THE BRITISH ASSOCIATION OF GYNAECOLOGICAL PATHOLOGISTS

Protocols for Pathological Processing of Sentinel Lymph Nodes in Endometrial, Vulval and Cervical Carcinomas

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Background:

- The sentinel lymph node (SLN) is the first lymph node to receive lymphatic drainage from a carcinoma and theoretically the first site of nodal metastasis.
- A tumour-free SLN has high negative predictive value for metastasis in non-sentinel lymph nodes.
- A SLN procedure (SLNP) is therefore currently believed to be a viable and attractive alternative to pelvic/inguinal lymphadenectomy in endometrial, vulval and cervical cancers.
- The process spares the patient from unnecessary surgical risk and morbidity secondary to pelvic/inguinal lymph node dissection and reduces healthcare costs.
- A successful SLNP in gynaecological malignancies is underpinned by many factors, principally surgical experience and the use of a standardised approach to correctly select cases, together with standardised pathological handling using a protocol that includes ultrastaging.
- SLN ultrastaging methods are often based on experience from other tumour sites. However there are variations in tumour histology, location of SLNs and in patient cohort in different gynaecological malignancies. Except for vulval cancer, where sentinel node sampling is standard of care in selected tumours, the indications for a SLNP and the impact on patient management is still in evolution in gynaecological oncology. [Cross reference to BGCS consensus document when published in IJGC]
- Ultrastaging of SLNs is labour-intensive for pathology laboratories (medical and technical staff) and it is essential for laboratory costs and workload impact to be addressed before this procedure undertaken in any unit. Pathology departments should be under no obligation to provide ultrastaging if adequate resources have not been allocated (Table 1 provides a rough guide to assist in quantifying the additional resources needed).
- The purpose of this document is to provide recommendations for standardised protocols for the pathological handling, including ultrastaging, of SLN in endometrial, vulval and cervical cancers.

- **SLN in endometrial carcinoma (EC)**
- Attitudes to lymphadenectomy as part of the surgical management of EC vary widely worldwide, from no nodal assessment to full pelvic and para-aortic lymphadenectomy.
- Lymph node involvement upstages about 10-15% of apparent stage I EC, which would have been deemed low-risk on pre-operative assessment.
- The benefit of SLNP in centres where lymphadenectomy is not routinely performed is that this results in accurate staging and thereby appropriate adjuvant treatment of patients with positive LN.
- The benefit of SLNP in centres where the routine management includes a full lymphadenectomy is that the same staging information can be achieved with a more limited procedure, since lymphadenectomy is not of proven therapeutic value.
- Ultrastaging of SLN in EC significantly increases the detection of micrometastases and isolated tumour cells.
- The greatest improvement in detection of micrometastases is in grade 1 stage IA EC; the detection of micrometastases is increased by 50%. Even then, the overall frequency of micrometastases after ultrastaging in grade 1 endometrioid carcinoma with less than 50% myometrial invasion is <4%.

- **SLN in vulval squamous cell carcinoma (VSCC)**
- SLNP is considered to be the standard of care for unifocal VSCC <4cm.
- Currently the optimal management of patients after a positive SLN is not established. In published trials this has been to proceed to a full lymphadenectomy; results of a trial determining whether lymphadenectomy can be safely replaced by radiotherapy are awaited.
- SLN involvement increases the likelihood of local and groin recurrence; the level of risk is proportional to the size of the metastasis.

- **SLN in cervical cancer (CaCx)**
- Of the 3 cancer sites under discussion, this is the area with the least prospective and long-term survival data.
- In CaCx, SLNP is used in two ways:
    - Intra operative frozen section to avoid an unnecessary radical hysterectomy and lymphadenectomy; this is resource intensive, has a high false negative rate, uses up/distorts nodal tissue, and is thereby less likely to become widely adopted.
    - SLNP as a replacement for lymphadenectomy for CxCa staging; this is a highly attractive option for women with low stage disease.

SLN ultrastaging

- SLN ultrastaging (SLN-US) refers to additional sectioning and immunostaining to detect small volume metastases within lymph nodes, as defined below.
- **Definitions of nodal involvement:** The size of the metastases in the lymph node affects the TNM stage allocated; current FIGO staging systems do not

take into account the size of nodal metastasis. There is currently no consensus on oncological management based on size of metastases.

- Macrometastasis (MAC): Metastases >2mm in size (pN1)
- Micrometastasis (MIC): Metastases >0.2 mm to </=2mm (pN1(mi))
- Individual tumour cells (ITC): Microscopic clusters and single cells </= 0.2 mm (pN0(i+))

Recommended SLN Pathology Protocols:

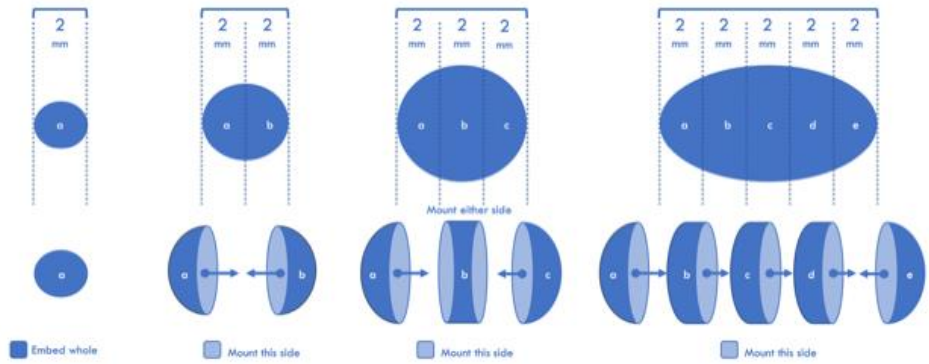
- Many different protocols have been advocated in the published literature.
- It is the purpose of this document to provide recommendations for standardised protocols.
- It is documented that in endometrial carcinoma a more limited SLN-US protocol performs with comparable accuracy to a detailed one and recurrences in low risk EC are rare. Furthermore in the UK the change in the vast majority of centres would be to move from ***no*** nodal sampling to SLNP; for these reasons a less labour-intensive protocol is advocated for endometrial carcinoma.
- In contrast, recurrences in VSCC and CxCa are potentially fatal. Current guidelines in VSCC and CxCa advocate examination of the entire node at regular intervals to maximise the detection of small volume metastases; for these reasons a more extensive ultrastaging protocol is advocated for these cancer sites.

Macroscopic handing

Macroscopic handling of the SLN is the same across all cancers.

- Examine the lymph node and adherent fat.
- Record the presence and colour of any visible dye.
- Lymph nodes up to 2mm can be embedded whole. Nodes 2-4mm in size should be bisected and both halves submitted. Nodes that are 4 mm or more in largest dimension should be bisected or sliced at 2 mm intervals, in a plane perpendicular to the longest axis (Figure 1).

Figure 1: Embedding of lymph nodes in SLN_US



- It is preferable to avoid any trimming of surrounding fat; however, if there is excessive fat around the nodes after slicing, the fat can be carefully trimmed away and placed in a separate cassette to enable as many nodal slices as possible, and preferably the entire node, to fit in one cassette.
- Overcrowding the cassette (over 80% of the area) must be avoided.
- When a node is trisected, the two end pieces should be placed cut surface down; the middle slice is placed randomly unless gross examination identifies a suspicious lesion.
- When more than 3 slices from a lymph node are submitted, the non-apposing faces should form the cutting surface ensuring that microscopic sections are 2.0 mm apart and ensuring a high probability of detecting all macrometastases.
- A block index must be maintained.

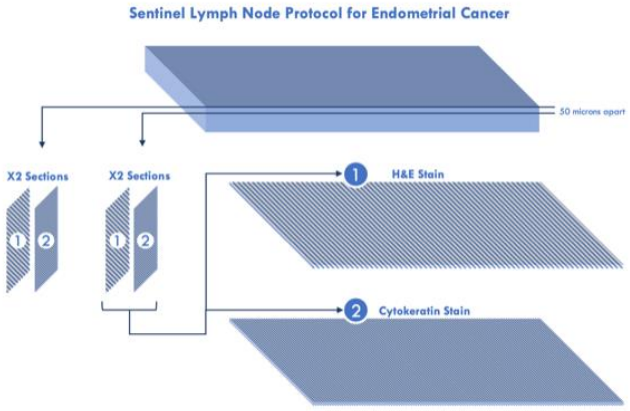
Ultrastaging protocols

- A single H&E section of each slice of SLN, with minimal trimming to avoid tissue wastage, should be examined in the first instance.
- If this is negative for tumour, SLN-US should be carried out following the appropriate protocol as below.
- In a bilateral procedure, if one SLN is positive on the initial H&E, but the node(s) on the contralateral side are negative, ultrastaging should be carried out only on the contralateral node(s)
- If SLN is accompanied by a full lymphadenectomy, there is no need to carry out SLN-US.

<u>SLN Protocol 1 (For EC) (Figure 2)</u>

- Two additional pairs of sections are cut at 50 micron intervals.
- One section from each of the two pairs is stained with H&E and the other with immunohistochemistry (IHC) for a broad spectrum cytokeratin (CK), AE1/AE3 is currently widely used.

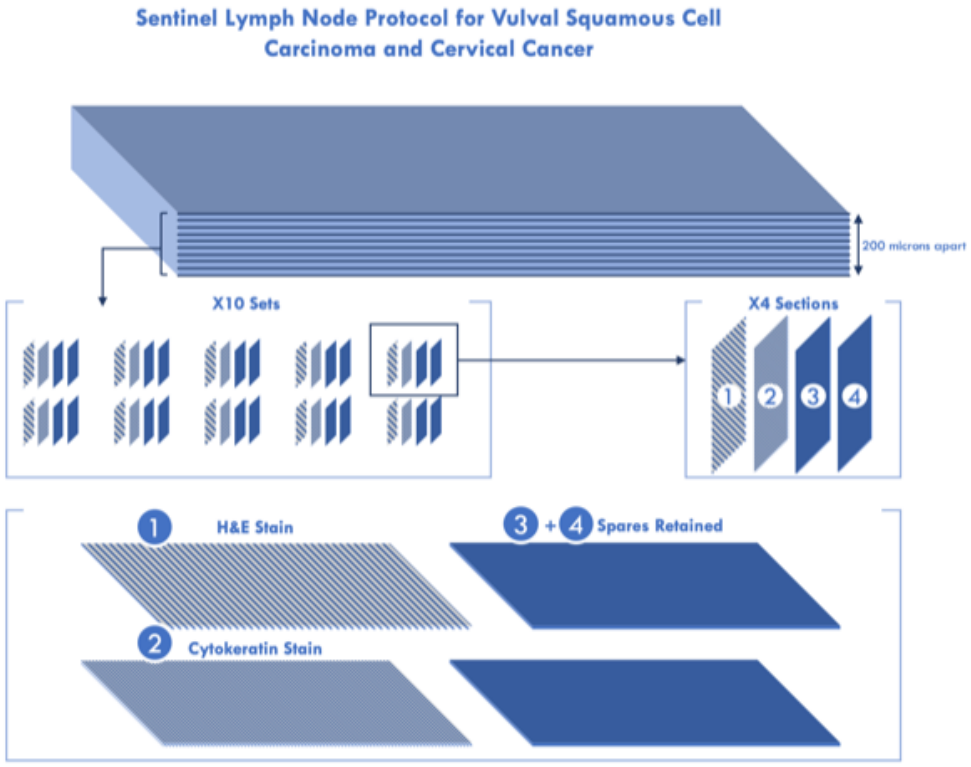
Figure 2: SLN protocol for EC



SLN Protocol 2 (For VSCC and CaCx) (Figure3)

- 4 sections are cut at 200 micron intervals through the block until all nodal tissue is exhausted; theoretically this should result in 10 additional sets of 4 slides.
- One section from every set of 4 is stained with H&E and one with CK.
- Two additional sections are retained at each level in case there is a problem with H&E or IHC staining, to see if the focus becomes larger on additional levels, or to use additional levels for further IHC to determine the nature of indeterminate CK-positive cells. If not used these additional sections should be stored for possible future use.

Figure 3: SLN protocol for VSCC and CaCx



Recommendations for reporting

Standardised reporting of SLN specimens is recommended in the following text format.

- Total number of lymph nodes on each side. If no lymph node is recovered this should be stated.
- Presence of dye or surgeon notes regarding tracer
- If no metastases are present: No metastatic carcinoma is seen in x lymph nodes.
- If metastases are present:
    - For macro or micrometastases: Metastatic carcinoma is identified in y of z lymph node(s). The largest deposit measures x mm in maximum dimension.

- For ITC: Metastatic carcinoma in the form of single cells and/or microscopic clusters measuring up to x mm are identified in y of z lymph node(s). Use of the term widespread ITC is strongly discouraged.
- Indeterminate CK positive cells should not be reported; an effort should be made to determine their nature, eg dendritic cells, mesothelial cells, macrophages, mast cells, endosalpingiosis, endometriosis.
- Presence/absence of extranodal extension should be stated in all node-positive cases.

Table 1: Guide to Calculation of Histopathology Laboratory Resources Needed for Sentinel Lymph Node Ultrastaging

Sentinel node examination has an impact on laboratory resources including increase in time taken to process by the laboratory, use of materials and IHC and time taken for reporting (monetary equivalent should be calculated locally). The BAGP would like to provide an estimate of the additional resources required by laboratories when sentinel node procedure is implemented.

SLN-US Protocol	Resources per SLN			**Resources per SLN procedure, based on 4 SLN[a]**		
	BMS, grade 6 or higher	Consultant pathologist[b]	Additional slides	**BMS, grade 6 or higher**	**Consultant pathologist[b]**	**CK IHC**
SLN1	5 minutes	4 minutes	2 H&E, 2 IHC	**20 minutes**	**16 minutes**	**8 H&E, 8 IHC**
SLN2	15 minutes	20 minutes	10 H&E, 10 IHC	**60 minutes**	**80 minutes**	**40 H&E, 40 IHC**

SLN = Sentinel lymph node
US = Ultrastaging
BMS = Biomedical scientist
SLN1 = SLN-US protocol 1, recommended for endometrial carcinoma
SLN2 = SLN-US protocol 2, recommended for vulval squamous cell carcinoma and cervical carcinoma

[a] Average number of SLNs per side is 2 based on published literature; this should be monitored as there can be considerable variation by surgeon/centre/tracer, and resource allocation adjusted as necessary
[b] SLN-US pathology would be amenable to reporting by trained advanced practitioner/biomedical scientists. Pre-screening by biomedical scientists can also be considered by laboratories and is not a part of the calculation provided.

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