascular endothelial growth factor (VEGF) and some G2/M pathway regulators, as well as, loss of metastasis suppressor NM23-H1 gene, seem to promote local and metastatic growth. However, not all these findings correlate with clinical prognosis [1, 8].

Studies in animal models and humans have shown that lymphangiogenesis in a primary tumor increases nodal metastasis [9]. Even before the metastasis actually takes place, the lymph nodes draining straight from the tumor – so called sentinel lymph nodes (SLNs) - undergo remodelling processes including lymphangiogenesis, alterations in structure, lymphatic flow and immune cell composition, and increases in chemokine and cytokine production, thus creating a premetastatic niche [10]. Vascular endothelial growth factor C (VEGF-C) secreted by the primary tumor is the most important lymphangiogenic factor causing the remodelling. It alters the lymphatic vessels around the primary tumor, increases the lymphatic flow and causes expansion of lymphatic network in SLNs, all this promoting lymphatic spread [11].

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In many human cancers, over-expression of VEGF-C by primary tumor is associated with poorer prognosis. It correlates with shorter progression free survival (PFS), overall survival (OS) and lymph node metastasis in melanoma [12], and is a poor prognostic factor in non-small-cell lung cancer and adenocarcinoma of the lung [13, 14]. High levels of VEGF-C expression in gastric cancer tissue imply worse overall prognosis than low VEGF-C levels [15]. Primary tumor VEGF-C expression has been reported to correlate with the possibility of lymph node metastasis in lung, oesophageal, prostate, thyroid and colorectal cancers [11].

To our knowledge, the influence of VEGF-C on the clinical course of vulvar cancer has not been studied, but one report of VEGF-C expression in 10 tissue samples has been published [16]. The aim of this study was to explore the presence of VEGF-C expression in vulvar cancer (primary tumor and SLN metastasis), and its influence on patients' surgical Stage, risk of recurrence and prognosis.

Materials and methods

Patients and tissue samples

Tissue samples from 44 vulvar cancer patients that had previously undergone vulvar surgery and a SLN mapping before complete lymph node dissection in Tampere University Hospital were used for this study. Under a 4.5-year familiarization period, a SLN mapping had been performed to all surgically treated vulvar cancer patients, and has been described elsewhere [17]. The specimens were obtained from the Tissue Biobank and Research Services FinTiB (Fimlab Laboratories Inc., Tampere, Finland). Forty-six tumor samples with representative malignant growth as well as 17 metastatic SLN samples were available for analysis.

The clinicopathological history and follow-up data of all patients were retrospectively collected from the hospital records. The history included the age at the time of the surgery, the date of the surgery, the site of the primary tumor, the type of surgery, the surgical Stage of the disease, the histopathology report, the status of the SLN (positive or negative for metastasis) and other regional lymph nodes, and whether or not other metastases were present. The follow-up data included also information of a potential adjuvant treatment and its duration, the observation date and location(s) of a recurrence, if any, the final date of the follow-up, and the date and cause of death, if it happened during the follow-up period. This data was combined with the results of the VEGF-C immunostaining for the final analysis.

Immunohistochemistry

The VEGF-C protein expression in vulvar tumors and SLN samples were evaluated by using immunohistochemistry (IHC). Representative samples from the invasive edges of the primary tumors and SLN metastases were selected for the study by an experienced pathologist (M.L.). 4 µm thick sections were cut from paraffin-embedded tissue blocks using a standard microtome. For IHC staining, the slides were then deparaffinized, rehydrated, and subsequently pretreated with a PT-Module (Lab Vision, Fremont, CA) at 98°C for 15 min in 0.05 M TrisHCl buffer, pH 9.0 containing 0.001 M EDTA. The primary VEGF-C antibody (Rabbit anti-VEGF-C; Invitrogen, Camarillo, CA) was visualized with a PowerVision + polymer kit (Leica Biosystems Newcastle Ltd., Newcastle, UK) and diaminobenzidine as chromogen (DABImmPact, Vectorlabs, Burlingame, CA). The tissue sections were counterstained with hematoxylin (Mayer's hematoxylin, Oy FFChemicals Ab, Haukipudas, Finland). Human colon carcinoma samples, known to have a strong VEGF-C expression, were used as a positive control. Negative controls were made by omitting the primary VEGF-C antibody from the procedure.

Analysis of the immunostaining

Immunostained sections were scanned with an Aperio Scanscope XT (Aperio Technologies, Vista, CA) and visually analysed on a computer screen. Two observers (R.N. and S.S.) assessed them, blinded to the clinicopathological data of the patients. In the first round, the assessments were performed independently and the results then pooled. If the assessments of two observers were contradictory, the staining was assessed again in consensus. In all tissue samples, IHC staining intensity was scored semiquantitatively as negative (no staining at all), weak (some scattered stained cells or faint more widespread staining), moderate (more abundant widespread staining or focal intensive staining) or strong (almost all cells intensively stained). For the statistical analysis, negative and weak staining were combined as "negative" and similarly, moderate and strong staining as "positive" staining.

Statistical analysis

The concordance between two observer's assessments of the VEGF-C expression in the first evaluation round was assessed using Cohen's unweighted kappa test [18]. Associations between VEGF-C staining and clinicopathological parameters were analyzed using the Fisher's exact test, odds ratio and relative risk. Disease-specific and progression free survival curves were calculated using Kaplan-Meier survival analysis, and compared using the log rank test. A p-value less than 0.05 was considered statistically significant. SPSS Statistics for Windows (version 19.0 released 2010, IBM Corp., Armonk, NY, USA) was used in calculation of statistical analysis.

Ethical considerations

The use of archived tissue specimens for IHC was approved by Valvira, National Supervisory Authority for Welfare and Health (6746/05.01.00.06/2009). The retrospective collection of patient data from the hospital records was approved by the Ethics Committee of Pirkanmaa Hospital District (R09066).

Results

Patient and follow-up data

The median age of patients was 76 years (range 44-93). The median follow-up time was 39 months (range 0.6 - 109 months). Tumor characteristics with Stage, adjuvant treatment and follow-up data are presented in Table 1.

During the follow-up, one patient died of cancer less than three weeks after surgery and two patients during the

Table 1 Data on disease and tumor characteristics in all 44 patients.

Variable	*Definition*	*Number of patients (percentage of all)*
FIGOa Stage	I	19 (43%)
	II	4 (9%)
	III	20 (46%)
	IV	1 (2%)
Histology and Grade of the primary tumor in vulva	SCC[b]	43 (98%)
	Grade 1	22 (50%)
	Grade 2	14 (32%)
	Grade 3	7 (16%)
	Anaplastic carcinoma	1 (2%)
	Grade 3	1 (2%)
Sentinel node metastasis	No	23 (52%)
	Yes	20 (46%)
	No SLN detected	1 (2%)
Postoperative adjuvant treatment	No adjuvant treament	22 (50%)
	RT[c]	21 (48%)
	Concurrent CRT[d]	1 (2%)
Alive at the end of the follow-up	Yes	22 (50%)
	No	22 (50%)
Cause of death	Vulvar cancer or related	15 (34%)
	Other cause	7 (16%)

[a]The International Federation of Gynecology and Obstetrics; [b]squamous cell carcinoma; [c]radiation therapy; [d]chemoradiotherapy

adjuvant radiotherapy before completion of the treatment (7%, 3/44). Thirteen patients (30%) had a recurrence after the completion of the treatment, 7 in the vulva and 6 outside the vulva. Three out of 7 patients (43%) with vulvar recurrence were salvaged by reoperation and were alive at the end of the follow-up, while all six patients with recurrences outside vulva died of their disease. At the end of the follow-up, half of the patients were still alive.

Interobserver agreement on IHC

The interobserver agreements on the VEGF-C expression in primary tumor and SLN samples were substantial; Cohen's unweighted kappa for concordance in tumor samples was 0.69 (95% CI 0.48-0.90) and in SLN samples 0.72 (95% CI 0.50-1.06).

VEGF-C expression in primary tumors and SLN metastases

Of 46 primary tumor samples, only 7% (3/46) of the invasive edges of vulvar tumors did not express any VEGF-C. The expression was weak in 26% (12/46). Thus, altogether 15 tumors (33%) were classified as VEGF-C negative (Figure 1a). The staining was moderate or strong in 53% (25/46) and 13% (6/46) of the tumor edges, respectively, and a total of 31 (67%) tumors classified as VEGF-C positive (Figure 1b). There was no difference in median age of patients with either VEGF-C negative or VEGF-C positive tumors (75.5 vs. 76 years, p = 1.00). The high- Grade tumors tended to express VEGF-C more often than the low-Grade tumors but

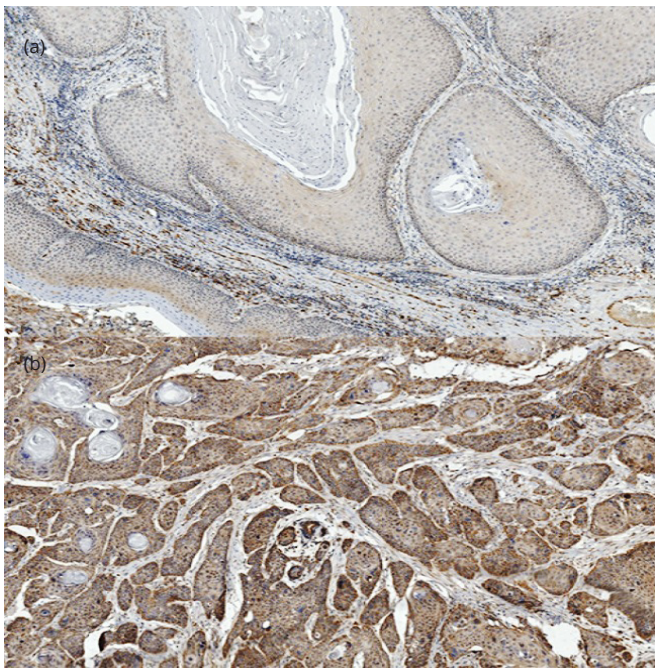

Figure 1 Examples of (a) a weak and (b) a strong VEGF-C immunostaining in squamous cell carcinoma of the vulva (magnification x 20).

the difference was not statistically significant (p = 0.36), see Figure 2. For one tumor, the Grade was not available.

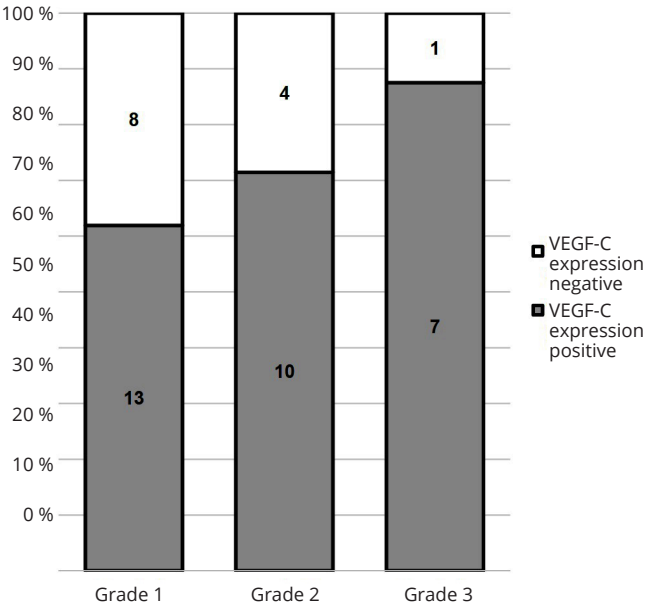

Figure 2 VEGF-C expression in invasive edges of vulvar cancer according to the histological Grade.

Tumoral VEGF-C expression and surgical Stage

At the time of the surgery, 17 out of 30 (57%) VEGF-C positive and 8 out of 14 (57%) VEGF-C negative tumors were advanced (> FIGO Stage I). The risk for more advanced surgical Stage was the same with VEGF-C positive and negative tumor groups (OR 0.98, 95% CI 0.27-3.53, p = 0.98). Also, the risk of having SLN metastasis at the time of surgery did not significantly differ between VEGF-C positive or negative tumors (47%, 14/30 and 46%, 6/13, respectively; OR 1.02, 95% CI 0.28-3.77, p = 0.98).

VEGF-C expression in SLN metastasis

The SLN metastases were VEGF-C negative in 24% (4/17) and VEGF-C positive in 76% (13/17) of the cases (Figure 3). When the primary tumor was VEGF-C positive, the SLN metastasis expressed VEGF-C in 91% (10/11) of the cases as compared to 50% (3/6) of the SLN metastases in the VEGF-C negative vulvar tumors, but the difference did not reach a statistical significance (p = 0.099).

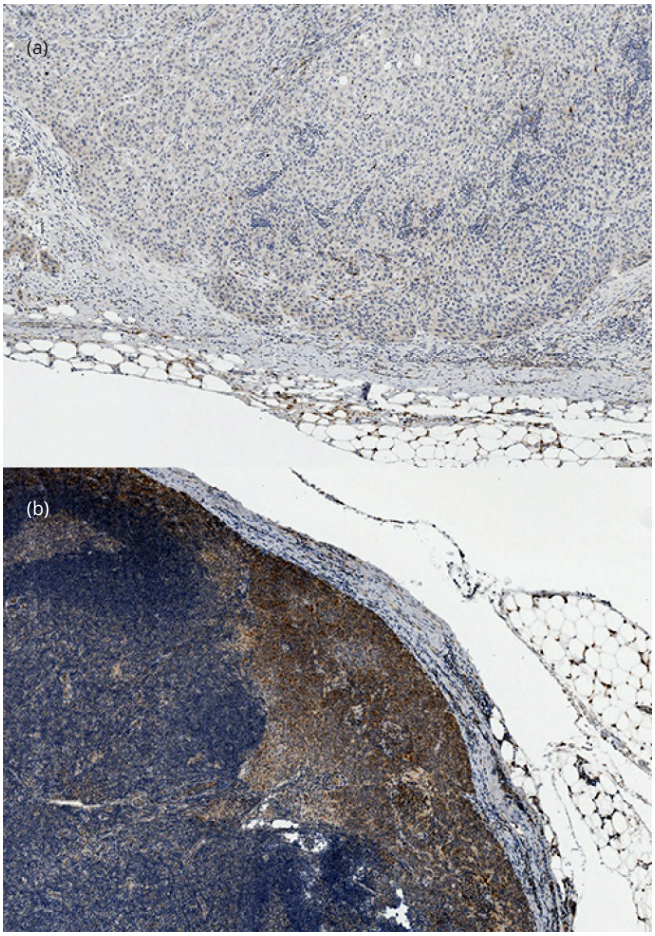

Figure 3 Examples of (a) a weak and (b) a strong VEGF-C immunostaining in sentinel lymph node metastases (magnification x 20).

When the SLN metastasis expressed VEGF-C, in 5 cases out of 13 (38%) metastatic non-SLNs were also found. However, in four cases when the SLN metastasis was VEGF-C negative, no other LN metastases were found (0/4; OR 5.82, 95% CI 0.26-130.89, p = 0.267). The positive predictive value of VEGF-C expression in the SLN metastasis in relation to the non-SLN metastases was 38 % and the negative predictive value 100%, bearing in mind the small number of VEGF-C negative SLN metastases.

VEGF-C expression and the clinical course of the disease

In primary tumors: Excluding three patients that died before the completion of the primary treatment, the patients with VEGF-C positive primary tumors seemed to relapse more often (39%, 11/28) during the follow-up than the patients with VEGF-C negative tumors (15%, 2/13), although the risk was not statistically significant (RR 2.55, 95% CI 0.66-9.90, p = 0.18). The VEGF-C positive tumors recurred mostly in the vulvar area (64%, 7/11) while the VEGF-C negative tumors recurred in the inguinal area (100%, 2/2). The risk of groin recurrence was significantly lower, when the tumor expressed VEGF-C (RR 0.36, 95% CI 0.16-0.79, p = 0.01).
The disease-specific survival (DSS) as a function of the VEGF-C expression of the primary tumors is shown in Figure 4. No difference was observed (Log rank test, p = 0.83). There seemed to be a trend towards a better PFS in patients with VEGF-C negative tumors when compared to VEGF-C positive tumors, see Figure 5. However, this trend did not reach a statistical significance (Log rank test, p = 0.19).

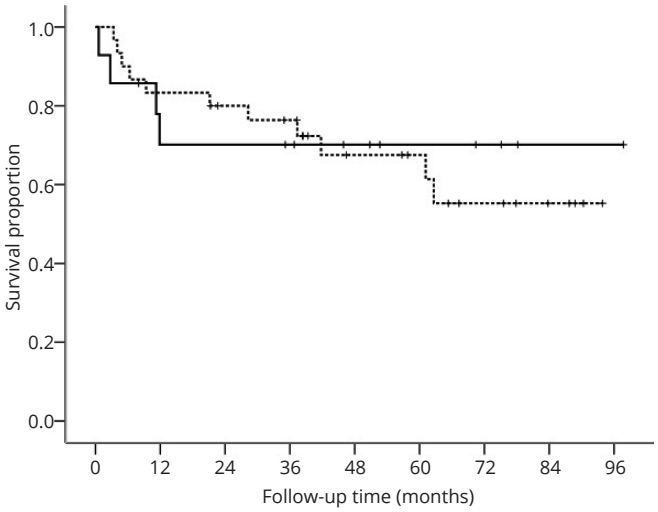

Figure 4 Disease-specific survival analysis according to VEGF-C expression of vulvar tumors (log rank test p=0.83).
Footnote: — negative VEGF-C expression; --- positive VEGF-C expression; + censored

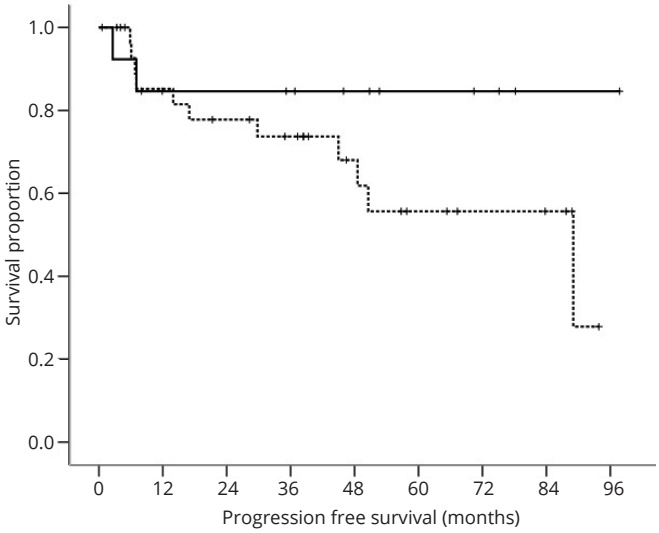

Figure 5 Progression-free survival analysis according to VEGF-C expression of vulvar tumors (log rank test p=0.19).
Footnote: — negative VEGF-C expression; --- positive VEGF-C expression; + censored

In SLN metastases: There was no difference in the risk of recurrence between patients with VEGF-C positive and negative SLN metastases (5/12 and 1/3, respectively, RR 1.25, 95 % CI 0.22-7.08, p = 0.80), respectively. The only recurrence in the group with VEGF-C negative SLN metastasis appeared in vulvar area whereas three out of five recurrences (60%) in the group with VEGF-C positive SLN metastasis appeared in inguinal area and two

recurrences (40%) in vulvar area. These groups were too small for statistical analysis.

Discussion

According to the results of this study, the primary tumors of most vulvar cancers express VEGF-C on their invasive edges. The frequency of expression tended to correlate positively with histological Grade, but the difference did not reach statistical significance. VEGF-C was also expressed in three quarters of the SLN metastases, more often when the primary tumor expressed it, although the difference was again not significant. VEGF-C expression of the primary tumor was not associated with higher surgical Stage or risk of nodal metastasis, nor did it have any statistically significant impact on DSS or PFS. A negative VEGF-C expression in a SLN metastasis could be a favourable indicator of cancer-free non-SLNs.

When considering the rarity of vulvar cancer, our sample size of 44 tumors was representative. However, it was still not large enough to show statistical significance between different groups even when a trend was observed. The strengths of this study were a long follow-up time and its clinical orientation. Our median follow-up of 39 months was long enough for relapses to become evident. We chose to keep the IHC scoring simple, bearing in mind its potential application to clinical patient care.

Our finding of the frequency of VEGF-C expression in vulvar cancer differs from the only other published report by Jach et al. In their much smaller population, VEGF-C expression was observed only in 10% (1 out of 10) of vulvar squamous cell cancer (SCC) cases. The carcinoma specimens they used for the IHC analysis were individually selected [16]. However, the authors did not specify, which part of the tumor these specimens represented nor did they tell the histological Grades of vulvar tumors – a feature that in our study seemed to effect on the VEGF-C expression. When the expression of VEGF-C was studied from 111 cervical SCC samples by Gombos et al., it was found to be heterogeneous within the tumors. The expression was significantly higher in the marginal portions of carcinomas compared with the central regions [19]. We also focused on the invasive edge of vulvar tumors and noticed the same phenomenon as Gombos et al. Furthermore; the semiquantitative scoring system Jach et al. used to assess VEGF-C expression took into account the percentage of VEGF-C positive cells. If central parts of the vulvar tumors were used for the immunostaining analysis, it might have diminished their scores even when the staining was strong.

According to literature, SCCs in many different organs express VEGF-C, i.e., tumors of oral cavity [20], oesophageal cancer [21] and cervical cancer [22]. The positive expression has been associated with poorer prognosis and higher risk of lymphatic metastasis. However, in our study tumoral VEGF-C expression did not predict surgical Stage or frequency of the SLN involvement.

We observed that VEGF-C positive cancers tended to recur more often than VEGF-C negative cancers, and therefore also PFS seemed to be more favorable in patients with VEGF-C negative tumors, albeit not significantly. In our study population, 43% of patients with a vulvar recurrence were successfully salvaged, while all groin recurrences were fatal. The risk of groin recurrence was significantly lower in VEGF-C positive tumor group than in VEGF-C negative tumor group. Better prognosis of the local recurrences compared to the groin recurrences might partly explain why the positive tumoral VEGF-C expression had no impact on the disease-specific survival. The VEGF-C expression in SLN metastasis was not associated with the recurrence rate.

At the moment, the only known prognostic factor for non-SLN metastasis in vulvar cancer in relation to characteristics of a SLN metastasis is the size of the metastasis. GROINSS-V study showed that the risk for non-SLN metastasis increases with the size of SLN metastasis. No size cut-off existed below which chances of non-SLN metastases would be close to zero. Therefore, additional treatment was recommended to all SLN positive patients, but it always increases side effects and lowers quality of life [23]. Our study suggests that a negative VEGF-C expression in SLN metastases could act as an indicator of cancer-free non-SLNs. However, the small number of metastatic SLN samples (four) limits drawing conclusions and this finding should be tested in a larger population. If reproducible, VEGF-C expression in SLN metastasis could serve as another prognostic factor when considering additional treatment.

Conclusion

VEGF-C expression was frequent in the invasive edges of malignant vulvar tumors and their SLN metastases. If verified in a larger population, the lack of VEGF expression in SLN metastasis may in the future prove to be a useful indicator of lower risk for non-sentinel lymph node metastasis. Otherwise, VEGF-C expression in primary tumors did not seem to function as a helpful indicator of surgical Stage or prognosis in vulvar cancer.

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Conflicts of interest

Authors declare no other conflicts of interest than the abovementioned Grants (J.M., R.N.).

References

[1] Gadducci A, Tana R, Barsotti C, Guerrieri ME, Genazzani AR. Clinico-pathological and biological prognostic variables in squamous cell carcinoma of the vulva. Crit Rev Oncol Hematol. 2012; 83(1):71–83.

[2] Ayhan A, Velipasaoglu M, Salman MC, Guven S, Gultekin M, et al. Prognostic factors for recurrence and survival in primary vulvar squamous cell cancer. Acta Obstet Gynecol Scand. 2008; 87(11):1143–1149.

[3] Woelber L, Eulenburg C, Choschzick M, Kruell A, Petersen C, et al. Prognostic role of lymph node metastases in vulvar cancer and implications for adjuvant treatment. Int J Gynecol Cancer. 2012; 22(3):503–508.

[4] Sznurkowski JJ, Milczek T, Emerich J. Prognostic factors and a value of 2009 FIGO staging system in vulvar cancer. Arch Gynecol Obs. 2013; 287(6):1211–1218.

[5] Hinten F, van den Einden LC, Cissen M, IntHout J, Massuger LF, et al. Clitoral involvement of squamous cell carcinoma of the vulva: localization with the worst prognosis. Eur J Surg Oncol. 2015; 41(4):592–598.

[6] Näyhä V V, Stenbäck FG. Increased angiogenesis is associated with poor prognosis of squamous cell carcinoma of the vulva. Acta Obstet Gynecol Scand. 2007; 86(11):1392–1397.

[7] Zanvettor PH, Filho DF, Soares FA, Neves AR, Palmeira LO. Study of biomolecular and clinical prognostic factors in patients with cancer of the vulva undergoing surgical treatment. Int J Gynecol Cancer. 2014; 24(4):766–772.

[8] Wang Z, Slipicevic A, Førsund M, Trope CG, Nesland JM, et al. Expression of CDK1Tyr15, pCDK1Thr161, Cyclin B1 (Total) and pCyclin B1Ser126 in vulvar squamous cell carcinoma and their relations with clinicopatological features and prognosis. PLoS One. 2015; 10(4):e0121398.

[9] Podgrabinska S, Skobe M. Role of lymphatic vasculature in regional and distant metastases. Microvasc Res. 2014; 95:46–52.

[10] Pereira ER, Jones D, Jung K, Padera TP. The lymph node microenvironment and its role in the progression of metastatic cancer. Semin Cell Dev Biol. 2015; 38:98–105.

[11] Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, et al. Lymphangiogenesis and lymphatic vessel remodelling in cancer. Nat Rev Cancer. 2014; 14(3):159–172.

[12] Boone B, Blokx W, De Bacquer D, Lambert J, Ruiter D, et al. The role of VEGF-C staining in predicting regional metastasis in melanoma. Virchows Arch. 2008; 453(3):257–265.

[13] Kilvaer TK, Paulsen EE, Hald SM, Wilsgaard T, Bremnes RM, et al. Lymphangiogenic markers and their impact on nodal metastasis and survival in non-small cell lung cancer - A structured review with meta-analysis. PLoS One. 2015; 10(8):e0132481.

[14] Kojima H, Shijubo N, Yamada G, Ichimiya S, Abe S, et al. Clinical significance of vascular endothelial growth factor-C and vascular endothelial growth factor receptor 3 in patients with T1 lung adenocarcinoma. Cancer. 2005; 104(8):1668–1677.

[15] Cao W, Fan R, Yang W, Wu Y. VEGF-C expression is associated with the poor survival in gastric cancer tissue. Tumour Biol. 2014; 35(4):3377–3383.

[16] Jach R, Dyduch G, Radon-Pokracka M, Przybylska P, Mika M, et al. Expression of vascular endothelial growth factors VEGF-C and -D, VEGFR-3, and comparison of lymphatic vessels density labeled with D2-40 antibodies as a prognostic factors in vulvar epithelial neoplasia (VIN) and invasive vulvar cancer. Neuro Endocrinol Lett. 2011; 32(4):530–539.

[17] Nyberg RH, Iivonen M, Parkkinen J, Kuoppala T, Mäenpää JU. Sentinel node and vulvar cancer: A series of 47 patients. Acta Obstet Gynecol Scand. 2007; 86(5):615–619.

[18] Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960; 20(1):37–46.

[19] Gombos Z, Xu X, Chu CS, Zhang PJ, Acs G. Peritumoral lymphatic vessel density and vascular endothelial growth factor C expression in early-stage squamous cell carcinoma of the uterine cervix. Clin Cancer Res. 2005; 11(23):8364–8371.

[20] Karatzanis AD, Koudounarakis E, Papadakis I, Velegrakis G. Molecular pathways of lymphangiogenesis and lymph node metastasis in head and neck cancer. Eur Arch Otorhinolaryngol. 2012; 269(3):731–737.

[21] Peng J, Shao N, Peng H, Chen LQ. Prognostic significance of vascular endothelial growth factor expression in esophageal carcinoma: A meta-analysis. J Balk Union Oncol. 2013; 18(2):398–406.

[22] Zhang J, Liu J, Zhu C, He J, Chen J, et al. Prognostic role of vascular endothelial growth factor in cervical cancer: A meta-analysis. Oncotarget. 2017; 8(15):24797–24803.

[23] Oonk MH, van Hemel BM, Hollema H, de Hullu JA, Ansink AC, et al. Size of sentinel-node metastasis and chances of non-sentinel-node involvement and survival in early stage vulvar cancer: Results from GROINSS-V, a multicentre observational study. Lancet Oncol. 2010; 11(7):646–652.

Ovarian Sentinel Node
Is It Feasible?

Reita H. Nyberg, MD, Pasi Korkola, Lic Tech, MD, PhD,† and Johanna Mäenpää*‡*

Objective: To examine whether the intraoperative combined injection technique is feasible in locating the sentinel node(s) of the ovary.

Methods/Materials: In 16 patients with high-risk uterine cancer and normal postmenopausal ovaries, technetium isotope and blue dye were injected in the right or left ovary during laparotomy, respectively. During the operation, the pelvic and para-aortic lymphatic areas were searched, and the number, method of detection, and location(s) of the hot and/or blue node(s) were recorded.

Results: One to 3 sentinel nodes per patient were identified in all but 1 patient (15 of 16, 94%). The sentinel nodes (n = 30) were all located in the para-aortic area. The sentinel nodes of the left ovary were mainly (9 of 14, 64%) located above the inferior mesenteric artery level, as the most sentinel nodes of the right ovary (15 of 16, 94%) were found below the inferior mesenteric artery level (P = 0.001). There were no contralateral or bilateral sentinel nodes.

Conclusions: The combined intraoperative injection technique with radioisotope and blue dye is fast enough to identify the ovarian sentinel node(s). The stained nodes were consistently located on a certain lymphatic area. The sentinel node concept for the early ovarian cancer deserves more attention.

Key Words: Sentinel lymph node, Ovary, Early ovarian cancer, Blue dye, Radioisotope

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In epithelial ovarian cancer macroscopically defined to the ovary/ovaries (International Federation of Gynecology and Obstetrics [FIGO] stage I), an occurrence of occult lymph node metastasis upstages the disease to FIGO stage IIIC. Lymph node involvement is an early phenomenon during the course of the disease. In a recent review, the total prevalence of pelvic and para-aortic node metastases in 689 patients with a suspected early-stage ovarian cancer was 20%.[1] The more nodal areas are explored and the more lymph nodes harvested, the higher is the incidence of metastatic nodes.[2]

An accurate surgical staging with systematic pelvic and para-aortic lymphadenectomy (LAE) is considered to be an important prognostic factor in early-stage ovarian cancer, although its therapeutic value and impact on survival are under discussion.[3] However, compared with the lymph node sampling, systematic LAE takes approximately 90 minutes longer to complete (P < 0.0001), the median blood loss is 300 mL higher (P < 0.0001), the median hospital stay is 1 day longer (P = 0.003), and the proportion of patients transfused is 36% versus 22% (P = 0.012).[4]

With the introduction of mini-invasive surgery to the gynecologic oncology, the concept of sentinel lymph node (SN) biopsy for assessing the regional lymph node status has been studied in other gynecologic cancers. In vulvar cancer, the accuracy of the concept has been proven with a total

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number of 617 patients, the SN being false negative in 1.3% of them.[5] A large multicenter observational study (Groningen International Study on Sentinel Nodes in Vulvar Cancer) has shown that in early vulvar cancer, inguinofemoral LAE can safely be omitted in patients with negative SN, leading to less surgery-related morbidity. The results of GOG 173 are pending, but the first reports support the use of SN procedure.[5] In cervical cancer, the accuracy of the SN concept is under investigation. The GOG 206 will hopefully answer that question in early-stage cervical cancer, and the validation of the method can begin. In endometrial cancer, the feasibility studies are ongoing concerning, for example, the technique of the tracer injection.[6]

In early-stage ovarian cancer, the reports of the SN concept with conventional blue dye and radioisotope tracers are missing in the literature. This may be due to perceptions that the ovary is an inconvenient target to the tracer injections, the radioisotope will require a too long time to be taken up to the lymphatic system and, moreover, that a lymphoscintigram is always needed for localizing the SNs. There is 1 report by Negishi et al[7] of ovarian sentinel node identification and lymphatic mapping in patients with endometrial and tubal carcinoma, but this was performed with activated charcoal solution, which is not the standard tracer regarding the sentinel node concept.

We wanted to examine whether a combined technique of radioisotope and blue dye injections to the ovary is feasible to use during laparotomy to locate lymph nodes that are the first to receive the tracers. Another objective was to find out if there are differences related to the side of the injection (right or left ovary, respectively).

MATERIALS AND METHODS

From October 2008 to July 2010, 16 postmenopausal women with histologically proven high-risk uterine carcinoma (endometrioid adenocarcinoma grade 3 [n = 9], uterine papillary serous carcinoma [n = 2], clear cell carcinoma [n = 3], or other risk factor(s) [n = 2]) and normal-looking postmenopausal ovaries were enrolled in this study. They were scheduled for total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), and pelvic and para-aortic LAE. In patients with suspected uterine papillary serous carcinoma, infracoelic omentectomy also was performed.

High-risk endometrial cancer patients were chosen to be recruited because of extensive LAEs required as a staging procedure for them. Partly apart from the fundal region of the uterus, the lymphatic drainage of the uterine body and the ovaries are different, with the uterus primarily draining to pelvic nodes and the ovaries to para-aortic nodes, respectively.[8] Consequently, we assumed that the uterine cancer and its possible lymphatic spread would not markedly interfere with our study.

At the beginning of laparotomy, as soon as the pelvic organs were reached, ^{99m}technetium (^{99m}Tc)–labeled human albumin colloid (Nanocoll; GE Healthcare, Saluggia, Italy) and patent blue dye (Bleu Patenté V; Guerbet, Paris, France) were slowly injected near the hilum of 1 ovary with a 22-gauge needle. The preparation of the radiopharmaceutical was made in the Department of Nuclear Medicine at the same day. The side of the injections was chosen in order of entrance to the study; in patients with uneven ordinal number, the injections were performed to the right ovary, and in patients with even ordinal number, the injection site was the left ovary. Therefore, for 8 patients, the tracers were injected to the right ovary, and for the 8 patients, the tracers were injected to the left ovary.

After injections, we waited for 10 to 21 minutes (median, 15 minutes) before starting TAH and BSO. During this time, abdominal cavity was thoroughly explored for any evidence of extrauterine malignancy, and a peritoneal cytological sample was taken. At this time, it often was possible to visualize the blue afferent lymph vessels leading to the blue-stained lymph node (Fig. 1). The location of the blue dye was noted. After this, the uterus and adnexa were removed, followed by a systematic pelvic and para-aortic LAE at least to the level of inferior mesenteric artery (IMA). During LAEs, blue-stained nodes were located visually. A hand-held detector was used to locate the radioactive or "hot" nodes (Neo2000; Neoprobe, Dublin, Ohio). A count rate of at least 100 times the background radiation was set to represent the threshold for a node to be hot. The number, the method(s) of detecting the node, the exact locations of the detected nodes, the timing of the injections, the start of the TAH and BSO, and the identification of the SNs were recorded. For each patient, demographic data, intraoperative and postoperative complications, and the results of the final pathological examination also were recorded.

The 2-sided Fisher exact test was used to assess the differences between the locations of the right and left ovarian SNs.

The study protocol was approved by the local ethics committee of Pirkanmaa Hospital District. A written informed consent was obtained from all patients.

RESULTS

The median age of the patients was 69 years (range, 58–77 years), and the median body mass index (BMI) was 27

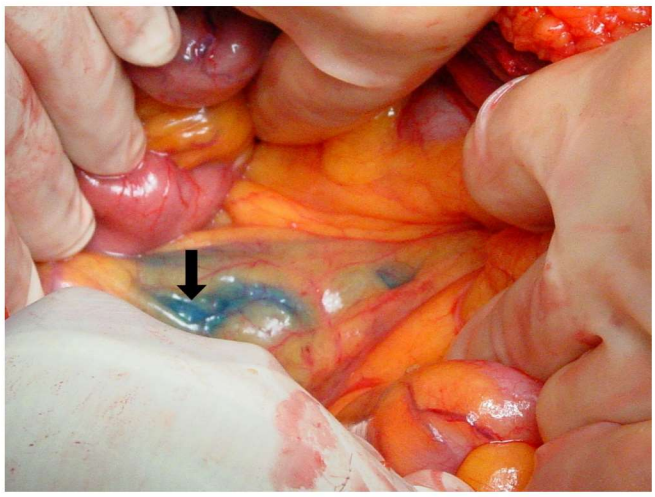

FIGURE 1. The blue afferent lymphatic vessels and a blue sentinel node (black arrow) on the left side of the aorta 10 minutes after tracer injections.

(range, 20–42). Four patients had had previous abdominal surgery (1 cholecystectomy and 3 appendectomies) but no previous gynecological surgery.

The median volume of the injected ^{99m}Tc-isotope was 0.8 (0.2–1.0) mL, and the median measured and calculated net activity was 19 MBq (range, 4.3–25.8 MBq). Therefore, the induced effective radiation dose to a patient by this procedure was less than 0.1 mSv. The count rates of the hot nodes varied between 230 and 20.300 counts per second. The median injected volume of the blue dye was 2.0 mL (range, 1.0–2.0 mL). The median number of the removed lymph nodes per patient was 28 (range, 14–52).

A total of 30 blue and/or hot lymph nodes were located in all but 1 patient (15 of 16, 94%), of which, 24 (80%) were both stained with blue dye and expressed radioactivity. One SN was detected only visually (total of 25 blue nodes) and 5 only because of increased radioactivity (total of 29 hot nodes). The median number of SNs per patient was 2 (range, 1–3). There was no difference in the median number of SNs per patient according to the site of injection (right vs left ovary; 2 [range, 1–3] vs 2 [range, 1–3], respectively).

The median interval from the tracer injection to the detection of all SN(s) was 138 minutes (range, 105–215 minutes). Even then was the blue dye visible in most of the SNs (25 of 30, 75%). The time intervals between tracer injections and identifications of the SNs are shown in Figures 2 and 3 according to the number of blue and hot nodes per patient, respectively. There did not seem to be any correlation between the time interval and the number of detected SN per patient. Although there was a tendency toward a longer time interval with a higher BMI, the BMI did not seem to affect the number of detected SNs. Unfortunately, the sample size is too small for a correlation analysis.

All the detected SNs were found in the para-aortic area ipsilaterally to the injection site. A total of 20 SNs (67%) were found below the level of IMA and 10 (33%) above that level. There was a statistically significant difference in the level of the right- and left-sided SNs in relation to IMA. When the tracers were injected to the right ovary, 94% (15 of 16) of the SNs were located below the IMA level. The left

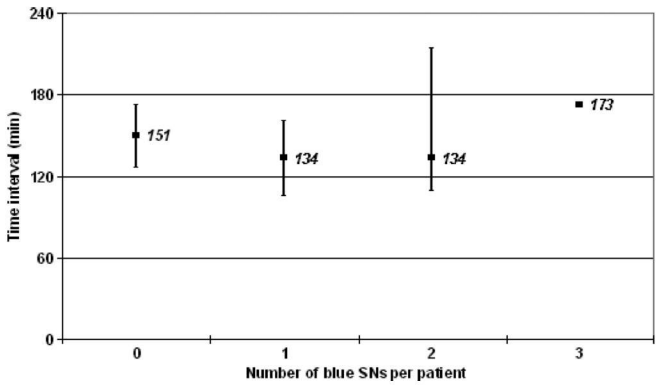

FIGURE 2. Time interval between the injection of blue dye and the detection of blue SNs according to the number of blue SNs per patient (■, median; I, range). Only 1 patient had 3 blue SNs, range not given.

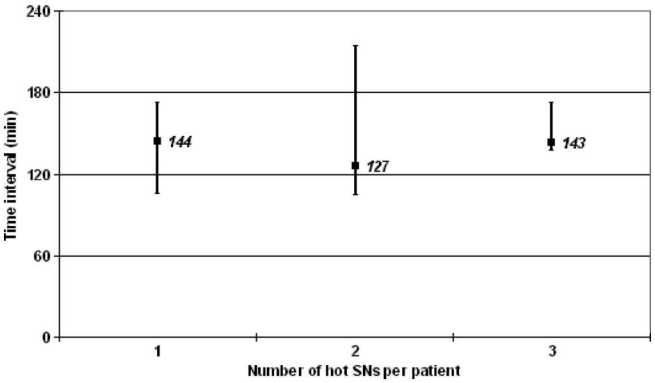

FIGURE 3. Time interval between the injection of technetium colloid and the detection of hot SNs according to the number of hot SNs per patient (■, median; I, range).

ovary being the injection site, 64% (9 of 14) of the SNs were located above the IMA level ($P = 0.001$). The exact locations are shown in Figure 4.

In the 1 patient (patient 2: a 69-year-old woman with BMI of 31) from whom the SN was missed, the tracers (0.7 mL, 17.0 MBq technetium isotope, and 1.0 mL blue dye) were injected to the left ovary. The interval from the tracer injection to the beginning of the TAH and BSO was 21 minutes. She had a final diagnosis of endometrial clear cell cancer FIGO (2009) stage IIIC1 with bilateral pelvic lymph node metastases. In the final histopathological report, 3 additional patients (patients 1, 3, and 10) were found to have lymph node metastases either in the pelvic (n = 2) or pelvic and para-aortic (n = 1) nodes, respectively. One patient without lymph node metastases (patient 8) had a metastasis in the left fallopian tube. In the remaining 11 patients (69%), the disease was limited to the uterus (FIGO 2009 stages IA-IB).

One patient (patient 7: age of 69 years and BMI of 42) had an intraoperative bleeding of 1000 mL during hysterectomy, but she did not require any transfusions perioperatively or postoperatively. There was 1 allergic reaction during the operation (patient 16: age of 68 years and BMI of 24), in the form of resilient hypotension and urticaria in the patient's left arm (where the intravenous line was placed), observed 15 to 20 minutes after tracer injections. She was given several boluses of etilephrine and hydrocortisone intravenously before an intravenous infusion of norepinephrine was started. In the recovery room, she still had some faint rash on her upper body and arms but no other symptoms. Two months after laparotomy, immediate-type hypersensitivity toward blue dye was confirmed by skin tests.

DISCUSSION

Our series of 16 patients shows that it is possible to use intraoperative injections of radioisotope and blue dye for the identifying sentinel node(s) of the ovary. The detection rate in our study was 94% with a total number of 30 identified SNs. Most of the SNs (80%) were identified by both tracers. Approximately 83% (25 of 30) of the SNs were blue stained,

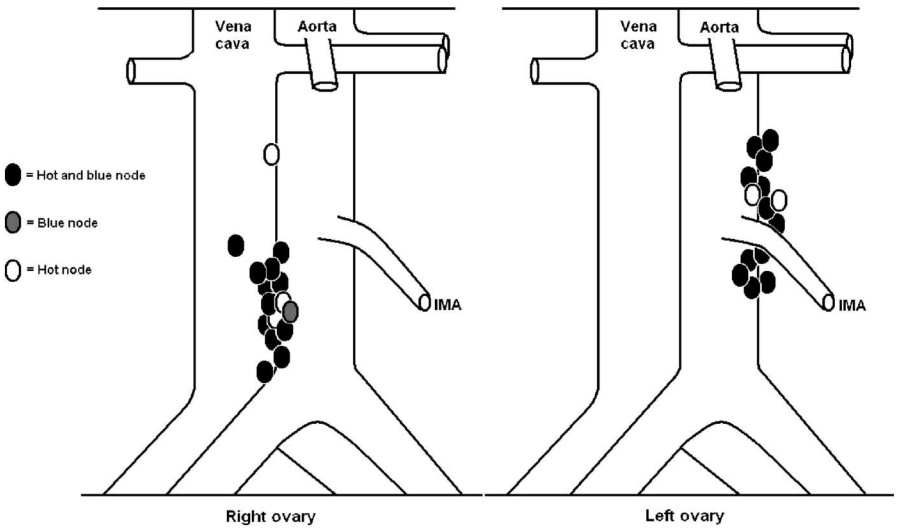

FIGURE 4. The sentinel node locations of the right and left ovary.

and 97% (29 of 30) expressed radioactivity. Either of the single tracers was identifiable in all detected SNs that favor the use of a combined technique.

Patent blue is known to be rapidly transported to the lymphatic channels. The ^{99m}Tc-labeled albumin colloid also seems to have a swift uptake. It also has been shown to be useful in breast cancer, where it has been a trend to start using intraoperative radiocolloid injections to minimize the pain related to the injection. Even waiting for just 15 minutes after injection, the results of lymphoscintigraphy have been similar as compared with the delayed imaging (18–24 hours after injection).[9]

The particle size of the colloid tracer is purported to determine the rate of transportation through the lymphatic system. The smaller the diameter is, the faster are the particles taken up and travelling across the lymphatic capillaries. The smallest particles (diameters of a few nanometers) also are exchanged through blood capillaries. Larger particles (diameters of hundreds of nanometers) are trapped in the interstitial space and drain slowly to the lymphatic channels. The medium-sized particles (diameters of tens of nanometers) travel across the lymphatic channels with ease and are trapped in the first lymph node.[10] The colloid particles smaller than 20 nm tend to spill over further upstream, thus showing too many "sentinel nodes." For localizing just the first one, a slightly larger diameter (50–200 nm) is required.[11] The pharmaceutical product of Nanocoll that we used includes particles that have a diameter of less than 80 nm (at least 95% of the particles) and seems to be suitable for the intraoperative use.

When considering surgery for early ovarian cancer, the concept described here offers plenty of time for tracer injections, for an evaluation of the abdominal cavity, for a removal of the ovarian tumor(s), and for a frozen section analysis of the tumor before the search for an SN must begin. The shortest possible interval from the injection to the detection of both tracers in the nodal areas was not determined in this study.

An important question is how many ovarian SNs can be expected to be found. In our study, the median number of the detected nodes per patient was 2 (range, 1–3). Whether the total number of the SNs is influenced by the time interval of injection and detection of the nodes remains unclear because the small sample size did not allow us to perform a statistical analysis on the matter. However, the longer interval did not seem to correlate with any higher number of the detected SNs.

In our study, we described an uptake of the used tracers only in the para-aortic nodal area. In previous works, para-aortic area has been found to be an important site for lymph node metastases. According to a review by Benedetti Panici and Angioli, in years 1983–2000, the prevalence of para-aortic metastasis alone in apparent early-stage ovarian cancer was 6% compared with pelvic metastasis alone (8%) and pelvic and para-aortic metastasis together (5%). However, they described precaval and paracaval and preaortic and para-aortic lymph nodes to be the most important single sites of metastasis in tumors seemingly confined to the ovary.[12] In a recent series of 355 patients, the main site for metastasis in early ovarian cancer was para-aortic area only (64% of metastatic nodes).[2] In a summary by Nomura et al,[13] in unilateral ovarian tumor cases (n = 67), only ipsilateral lymph nodes were positive in 54% of patients. Contralaterality of the lymph node metastasis was found in 13% of patients and bilaterality in 33% of patients.

In the previously mentioned series of 11 patients by Negishi et al,[7] the activated charcoal solution (CH40) injected to 1 ovary deposited to the para-aortic sites in all patients and also to the common and external iliac nodes in 26% and 9%, respectively, of the patients (when injected to the right ovary). The number of detected nodes per patient was not specified. They reported bilateral staining in 60% of patients after the right-sided injection and 33% after the left-sided injection.

In our series, the location of the SNs in relation to IMA seemed to vary according to the side of injection. The SNs of the left ovary often were more found above the level

of the IMA than the SNs of the right ovary ($P = 0.001$). It is consistent with the anatomy of the main ovarian lymphatic routes that run bilaterally along the ovarian blood vessels and terminate in the aortic nodes asymmetrically, where the ovarian veins reach the vena cava on the right side or the left renal vein on the left side.[12] Similarly, in the work of Negishi et al,[7] the uptake of CH40 was restricted to the para-aortic area and especially above the IMA when injected to the left ovary.

Despite previous reports mentioned above, we found no contralateral, bilateral, or pelvic uptake. Early in the learning curve, the remnants of the tracers in the stump of ovarian vessels might interfere with the search of the pelvic ipsilateral SN. Therefore, the small sample size in our study is likely to have affected our results in this respect. However, we reached the purpose of this study, which was to test whether the intraoperative injection technique is feasible in demonstrating ovarian sentinel node(s).

The only patient (patient 2) from whom we could not identify any SN had bilateral pelvic lymph node metastases of uterine clear cell carcinoma. The tracer injection technique and the amounts of isotope and blue dye were comparable to the other cases. The early stage of learning curve of this particular procedure is likely to have influenced the inability to detect SNs because she was our second patient. Another explanation is that lymph node metastases can obstruct lymph flow and thereby cause a failure of SN detection. This phenomenon has been described at least in vulvar cancer.[14] On the other hand, in 3 other FIGO stage IIIC patients, we were able to detect 1 to 2 SNs.

There was one injection-related complication—a relatively mild allergic reaction—in our series, which was diagnosed as immediate-type hypersensitivity to blue dye. According to literature, the rate of all allergic reactions toward isosulfan blue (Lymphazurin 1%; US Surgical Corp, Norwalk, Conn) in sentinel node procedures has been 1.4%, and the rate of serious grade III reactions has been 0.4%. Isosulfan blue is a structural isomer of patent blue, which we used and which, according to a recent publication, is even less allergenic; the rate of all reactions was 0.9%, and that of the serious reactions was 0.06%.[15] However, the possibility of an allergic reaction is important to remember because vital blue dyes also are widely used in everyday life and allergic cross-reactions between them do occur.[16]

We have here presented the first feasibility study of ovarian SN with the conventional combined method of radioisotope and blue dye. The use of both tracers during laparotomy seems feasible. No markedly prolonged time interval between tracer injections and beginning of the TAH and BSO is required. The blue dye gives the direction where to look for the SN, and both tracers are identifiable even after extended surgery. The pattern of SN locations was constant and relevant to the previous knowledge of ovarian lymphatic drainage. Our results cannot directly be translated into the surgical treatment of ovarian cancer patients. However, we are encouraged to proceed to the research on the SN(s) of ovarian malignant tumors per se.

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REFERENCES

1. Angioli R, Plotti F, Palaia I, et al. Update on lymphadenectomy in early and advanced ovarian cancer. *Curr Opin Obstet Gynecol*. 2008;20:43–39.
2. Fournier M, Stoeckle E, Guyon F, et al. Lymph node involvement in epithelial ovarian cancer. *Int J Cynecol Cancer*. 2009;19:1307–1313.
3. Kim HS, Ju W, Jee BC, et al. Systematic lymphadenectomy for survival in epithelial ovarian cancer: a meta-analysis. *Int J Gynecol Cancer*. 2010;20:520–528.
4. Maggioni A, Benedetti Panici P, Dell'Anna T, et al. Randomised study of systematic lymphadenectomy in patients with epithelial ovarian cancer macroscopically confined to the pelvis. *Br J Cancer*. 2006;95:699–704.
5. Oonk MHM, van de Nieuwenhof HP, van der Zee AGJ, et al. Update on the sentinel lymph node procedure in vulvar cancer. *Expert Rev Anticancer Ther*. 2010;10:61–69.
6. Oonk MHM, van de Nieuwenhof HP, de Hullu JA, et al. The role of sentinel node biopsy in gynecological cancer: a review. *Curr Opin Oncol*. 2009;21:425–432.
7. Negishi H, Takeda M, Fujimoto T, et al. Lymphatic mapping and sentinel node identification as related to the primary sites of lymph node metastasis in early stage ovarian cancer. *Gynaecol Oncol*. 2004;94:161–166.
8. Creasman WT. Adenocarcinoma of the uterus. In: DiSaia PJ, Creasman WT, eds. *Clinical Gynecologic Oncology*. Philadelphia, PA: Mosby Elsevier; 2007:147–184.
9. Babiera GV, Delpassand ES, Breslin TM, et al. Lymphatic drainage patterns on early versus delayed breast lymphoscintigraphy performed after injection of filtered Tc-99m sulfur colloid in breast cancer patients undergoing sentinel lymph node biopsy. *Clin Nucl Med*. 2005;30:11–15.
10. Jimenez IR, Roca M, Vega E, et al. Particle sizes of colloids to be used in sentinel lymph node radiolocalization. *Nucl Med Commun*. 2008;29:166–172.
11. Keshtgar MRS, Ell PJ. Sentinel lymph node detection and imaging. *Eur J Nucl Med*. 1999;26:57–67.
12. Benedetti Panici P, Angioli R. Role of lymphadenectomy in ovarian cancer. *Best Pract Res Clin Obstet Gynaecol*. 2002;16:529–551.
13. Nomura H, Tsuda H, Susumu N, et al. Lymph node metastasis in grossly apparent stages i and ii epithelian ovarian cancer. *Int J Gynecol Cancer*. 2010;20:341–345.
14. 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.
15. Barthelmes L, Goyal A, Newcombe RG, et al. NEW START and ALMANAC study groups. Adverse reactions to patent blue v dye—the NEW START and ALMANAC experience. *Eur J Surg Oncol*. 2010;36:399–403.
16. Scherer K, Studer W, Figueiredo V, et al. Anaphylaxis to isosulfan blue and cross-reactivity to patent blue v: case report and nomenclature of vital blue dyes. *Ann Allergy Asthma Immunol*. 2006;96:497–500.

Sentinel Node and Ovarian Tumors
A Series of 20 Patients

Reita H. Nyberg, MD, Pasi Korkola,† and Johanna U. Mäenpää‡*

Objective: Intraoperative detection of ovarian sentinel nodes has been shown to be feasible. We examined the detection rate and locations of sentinel nodes in patients with ovarian tumors. We also aimed to assess the reliability of sentinel node method in predicting regional lymph node metastasis.

Methods: Twenty patients scheduled for laparotomy because of a pelvic mass were recruited to the study. In the beginning of the laparotomy, radioisotope and blue dye were injected under the serosa next to the junction of the ovarian tumor and suspensory ligament. The number and locations of the hot and/or blue nodes/spots were recorded during the operation. If the tumor was malignant according to the frozen section, systematic lymphadenectomies were performed, the sentinel nodes sampled separately, and their status compared with other regional lymph nodes.

Results: Eleven patients had a right-sided ovarian tumor, 7 patients a left-sided tumor, and 2 patients had bilateral tumors. A median of 2 sentinel nodes/locations per patient (range, 1–3) were found. Sixty percent of all sentinel nodes were located in the para-aortic region only, compared with 30% in both para-aortic and pelvic areas and 10% in pelvic area only. Both unilateral and bilateral locations were found. In 83% of the cases with more than 1 sentinel node location, they were located in separate anatomical regions. In 3 patients, systematic lymphadenectomies were performed. One of them had nodal metastases in 2 regions and also a metastasis in 1 of her 2 sentinel nodes in 1 of those regions.

Conclusions: In patients with ovarian tumor(s), the detection of sentinel nodes is feasible. They are located in different anatomic areas both ipsilaterally and contralaterally, although most of them are found in the para-aortic region. The reliability of the sentinel node concept should be evaluated in the framework of a multicenter trial.

Key Words: early ovarian cancer, lymphadenectomy, ovarian tumor, sentinel lymph node, surgical staging

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Pelvic and para-aortic (PA) lymphadenectomy is considered to be a mandatory part of surgical care in early ovarian cancer. Accurate surgical staging is associated with better prognosis, provided that at least 10 nodes are harvested from different and specific retroperitoneal sites, especially PA/paracaval (PC) nodes between the inferior mesenteric artery

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(IMA) and the renal vein, superficial iliac nodes, and nodes from the obturator fossa.[1] Moreover, larger number of removed nodes increases the likelihood of finding more lymph node metastasis.[2] On the other hand, the increasing extent of lymphadenectomy carries increasing risk of serious complications,[1] the blood loss and proportion of patients transfused, the duration of the surgery and the length of hospital stay.[3]

During the surge of mini-invasive surgery in gynecological cancer, sentinel node (SN) concept has been adopted into surgery of early vulvar, cervical, and endometrial cancer.[4] In early ovarian cancer, the concept has been mainly overlooked because of a laborious reachability of the intra-abdominal injection site, a presumption of the slowness of commonly used tracers, and a fear of spillage of tumor cells following the injection.

In 2011, Nyberg et al[5] reported a successful intraoperative use of blue dye and technetium isotope in detection of ovarian SNs after tracer injection into a normal postmenopausal ovary in patients with high-risk uterine cancer. The SN detection rate during systematic lymphadenectomies in a series of 16 patients was 94%, and all SNs were located in the PA regions. Three years later, Kleppe et al[6] described that the use of blue dye and radioisotope in ovarian tumor patients was successful in finding SN locations in all of their 21 patients. They injected the tracers into 2 sites in ovarian ligaments outside the suspicious tumor to prevent any potential dissemination of cancer cells. After injection, they located the hot SN sites transperitoneally with a gamma detector and retroperitoneally, if the tumors proved to be malignant and lymphadenectomies were carried out.[6]

With our patient series, we wanted to examine the detection rate and locations of SNs by conventional tracers (blue dye and radioisotope) in patients with suspicious ovarian masses. We also aimed to evaluate the reliability of SN method in predicting regional lymph node metastasis, in case the mass proved to be malignant in frozen-section analysis.

MATERIALS AND METHODS

From December 2010 through September 2013, 20 eligible women with either unilateral or bilateral ovarian masses were recruited to the study. The inclusion criteria were as follows: scheduled open surgery (total abdominal hysterectomy and bilateral salpingo-oophorectomy) to remove the suspicious mass(es), which was estimated to involve the ovary/adnexa only. In premenopausal women with intact uterus, also a negative pregnancy test within 24 hours before surgery was required. The exclusion criteria were as follows: previous allergic reaction to blue dye or human albumin and signs of malignant spread to the abdominal cavity in preoperative imaging. Ascites alone and/or elevated serum CA-125 without any other sign of dissemination were not exclusive.

The patient demographics, previous operative history, and the largest diameter of the ovarian tumor(s) from each patient were recorded.

In the beginning of each laparotomy, the adnexal mass (or masses) was exposed. This sometimes required liberation of adhesions and/or lifting the large tumor outside the abdominal cavity. Then, 1 mL of technetium Tc 99m–labeled human albumin colloid (Nanocoll; GE Heathcare, Saluggia, Italy) was slowly injected to 1 spot under the serosa next to the lateral junction of the ovarian tumor (mesovarium) with a 27-gauge needle (Fig. 1). The preparation of the radiopharmaceutical was performed in the Department of Nuclear Medicine on the same day. The needle was then kept in its place, the syringe changed, and 2 mL of patent blue dye (Bleu Patenté V; Guerbet, Paris, France) was injected to the same spot, to prevent the tracers to spill out from multiple needle holes and stain the operation field. In the case of bilateral tumors, the tracers were injected to both sides.

After a minimum of a 10-minute interval, during which the abdominal cavity was examined, the peritoneal cytology taken, and the passage of the blue dye noted, the adnexal mass or masses were removed, opened, and sent to a pathologist for frozen-section analysis. After that, the operation was continued with hysterectomy (unless previously removed). If the mass was benign or borderline not needing lymphadenectomies, the pelvic and PA areas were then closely examined transperitoneally for the visible blue dye and extra radiation without opening the peritoneum or removing the nodes. A count rate of at least 10 times the background radiation was thought to represent a "hot" spot and SN location, using a handheld gamma detector (Neo2000; Neoprobe, Dublin, OH). The number and locations of each blue and/or hot spots were marked on a map, which contained all significant lymphatic regions including upper PA and PC areas (above IMA), lower PA/PC area (below IMA), right and left common iliac area (upper pelvic area), right and left external iliac area, and right and left obturator area (lower pelvic area), as well as the method of their detection. In the case of a borderline tumor, a complete surgical staging (peritoneal biopsies, omentectomy, appendicectomy) was then performed. If the tumor was malignant, systematic

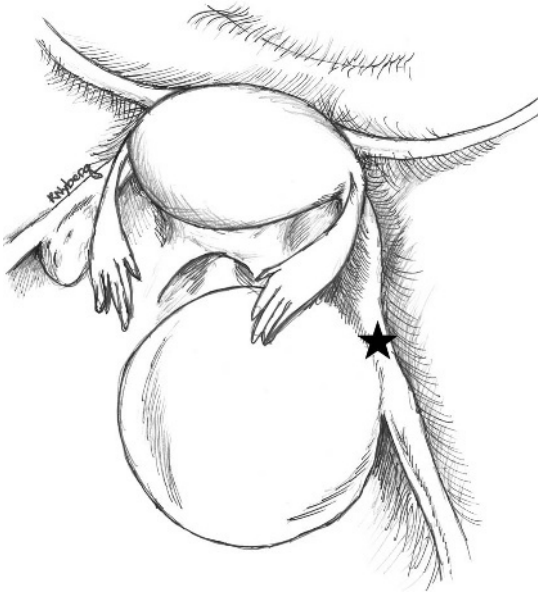

FIGURE 1. The injection spot of the tracers to the mesovarium.

pelvic and PA lymphadenectomies were carried out as a part of complete surgical staging, when technically possible. After opening the peritoneum, all retroperitoneally detected SNs (hot and/or blue) were sampled separately, and their location marked on the map similarly as above.

The non-SNs were processed according to a standard protocol for lymph node examination; they were cut into single sections or, if more than 1 cm in diameter, into 2 to 3 sections and stained with hematoxylin-eosin before microscopy. The SNs were cut into 2-mm sections for hematoxylin-eosin staining. Ultrastaging and immunohistochemistry were not used. All detected metastases and their locations were recorded, and the status of each SN was compared with the status of the non-SNs in the same region.

Pearson correlation coefficient was used to estimate the association between tumor size and SN number. $P < 0.05$ considered statistically significant.

The study protocol was approved by the local ethics committee of Pirkanmaa Hospital District (approval no. R10072). A written informed consent was obtained from all patients.

RESULTS

Twenty women, with median age of 63.3 years (range, 41.1–80.7 years) and body mass index (BMI) of 25.8 kg/m^2 (range, 21.5–36.1 kg/m^2), were enrolled. The patient data are shown in Table 1. In women with previous open surgeries, a careful liberation of adhesions had to be made before the injection site was properly exposed. Two tumors were torquated (patients 12 and 18), and the latter also buried under adhesions requiring liberation. The distribution of the tumor aspects is shown in Table 2. The median preoperative diameter of the right-sided tumors was 11 cm (range, 5–20 cm) and that of the left-sided tumors 7 cm (range, 3.5–21 cm). One right-sided tumor was found to be ruptured, and according to the symptoms of the patient, that had happened the night before surgery. Both tracers were successfully injected as described previously all 20 women, and no adverse or allergic reactions were noted. The median injected activity of Nanocoll to 1 side was 20.0 MBq (range, 17.0–26.6 MBq). The median interval between the tracer injections and the beginning of the removal of the tumor(s) was 12 minutes (range, 10–33 minutes).

TABLE 1. Patient demographics

Patient No.	Age, y	BMI, kg/m^2	Tumor Side	Previous Operations	Performed Operations (Cytology Was Taken in Every Case)	Final Diagnosis and FIGO Stage
1	80.5	32.0	Bilateral		TAH, BSO	Cystadenofibroma
2	57.7	28.3	Left	SVA, BS	BO, ADH	Serous cystadenofibroma
3	64.6	24.2	Right		TAH, BSO, OM, APP, BP	Mucinous BOT stage 1c2
4	70.2	27.5	Right		TAH, BSO	Serous cystadenofibroma
5	56.4	26.0	Right		TAH, BSO, OM, APP, BP	Serous BOT stage 1a
6	63.0	25.6	Left		TAH, BSO	Serous cystadenoma
7	41.1	24.3	Right	APP	TAH, BSO, LAE, OM	Endometrioid adenocarcinoma stage 1c2
8	80.7	28.7	Right		TAH, BSO, OM, APP	Serous adenocarcinoma grade 2 stage 3a2
9	62.5	22.9	Left	VH	BSO, OM, APP, BP	Mucinous BOT stage 1a
10	75.1	27.4	Right	TAH, BS, APP	BO, ADH	Mucinous cystadenoma
11	43.0	23.0	Right		TAH, BSO	Mucinous cystadenoma
12	63.8	24.9	Left		TAH, BSO	Fibroma
13	63.6	27.7	Left		TAH, BSO	Brenner tumor/mucinous cystadenoma
14	65.5	25.3	Left		TAH, BSO	Mucinous cystadenoma
15	43.6	23.7	Right	APP	TAH, BSO, LAE, OM	Serous cystadenocarcinoma grade 3 stage 3a1
16	55.6	27.6	Right	TAH	BSO, OM, APP, ADH	Metastatic breast cancer
17	62.1	21.5	Right		TAH, BSO, LAE, OM, APP	Serous BOT stage 1c3
18	58.1	25.1	Bilateral		TAH, BSO	Adenofibroma
19	68.9	25.9	Left		TAH, BSO	Serous cystadenoma
20	68.5	36.1	Right		TAH, BSO, OM, APP	Serous adenoma grade 3 stage 2a

ADH, liberation of adhesions; APP, appendicectomy; BO, bilateral oophorectomy; BOT, borderline ovarian tumor; BP, peritoneal biopsies; BS, bilateral salpingectomy; BSO, bilateral salpingo-oophorectomy; FIGO, International Federation of Gynecology and Obstetrics; LAE, pelvic and PA lymphadenectomies; OM, omentectomy; SVA, supravaginal uterine amputation; TAH, total abdominal hysterectomy; VH, vaginal hysterectomy.

TABLE 2. Distribution and laterality of SN locations in 20 patients according to the side of the ovarian tumor

Tumor Location	No.	The Region Of SN Site			Unilateral SNs		Bilateral SNs
		Para-aortic Only	Both PA and Pelvic	Pelvic Only	Ipsilateral	Contralateral	
Right ovary	11 (55%)	7 (64%)	2 (18%)	2 (18%)	9 (82%)	1 (9%)	1 (9%)
Left ovary	7 (35%)	5 (71%)	2 (29%)	0 (0%)	6 (86%)	0 (0%)	1 (14%)
Bilateral	2 (10%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Total	20 (100%)	12 (60%)	6 (30%)	2 (10%)	15 (75%)	1 (5%)	4 (20%)
					16 (80%)		

Tumor side was also the tracer injection site.

During the operation, 11 women had benign frozen sections, and the final histopathologic examination confirmed those results. Four women had borderline tumors. In 1 case (patient 17), the pathologist could not exclude invasion based on the frozen section. As there were also palpable prominent lymph nodes on the right PA region, systematic lymphadenectomies were carried out, and 3 hot and blue SNs were found and sampled on that area. The final diagnosis was serous borderline tumor, with endosalpingiosis present in 2 of 3 SNs.

Five women had a malignant disease (patients 7, 8, 15, 16, and 20). During the examination of the abdominal cavity, an unexpected carcinosis was found in 2 of them (patients 8 and 16). Patient 8 had a final diagnosis of International Federation of Gynecology and Obstetrics stage 3a2 serous ovarian cancer with suboptimal surgical result, and patient 16 had a breast cancer metastasis in the right ovary, spreading to the abdominal cavity. In both, SN locations (1 and 3, respectively) were transperitoneally detected and mapped. Patient 20 had a BMI of 36.1 kg/m^2, and because of technical difficulties, the lymphadenectomies were passed, but 1 hot SN location was found and mapped. Altogether, systematic lymphadenectomies with separate SN samplings were performed to 3 patients (15% of study population), and in the rest of the patients, a transperitoneal SN mapping was carried out.

We were able to locate 1 to 3 SNs (median of 2) from each woman, resulting in total of 36 SN sites. For both right- and left-sided tumors, the mean number of SNs was 2, with a range of 1 to 3 and 1 to 2, respectively. In the case of bilateral tumors, the median number of SNs was 2 (range, 2–3). There was no correlation between the size of the tumor and the number of detected SN sites per patient ($r^2 = 0.0012$, 2-sided $P = 1.00$). In 12 patients (60%), more than 1 SN was found, and in 10 cases (83%), they were located in the separate anatomical regions. The distribution of SN locations and laterality according to the tumor side are shown in Table 2. In 18 patients with unilateral tumors, most of the SN sites were ipsilateral (83%, 15 patients), and contralateral only in 1 patient (6%), whereas bilateral SN sites were found in 11% (2 patients). With bilateral ovarian masses, all the SN sites were detected bilaterally.

The regional distributions of all SNs are shown in Figure 2. Seventy-eight percent (28/36) of all SN sites were located in the PA region. In 12 (60%) of 20 patients, it was the only region where SNs were detected. In 6 (30%) of patients, both PA and pelvic SN locations were seen together. In only 2 patients (10%), the SNs were found solely in the pelvis.

The left-ovary–related SN sites seemed to be located higher than those related to the right ovary; 64% versus 30% were located above the IMA, respectively. Whereas 18% of the left-ovary–related SN sites were traced to the upper pelvic (common iliac) region, no SN sites were found in the lower pelvic area. Ten percent of the right-ovary–related SN sites were found in the upper pelvic region (common iliac region), and 10% in the lower pelvic region. The tracers seemed to cross the midline from left to right only above the IMA level, whereas crossover from right to left was observed in the upper pelvic, lower PA, and upper PA regions.

The results of the 3 patients with systematic lymphadenectomies are shown in Table 3.

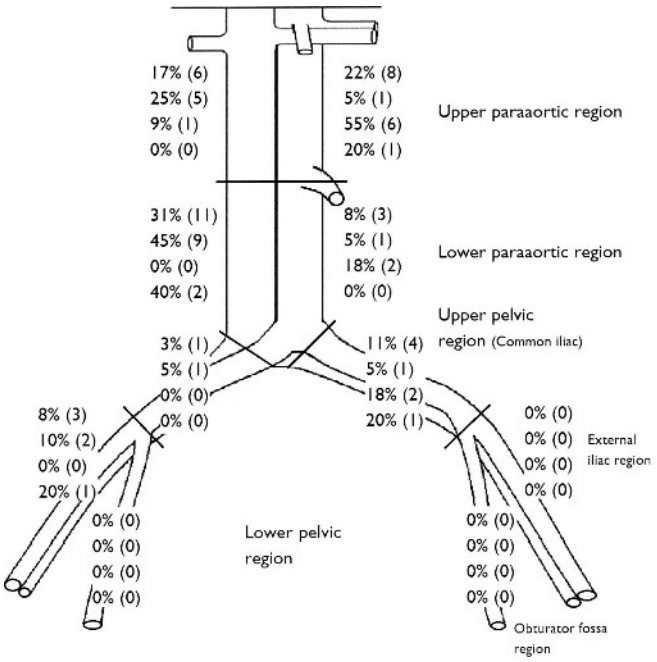

FIGURE 2. The distribution (percentages and numbers) of SNs in all anatomic regions according to the injection site: all SNs, right-ovary–related SNs, left-ovary –related SNs, and SNs of both ovaries, respectively.

Eighteen of all SNs (50%) were detected both visually and with gamma detector, 17 (47%) by means of radiation only, and 1 (3%) only visually. With transperitoneal mapping (28 SN locations), 43% (12 SNs) were found by combining dye and radiotracer, 54% (15 SNs) with radiation only, and 4% (1 SN) with dye only. Eight SNs were collected in lymphadenectomized patients, 75% (6 SNs) with combined method and 25% (2 SNs) by using gamma detector. The median intervals between injections and identification of SNs were 56 minutes (28–126 minutes) transperitoneally and 129 minutes (49–180 minutes) retroperitoneally.

DISCUSSION

With a detection rate of 100%, our results confirm that blue dye and radioisotope are swiftly transported from the mesovarium to the regional lymph nodes and that perioperative SN mapping is feasible in patients with ovarian tumors.

The distribution of SN sites in our study is in line with literature concerning lymph node metastases in apparent early ovarian cancer (EOC). According to a review with more than 1200 EOC cases, PA metastases alone were found in 50% of patients, both PA and pelvic metastases in 30% of patients, and pelvic metastases alone in 20% of patients. In case of a unilateral tumor, 56% of the metastases were ipsilateral, 21% contralateral, and 41% bilateral (2).

In the present study, the left-ovary–related SNs were located either in the PA (71%) or PA and upper pelvic (29%) areas, whereas the right-ovary–related SNs were located PA (64%), PA and pelvic (18%), or solely pelvic (18%) areas, including also the lower pelvis. The left-ovary–related SNs were more often found above the IMA level than the right-ovary–related SNs (64% vs 30%, respectively). With unilateral tumors, unilateral SNs were more common than bilateral SNs (89% vs 11%). Nyberg et al[5] reported 64% of the left-ovary–related SNs to be located above the IMA level, whereas 94% of the right-ovary–related SNs were located below the IMA level. In that series, no bilateral or contralateral SNs were found. Kleppe et al[6] reported most of their SN locations being traced to the PA/PC region (67%), as only 9% were traced to the pelvis and 24% to both regions. Ninety-three percent of SN locations were ipsilateral. In their study, half of transperitoneally detected SN sites related to the left ovary were located in the ipsilateral upper PA area, compared with 45% of the SN sites related to the right ovary. All their removed SNs after left-sided injections were found high in the upper left PA area, but the SNs after right-sided injections were mainly located at the IMA level (6). This asymmetry, present in all 3 studies, may be a reflection of the asymmetrical drainage of the right and left ovarian veins.

It has been purported that the tracers should be injected to both ovarian ligaments.[6,7] According to a recent article, 3 lymphatic drainage pathways from the ovaries can be distinguished. The 2 main routes drain the ovaries via the suspensory ligament toward the PA/PC areas and via the proper ligament of the ovaries toward the obturator fossa and the internal iliac artery. The third minor pathway drains the ovaries via the round ligament to the inguinal lymph nodes but is probably present only in a small percentage of people. This

TABLE 3. Histopathologic results of the retroperitoneal SN sampling and systematic lymphadenectomy

		Lymphadenectomy and SN Results According to Anatomic Region						
Patient No.	Tumor Side	Right PA SNs (metastatic)	Right PA non-SNs (metastatic)	Left PA SNs (metastatic)	Left PA non-SNs (metastatic)	Right pelvic SNs (metastatic)	Right pelvic non-SNs (metastatic)	Left pelvic SNs (metastatic)	Left pelvic non-SNs (metastatic)
7	Right	3 (0)	10 (0)	0 (0)	19 (0)	0 (0)	24 (0)	0 (0)	31 (0)
15	Right	2 (1)	6 (2)	0 (0)	9 (0)	0 (0)	10 (2)	0 (0)	12 (0)
17	Right	3 (0*)	7 (0)	0 (0)	7 (0)	0 (0)	8 (0)	0 (0)	7 (0)

*Endosalpingiosis in 2 SNs.

study did not find any connections between right and left ovarian drainage, nor did it explain the asymmetry of lymphatic networks between right and left ovaries. It, however, described that a considerable number of lymphatic vessels are present in mesovarium along the entire length of the ovary.[7] To our experience, the tracers leak out from the tiniest holes and smudge the operation field, hampering the visibility of blue-stained lymphatics and the use of gamma detector in pelvis. Our 1-spot injection to the mesovarium gave similar SN distribution results compared with the study by Kleppe et al,[6] although they injected the tracers to both ovarian ligaments. Although one should be cautious in making firm conclusions based on small materials, it seems unnecessary to use multiple injection spots.

In the present study, blue dye was observed transperitoneally in less than half of the cases. Retroperitoneally explored, 75% of the hot nodes were also blue stained. In the study of Kleppe et al,[6] blue staining was not recorded at all in transperitoneal mapping. In retroperitoneal exploration, they identified blue dye in only one third of the patients.[6] The visibility of blue dye depends on the interval between the injection and mapping and the thickness of the tissue around the lymphatic vessels. To our experience, blue dye shows where to seek for the radiation. However, a lack of blue staining does not exclude the possibility of hot nodes. A new tracer indocyanine green is reported to have an improved tissue penetration compared with blue dye. Replacing blue dye with it might enhance the visibility. The results in endometrial and cervical cancer have certainly been promising.[8,9]

Only 1 patient in our series (patient 15) had lymph node metastases, in PC and the right pelvic area. Two SNs were found in the lower PC area, one of which was metastatic and predicted correctly the surgical stage of the patient. However, there were no SNs detected in the right pelvic area. In the series by Kleppe et al,[6] also 1 patient had lymph node metastases in SNs and in other regional nodes. She, however, had a final diagnosis of ovarian and synchronous endometrial cancer, and it was not conclusive which cancer the metastases were related to.[6] The number of patients studied is still too small to allow for any conclusions about the reliability of SN concept in ovarian cancer. When adding our results to the study of Kleppe et al,[6] 41 ovarian tumor patients have been operated on, 9 lymphadenectomies have been performed, and only 2 patients (5% of all) have had lymph node metastasis. Based on the above figures, to collect data of 20 EOC patients with nodal metastasis, one should include approximately 400 ovarian tumor patients. It is not easy to find suitable patients with suspicious ovarian tumors but no signs of dissemination. Two of our patients (10%) had more advanced disease than was preoperatively assumed. For us, it took several years to gather a series even this large. Obviously only a multicenter study could solve this problem.

We conclude that perioperative 1-spot injection of blue dye and technetium isotope is feasible in detecting SNs in patients with ovarian tumor(s). One to 3 SNs per patient can be found with this method; they are usually ipsilateral, but both bilateral and contralateral SNs are conceivable. Our results confirm that the main lymphatic drainage route from the ovary is to the PA region, the SNs of the left ovary being usually located higher than those of the right ovary. Also, the pelvic SNs related to the left ovary seem to be located higher in the pelvis than those related to the right ovary. The reliability of the SN concept in ovarian cancer and its clinical applications should be evaluated in a larger multicenter study.

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REFERENCES

1. Trimbos JB. Lymphadenectomy in ovarian cancer: standard of care or unnecessary risk. *Curr Opin Oncol*. 2011;23:507–511.
2. Kleppe M, Wang T, van Gorp T, et al. Lymph node metastasis in stages I and II ovarian cancer: a review. *Gynecol Oncol*. 2011;123:610–614.
3. Maggioni A, Benedetti Panici P, Dell'Anna T, et al. Randomised study of systematic lymphadenectomy in patients with epithelial ovarian cancer macroscopically confined to the pelvis. *Br J Cancer*. 2006;95:699–704.
4. Cibula D, Oonk MHM, Abu-Rustum N. Sentinel lymph node biopsy in the management of gynecologic cancer. *Curr Opin Obstet Gynecol*. 2015;27:66–72.
5. Nyberg RH, Korkola P, Mäenpää J. Ovarian sentinel node—is it feasible? *Int J Gynecol Cancer*. 2011;21:568–572.
6. Kleppe M, Brans B, van Gorp T, et al. The detection of sentinel nodes in ovarian cancer: a feasibility study. *J Nucl Med*. 2014;55:1799–1804.
7. Kleppe M, Kraima AC, Kruitwagen RFPM, et al. Understanding lymphatic drainage pathways of the ovaries to predict sites for sentinel nodes in ovarian cancer. *Int J Gynecol Cancer*. 2015;25:1405–1414.
8. Handgraaf HJM, Verbeek FPR, Tummers QRJG, 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.
9. Darin MC, Rodrigues Gómez-Hidalgo N, Westin SN, et al. Role of indocyanine green in sentinel node mapping in gynecologic cancer: is fluorescence imaging the new standard? *J Minim Invasive Gynecol*. 2016;23:186–193.

