



The 4th MEMAGO Annual Congress in Association with the 1st Emirates Gynecological Oncology Conference



Sentinel lymph nodes in gynecologic cancers: Imprint, frozen or permanent sections or ultra-staging? A guide for the practicing gynecologist

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Learning Objectives

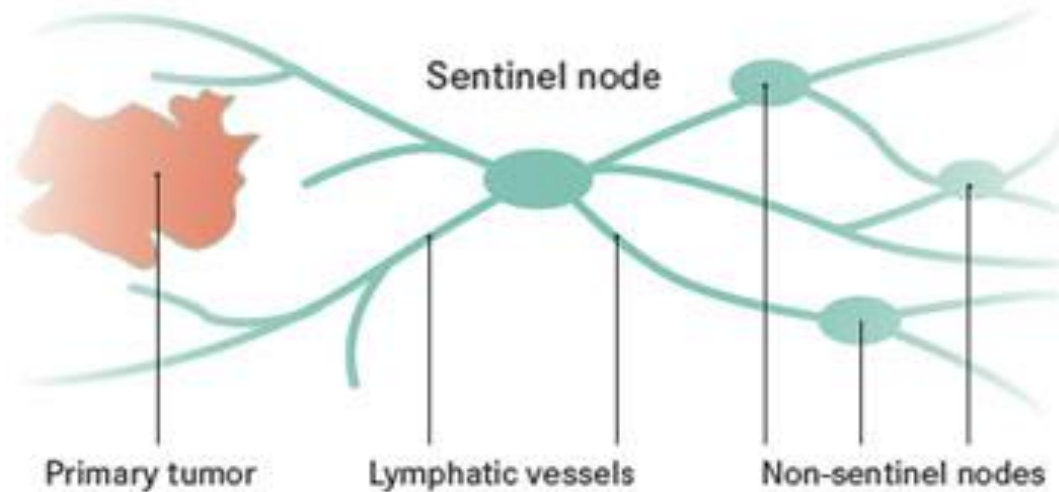
- Sentinel lymph node
 - Definition, aims of SLN biopsy
- Parameters that effects SLN biopsy
- Pathological evaluation methods of SLN
- Accuracy of pathological assessment techniques in different gyn cancers

Sentinel Lymph Node

Lymphatics of
tumoral region

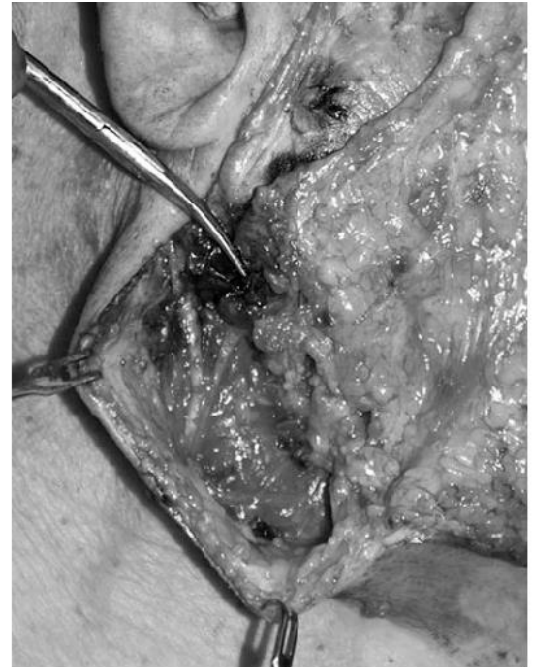


First drain into
SLN



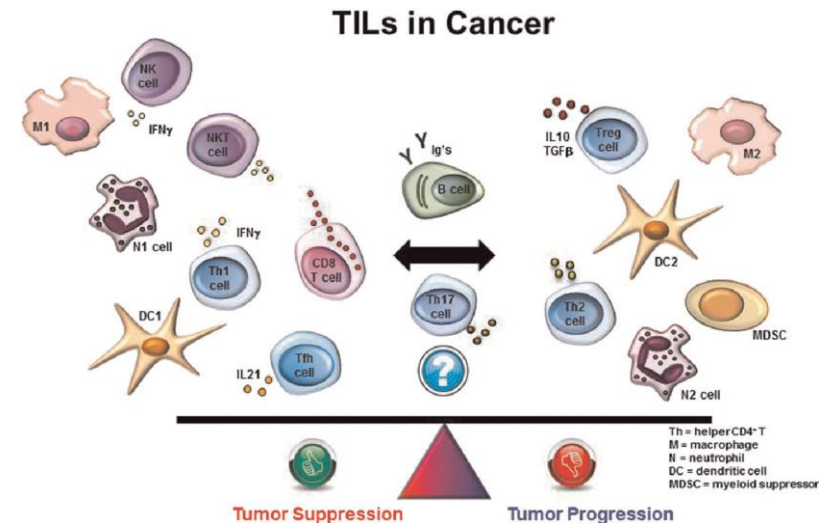
History

- 1960's parotid gland carcinoma
- 1977 penile cancer with lymphangiography
- Breast cancer, malignant melanoma
- Gyn Cancer
 - Vulva
 - Cervix
 - Endometrium

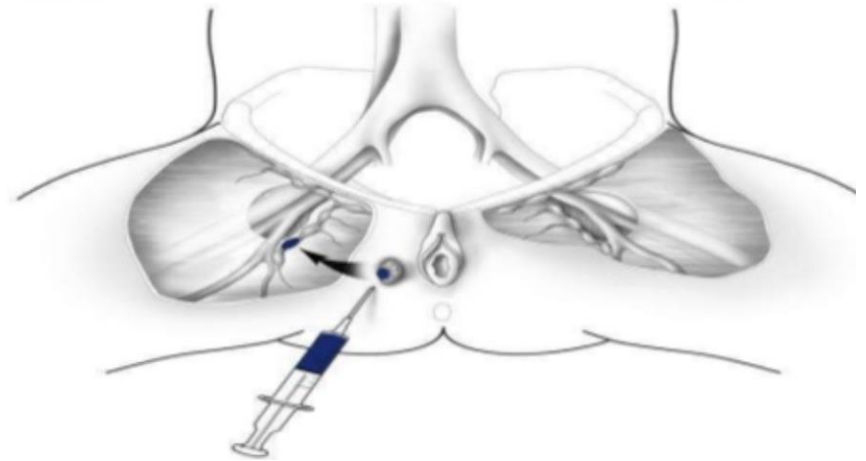


Aim

- To understand if lymphatic dissemination of tumor started, w/o excision of other (probably) normal LN
 - Due to their
 - Immunologic functions (antitumoral effects of lymphocytes)
 - Physiologic functions (lymphatic drainage)
 - Due to prevent intra / post operative complications



Success of SLN



- Patient selection
 - Tumor stage & size
- Injection site
- Dye
 - Colored (isosulfan blue, methylene blue, ICG...)
 - Radioactive (Tech-99,...)
- Detection rate, bilaterality
- Experience of the center
- **Pathologic evaluation of SLN**
- Interpretation of the pathology results
 - Significance of MIC/ITC
- Long term oncological outcomes

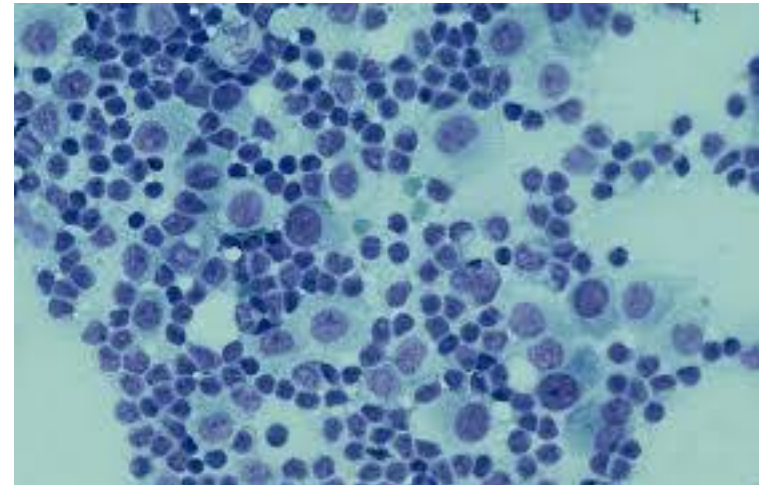
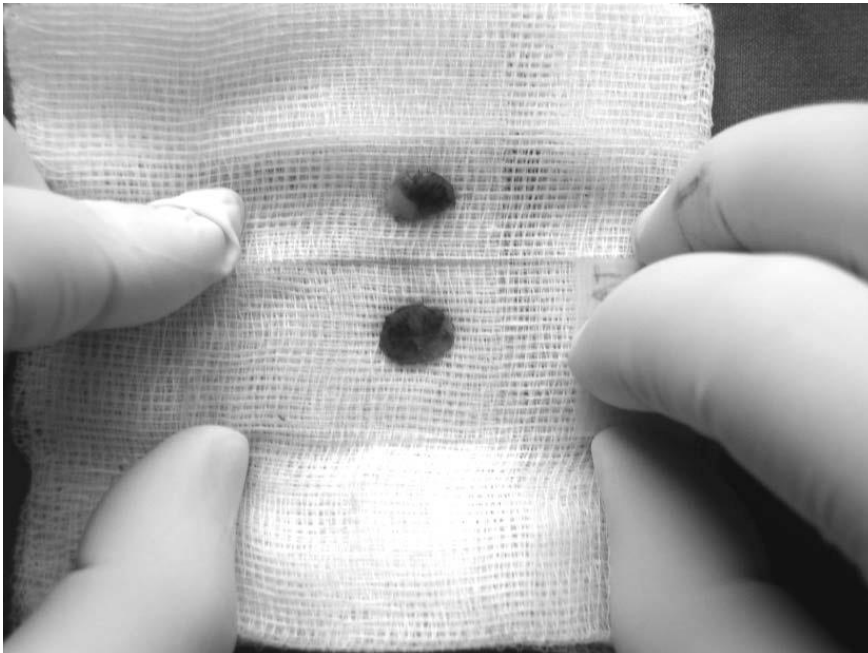
Pathologic Evaluation of SLN

- Intraoperative
 - Imprint / scrape cytology
 - Frozen section
 - R-IHC
 - Molecular methods
- Postoperative
 - Permanent H&E sections
 - Ultrastaging \pm IHC
 - Molecular methods



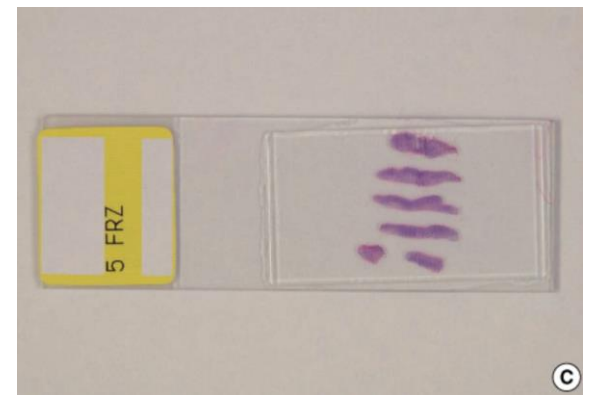
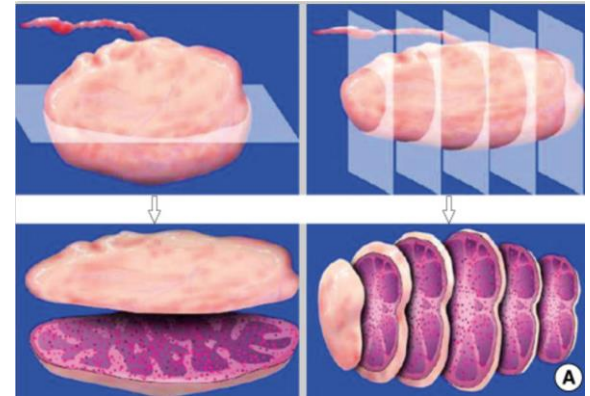
Imprint Cytology

- Touch cytology
- Advantage: No tissue loss as in FS / intra-op.
- Scrape cytology as an alternative or combination



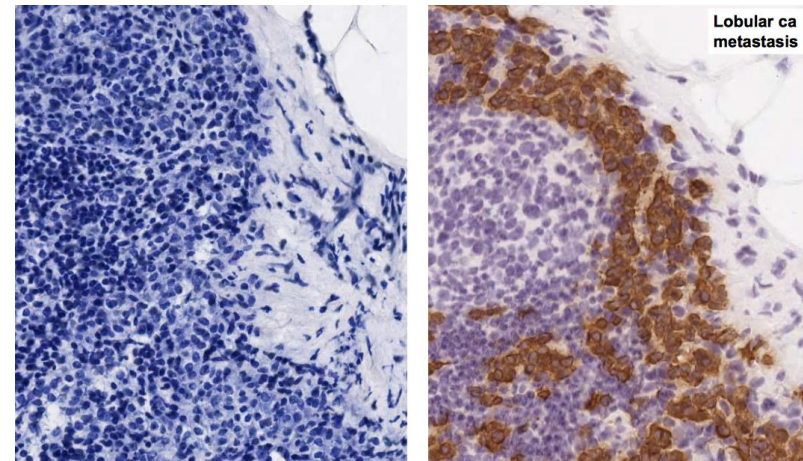
Frozen Section

- LN < 5mm, bisection
- LN > 5mm, 2mm slices, perpendicular to long axis
- Frozen rapidly in a cryostat to approximately -20°C
- 4-8 μm sections by microtome
- How many sections ? 4-6 ?
- H&E staining
- More expensive, artefacts, experience



R-IHC

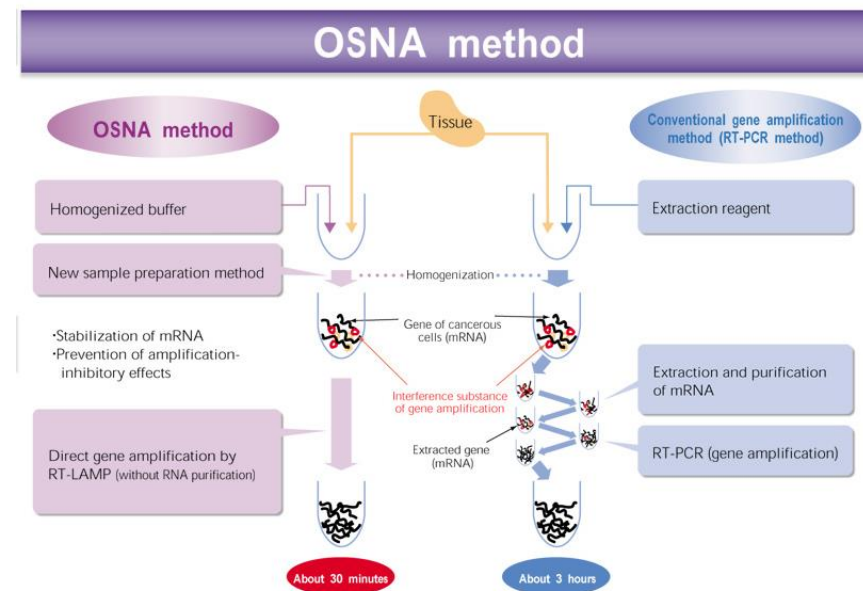
Cytokeratin (AE1/AE3)



- In case of suspicious or negative for metastasis in FS or IC
- Sections were fixed in cold acetone for 3 minutes
- Air-dried for 5 seconds and then incubated for 10 minutes at room temperature with anti-CK monoclonal antibody.
- After washing in tris-buffered saline solution at pH 7.4, the slides were incubated with the secondary antibody conjugated to peroxidase for 10 minutes.
- Sections were subjected to diaminobenzidine tetrahydrochloride and counter-stained with hematoxylin

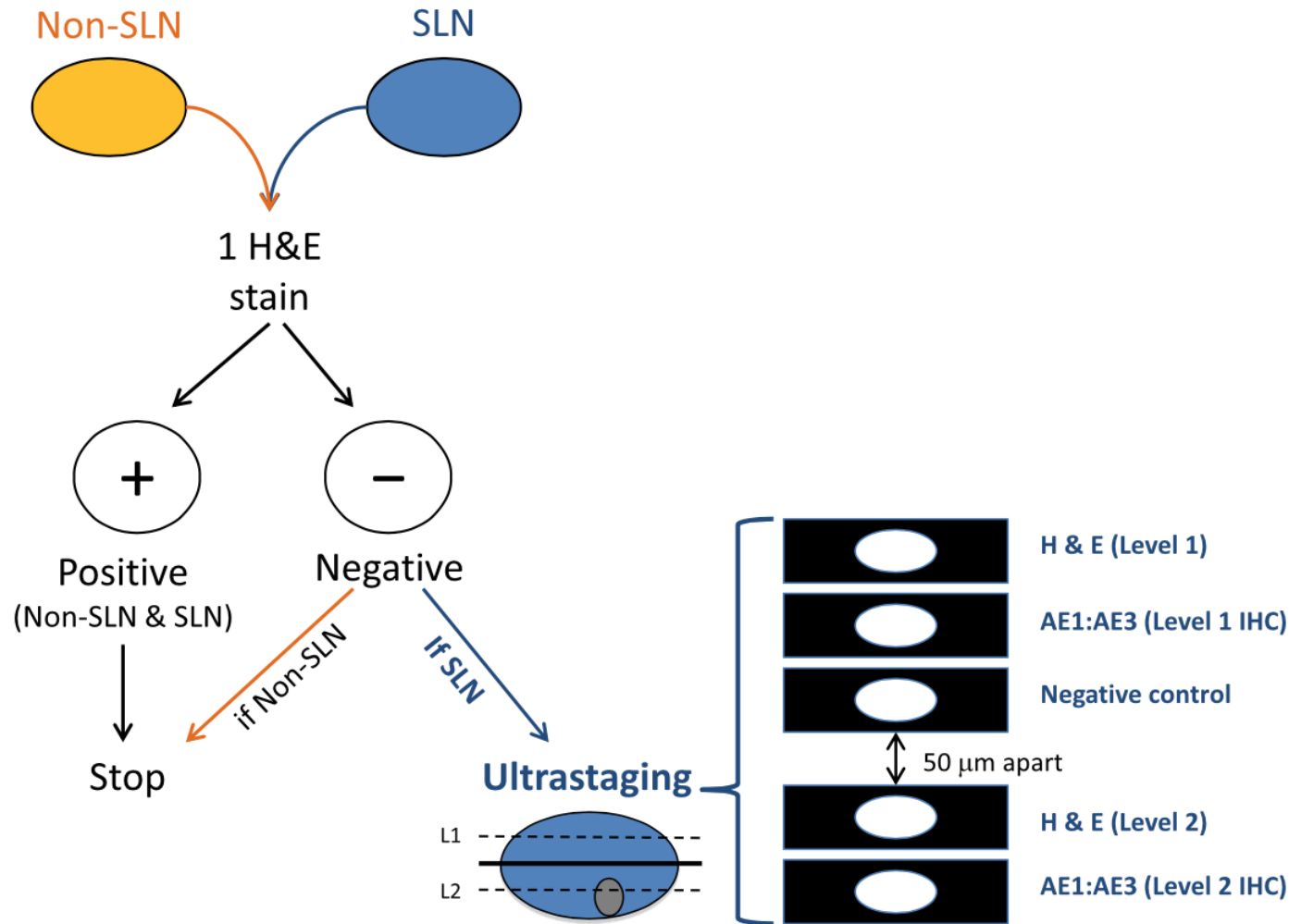
OSNA (One-Step Nucleic Acid Amplification)

- Quantitative measurement of the target mRNA: cytokeratin 19 mRNA (CK19)
- Homogenization of lymph node followed by reverse-transcription loop-mediated isothermal amplification (RT-LAMP)
- Analyzing whole part of the tissue in 30 min.
- Tissue loss, expensive



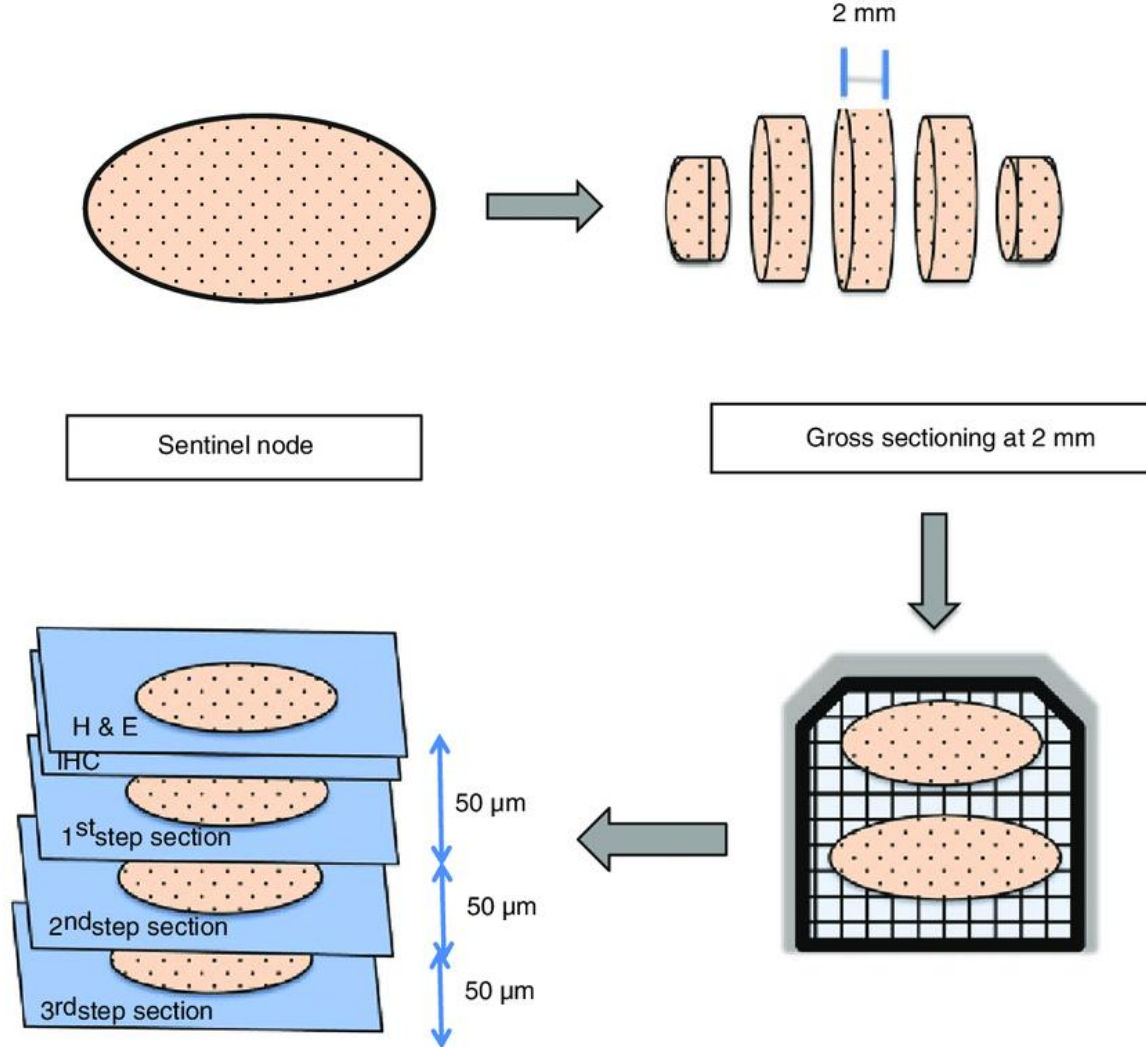
Ultrastaging

- More intense evaluation of SLN with higher number of slices with more frequent sections in order to detect low volume disease
- If SLN negative in standard H&E staining.
- No standard protocol



Memorial Sloan-Kettering Cancer Center's pathologic ultrastaging algorithm for sentinel lymph nodes

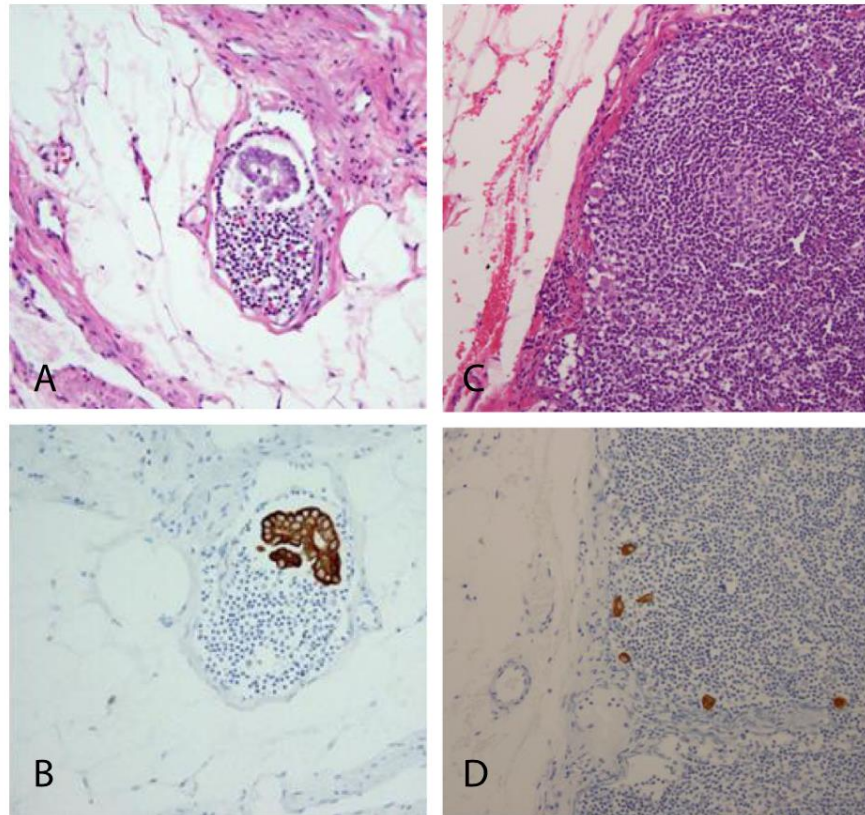
- 5 μm sections with 50 μm distance apart



- Interval should be $< 200 \mu\text{m}$
 - Micrometastasis: tumor cells measuring more than 0.2 mm and 2 mm or less
 - Isolated tumor cells: microscopic clusters and single cells measuring 0.2 mm or less

Ultrastaging + IHC

- Cytokeratin (AE1/AE3)



A,B: MIC; C,D: ITC

Performance of Intraoperative SLN Biopsy in BREAST Cancer (%)

	Accuracy	Sensitivity	Specificity	FNR	NPV	PPV
Imprint	82-98	30-96	90-100	14-70	74-99	100
MAC		81				
MIC		22				
Frozen	79-98	52-95	100	4-45	76-97	100
MAC		89-95				
MIC		27-40				
Fr + Imp	79-93	48-77	100	23-52	74-92	100
R-IHC		95	100			
OSNA				1.7		

Silverberg et al, Histopathology, 2000. Madsen et al, Wirschow Arch, 2012.



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Vulvar Cancer & SLN

What we know

Disease Extent	Percent of cases	5-Year Survival
Localized (confined to vulva)	59%	86.4%
Regional (spread to lymph nodes)	30%	56.9%
Distant (spread beyond lymph nodes)	6%	17.4%
Unknown/Unstaged	5%	56.2%

*Patients with clinically early stage disease: 25-35% has LN metastasis

Vulvar Cancer & SLN

What we know

- Candidates of SLN
 - Tumor diameter <4 cm
 - >1 mm depth of invasion
 - No palpable groin lymph nodes
 - Unifocal disease

Vulvar Cancer & SLN

What we know

- GROINSS-V I (2008)*
 - 403 patients
 - combined technique (radioactive tracer and blue dye)
 - **SLN negative: no further tx**
 - SLN positive (either intra or post operative)
 - Complete IF LND
 - 127 p: metastatic (31.5%), 276 p negative SLN (observation)
 - Median F-Up: 35 m; 3% groin recurrence in SLN negative p.
- Long-term F-up of GROINSS-V I (2016)**
 - Median F-Up: 105 m; 2.5% isolated groin recurrence in SLN negative p.

*Van der Zee et al. J Clin Oncol, 2008.

**Te Grootenhuis et al. Gynecol Oncol, 2016

Sentinel Node Dissection Is Safe in the Treatment of Early-Stage Vulvar Cancer

Ate G.J. Van der Zee, Maaike H. Oonk, Joanne A. De Hullu, Anca C. Ansink, Ignace Vergote, René H. Verheijen, Angelo Maggioni, Katja N. Gaarenstroom, Peter J. Baldwin, Eleonore B. Van Dorst, Jacobus Van der Velden, Ralph H. Hermans, Hans van der Putten, Pierre Drouin, Achim Schneider, and Wim J. Sluiter

SLN Pathology

- SLN cut in the middle for frozen section or cytologic specimen (both optional)
- 4 sections were cut from every half for H&E.
- Ultrastaging only if SLN negative in routine exam.
- For ultrastaging, additional pairs of sections were cut with 0.33 mm (333 μ m).
- One section of each pair was stained with HE, and the other section was immunostained with cytokeratin AE1:AE3
- **58.3% of met. groins detected with intraop + routine path.**
- **41.7% was detected by ultrastaging.**

GOG-173 (2012)

- 452p, 418 at least one SLN detected
- At least 1-mm invasion, and tumor size ≥ 2 cm and ≤ 6 cm, without clinical LN
- SLNB with isosulfan blue dye. preoperative lymphoscintigraphy and intraoperative radiolocalization were optional.
- Following SLN identification, **a completion lymphadenectomy was performed for all patients**
- 132 SLN positive
- Sensitivity 91.7%, NPV 96%
- False-negative predictive value [1-negative predictive value] 3.7%
- Tumors smaller than 4 cm, the false-negative predictive value was 2.0%

Lymphatic Mapping and Sentinel Lymph Node Biopsy in
Women With Squamous Cell Carcinoma of the Vulva:
A Gynecologic Oncology Group Study

*Charles F. Levenback, Shamshad Ali, Robert L. Coleman, Michael A. Gold, Jeffrey M. Fowler, Patricia L. Judson,
Maria C. Bell, Koen De Geest, Nick M. Spirtos, Ronald K. Potkul, Mario M. Leitao Jr, Jamie N. Bakkum-Gamez,
Emma C. Rossi, Samuel S. Lentz, James J. Burke II, Linda Van Le, and Cornelia L. Trimble*

SLN Pathology

- Frozen section evaluation was discouraged.
- At least two sections 40 microns apart
- If H&E negative, IHC with cytokeratin
 - IHC: 200 (71%) of the 285 women with negative SLNs on routine H&E.
 - **23% of cases detected only by IHC.**

Vulvar Cancer & SLN

Frozen Section

The Accuracy of Intraoperative Frozen Section of the Inguinal Sentinel Lymph Node in Vulvar Cancer

ANDREAS H. BRUNNER¹, STEPHAN POLTERAUER¹, CLEMENS TEMPFER¹, ELMAR JOURA¹,
ALEXANDER REINTHALLER¹, REINHARD HORVAT² and LUKAS HEFLER¹

ANTICANCER RESEARCH 28: 4091-4094 (2008)

- 44p, 54 groins
- If SLN negative: no further tx
- Frozen: 4µm sections from each 2-3 mm, 4-6 slides
- Final evaluation; ULTRASTAGING :4µm sections from each 400µm; H&E: if negative, IHC (cytokeratin)

Vulvar Cancer & SLN

Frozen Section

Table II. *False-negative rate in frozen sections analysis: literature review.*

Author	Number of patients	Number of resected sentinel nodes	Number of patients with metastatic inguinal lymph nodes	False-negative rate in frozen sections
Hauspy <i>et al.</i> (15)	41	145	16/41	1/41
Rob <i>et al.</i> (16)	43	98	13/43	2/43
Brunner <i>et al.</i> (present)	44	120	17/44	3/44

- False negative rate: 6/128 (4.7%)
- Sensitivity 88.5%, specificity 100%
- PPV 100% and NPV 93.2%

Vulvar Cancer & SLN

Summary

- Frozen Section
 - False negative rate: 6/128 (4.7%)
 - Sensitivity 88.5%
- Ultrastaging
 - 20-40% of patients detected only by ultrastaging
- Imprint, R-IHC, OSNA
 - No data available



Cervical Cancer & SLN

What we know?

- LN status is of note
 - Positive: to abandon hysterectomy for Ch-R Tx
 - Negative: to continue with fertility sparing method
- ESGO
 - T1a1, LVSI positive; T1a2
 - SLN alone acceptable (without additional P-LND)
 - T1b1-T2a1
 - Complete PLND (but SLN before PLND strongly recommended)

Cervical Cancer & SLN

What we know?

- NCCN (v5.2019, September)
 - T1a1, LVSI positive; T1a2
 - Consider SLN
 - SLN, better if tm <2 cm
 - Ultrastaging provide enhanced detection of micrometastases
 - Ipsilateral LND if not detected in a hemipelvis

SENTICOL Study (2011)

- 141p; 1a1 LVSI (+) to 1B1
- Technetium 99 lymphoscintigraphy and Patent Blue injection
- SLN + complete LND
 - Reference method: P-PA LND with histologic examination of all nodes.
- 23 true-positive & 2 false-negative in 136 p SLN detected
- 92.0% sensitivity and 98.2% NPV
- If SLN detected bilaterally, no FN result

David Cibula^a, Nadeem R. Abu-Rustum^b, Ladislav Dusek^c, Jiri Slama^a, Michal Zikán^a, Afra Zaal^d, Libor Sevcik^e, Gemma Kenter^f, Denis Querleu^g, Robert Jach^h, Anne-Sophie Batsⁱ, Grzegorz Dyduch^j, Peter Graf^e, Jaroslav Klat^e, Chris J.L.M. Meijer^f, Eliane Mery^g, Rene Verheijen^d, Ronald P. Zweemer^d

- **645p, SLN + standard P-LND**
- **72% bilateral SLN detection**
- **SLN**
 - FN 2.8% in whole group (sens: 91%)
 - FN 1.3% in bilaterally detected group (sens: 97%)
 - 23 cases MIC/ITC in SLN but MAC in non-SLN (3.6%)
 - These group could be missed if ultrastaging was not performed
- **Ultrastaging + IHC for all SLN**
 - **250µm apart, three consecutive sections (5µm) thick**
 - **H&E and IHC staining for cytokeratin.**
- **Pelvic non-sentinel nodes: single section, H&E**

Diagnostic value of intraoperative examination of sentinel lymph node in early cervical cancer: A prospective, multicenter study

Gynecologic Oncology 123 (2011) 230–235

Anne-Sophie Bats ^{a,b,*}, Annie Buénerd ^c, Denis Querleu ^d, Eric Leblanc ^e, Emile Daraï ^f, Philippe Morice ^g, Henri Marret ^h, Florence Gillaizeau ^{i,j}, Patrice Mathevet ^k, Fabrice Lécuru ^{a,b}
and for the SENTICOL collaborative group

- 102 p (97 FS, 5 IC)
- Tc99m + blue dye; SLN + full LND
- **Intraoperative evaluation**
 - SLN, first cut in to two. One half for paraffin
 - One section from the rest for FS or IC
- **Final evaluation**
 - Non-metastatic SLN in FS was cut each 200 μm
 - H&E and anti pan-cytokeratin AE1–AE3

Diagnostic value of intraoperative examination of sentinel lymph node in early cervical cancer: A prospective, multicenter study

Gynecologic Oncology 123 (2011) 230–235

Anne-Sophie Bats ^{a,b,*}, Annie Buénerd ^c, Denis Querleu ^d, Eric Leblanc ^e, Emile Daraï ^f, Philippe Morice ^g, Henri Marret ^h, Florence Gillaizeau ^{i,j}, Patrice Mathevet ^k, Fabrice Lécuru ^{a,b}
and for the SENTICOL collaborative group

- Positive LN
 - Intraoperative : 5
 - Postoperative: 22
 - False negative : 17
 - 4 macrometastasis, 4 micrometastasis and 9 ITC
- Sensitivity 20.7%, NPV 93%

Sentinel lymph node biopsy in early-stage cervical cancer: Utility of intraoperative versus postoperative assessment

Gynecologic Oncology 111 (2008) 13–17

A. Nickles Fader^a, R.P. Edwards^{a,b}, M. Cost^b, A. Kanbour-Shakir^c, J.L. Kelley^a, B. Schwartz^a,
P. Sukumvanich^a, J. Comerci^a, J. Sumkin^e, E. Elishaev^c, L. Cencia Rohan^{a,b,e,*}

- 38 p, FS + IC
- Tc99m + isosulfan blue; SLN + full LND
- **Intraoperative evaluation**
 - One section from each SLN for FS & IC
- **Final evaluation**
 - Section not clear
 - H&E and Cytokeratin AE1/AE3

Sentinel lymph node biopsy in early-stage cervical cancer: Utility of intraoperative versus postoperative assessment

Gynecologic Oncology 111 (2008) 13–17

A. Nickles Fader^a, R.P. Edwards^{a,b}, M. Cost^b, A. Kanbour-Shakir^c, J.L. Kelley^a, B. Schwartz^a,
P. Sukumvanich^a, J. Comerci^a, J. Sumkin^e, E. Elishaev^c, L. Cencia Rohan^{a,b,e,*}

- 6 metastasis out of 38p (15.7%)
- **Intraoperative detection 33%**

High false negative rate of frozen section examination of sentinel lymph nodes in patients with cervical cancer

J. Slama ^a, P. Dundr ^b, L. Dusek ^c, D. Cibula ^a

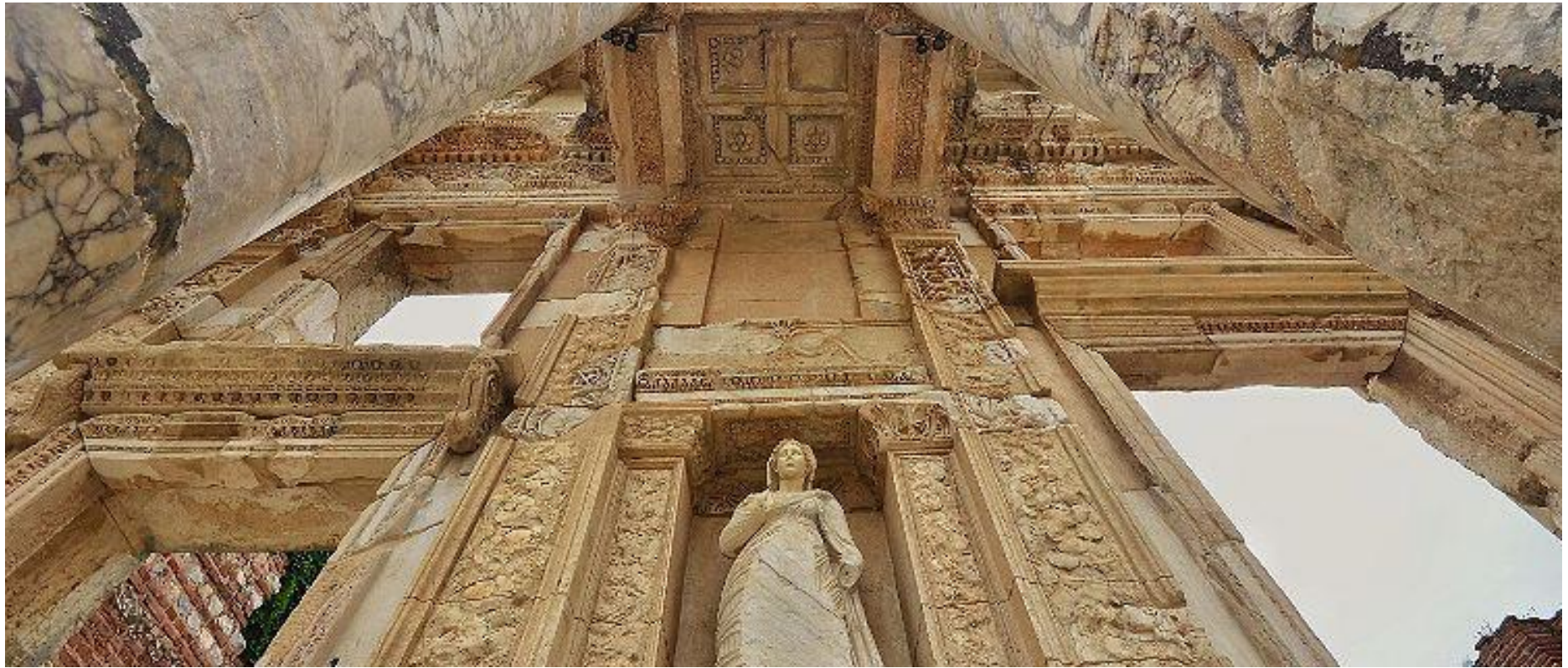
Gynecologic Oncology 129 (2013) 384–388

- 225p ,stage 1a2-2B
- SLN + full LND
- **Intraoperative evaluation**
 - One section from each SLN for FS
- **Final evaluation**
 - Non-metastatic SLN in FS was cut each 150 μm
 - H&E and anti pan-cytokeratin AE1–AE3
- 73 cases with metastasis (32.4%)
 - 48 MAC, 17 MIC and 8 ITC
- Intra-operative SN assessment correctly in 41 cases (**56.2%**)
 - In 49 cases (63%) if ITC had been excluded.
 - Final ultrastaging of intra-operatively negative SN
 - 8 MAC, 18 MIC and 8 ITC

Cervical Cancer & SLN

Summary

- Intraoperative evaluation
 - 20-56%
- Bilateral detection & ultrastaging
 - Sensitivity 92-97%
 - FN 0-1.3%
- Ultrastaging*
 - At least 200 μm apart
 - H&E, if negative cytokeratin
- Only one study with OSNA, compared with permanent sections (not ultrastaging)**
 - 96.2% agreement with 2 mm sectioned slides



Ephesus Ancient City, Izmir

Endometrial Cancer & SLN

What we know?

- NCCN v4.2019 (September)
 - Apparent uterine-confined disease
 - Intermediate/high risk histologies may be included*
 - Increased detection of lymph node metastasis and a low FN rate compared with systematic LND**
 - SLN algorithm
 - Side-specific nodal dissection in cases of failed mapping
 - Removal of any suspicious or grossly enlarged nodes regardless of mapping
 - Ultrastaging

*Ducie JA, et al. Gynecol Oncol, 2017.

Baiocchi G, et al. Ann Surg Oncol, 2017

** Rossi EC, et al. Lancet Oncol, 2017.

Ballester M, et al. Lancet Oncol, 2011

SENTI-ENDO study (2011)

- Prospective, multicenter, stage I-II End Ca
- SLN + full LND
- technetium and patent blue / ultrastaging SLN
- SLN detected at least at one-side 111 of 125 p
- 19p with LN metastasis
- 3 FN (2 contralateral side, 1 para-aortic)
 - All type 2 End Ca
- NPV: 97% (95% CI 91–99) Sensitivity: 84% (62–95)

Detection rate and diagnostic accuracy of sentinel-node biopsy in early stage endometrial cancer: a prospective multicentre study (SENTI-ENDO)

Lancet Oncol 2011; 12: 469-76

Marcos Ballester, Gil Dubernard, Fabrice Lécuru, Denis Heitz, Patrice Mathevet, Henri Marret, Denis Querleu, François Golfier, Eric Leblanc, Roman Rouzier, Emile Daraï

- Ultrastaging
 - 200 µm apart
 - H&E, if negative: anti-cytokeratin antibody
- **9 of 19 metastasis**
 - **undiagnosed by conventional histology**
 - **Diagnosed by ultrastaging**

Pathologic Ultrastaging Improves Micrometastasis Detection in Sentinel Lymph Nodes during Endometrial Cancer Staging

Christine H. Kim, MD¹, Robert A. Soslow, MD², Kay J. Park, MD², Emma L. Barber³, Fady Khoury-Collado, MD¹, Joyce N. Barlin, MD¹, Yukio Sonoda, MD^{1,4}, Martee L. Hensley, MD^{4,5}, Richard R. Barakat, MD^{1,4}, and Nadeem R. Abu-Rustum, MD^{1,4}

Int J Gynecol Cancer. 2013 June ; 23(5): 964–970.

- Retrospective, 2005-2011
- Blue dye, cervical injection
- SLN + full P-Pa-LND
- Ultrastaging 5- μ m sections with 50- μ m apart
- 508 (80.0%) of 635 eligible patients with at least one SLN detected
- Metastasis
 - 35 (6.9%) in initial routine H&E
 - **23 (4.5%) additional in ultrastaging (23/64, 35%)**
 - 4 MIC, 19 ITC
 - **FN: 6 (1.2%) non-SLN metastasis**

FIRES Trial (2017)

- **Multicenter, prospective**
- Clinical stage 1 endometrial cancer of all histologies and grades
- ICG / SLN followed by P-LND with or without PA-LND
- 293 out of 340p had at least one SLN
- 41p with metastasis
 - In 36 of 41, SLN detected

A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study

Lancet Oncol 2017; 18: 384-92

Emma C Rossi, Lynn D Kowalski, Jennifer Scalici, Leigh Cantrell, Kevin Schuler, Rabbie K Hanna, Michael Method, Melissa Ade, Anastasia Ivanova, John F Boggess

- **Ultrastaging**
 - Two paraffinembedded slides with 50 μm apart
 - H&E and pancytokeratin AE1 and AE3
- **SLN detected 36p with metastasis**
 - 35p positive SLN, 1 false negative
 - 19 out of 35p were detected only by ultrastaging (54%)

Endometrial Cancer & SLN Intraoperative Evaluation

Comparison of Diagnostic Accuracy of Frozen Section with Imprint Cytology for Intraoperative Examination of Sentinel Lymph Node in Early-Stage Endometrial Cancer: Results of Senti-Endo Study

Marcos Ballester, MD¹, Gil Dubernard, MD, PhD², Anne-Sophie Bats, MD, PhD³, Denis Heitz, MD⁴, Patrice Mathevet, MD, PhD⁵, Henri Marret, MD⁶, Denis Querleu, MD⁷, François Golfier, MD, PhD⁸, Eric Leblanc, MD⁹, Roman Rouzier, MD, PhD¹, and Emile Daraï, MD, PhD¹

- Frozen section vs. IC*
 - Retrospective analysis of SENTI-ENDO
 - stage I-II End Ca
 - SLN + full LND
 - technetium and patent blue
 - SLN detected at least at one-side 111 of 125 p
 - 87 of 111, intraoperative evaluation
 - 30 FS, 57 IC
 - 16 metastasis detected by SLN
 - 9 with intraop. evaluation (sens: 56%)
 - 7 FN: 6 MIC, 1 ITC
 - Frozen Section
 - **Sensitivity 85.7 % (95 % CI, 42–99.6),**
 - **NPV 96.8 % (95 % CI: 83.8– 99.9)**
 - Diagnostic accuracy 97.3 % (95 % CI: 85.8–99.9)
 - IC
 - **Sensitivity 33.3 % (95 % CI, 7.5–70)**
 - **NPV 87.2 % (95 % CI, 74.2–95.2)**
 - Diagnostic accuracy 88 % (95 % CI, 75.7–95.5 %)

Endometrial Cancer & SLN

Intraoperative Evaluation-OSNA

RESEARCH ARTICLE

One-Step Nucleic Acid Amplification (OSNA): A fast molecular test based on CK19 mRNA concentration for assessment of lymph-nodes metastases in early stage endometrial cancer

Francesco Fanfani^{1*}, Giorgia Monterossi², Viola Ghizzoni², Esther D. Rossi³,
Giorgia Dinoi², Frediano Inzani³, Anna Fagotti², Salvatore Gueli Alletti²,
Francesca Scarpellini³, Camilla Nero², Angela Santoro³, Giovanni Scambia², Gian
F. Zannoni³

PLOS ONE | <https://doi.org/10.1371/journal.pone.0195877> April 26, 2018

- 40 p, clinical stage I, all histology & grades
- OSNA vs ultrastaging
- 29p SLN detected
- 8 positive nodes, 1 FN at OSNA group
- 6 false positive results
- **Sensitivity 50% Specificity 94.4%**
- **PPV 14.3% and NPV 99%, accuracy 93.6%**

Endometrial Cancer & SLN

Intraoperative Evaluation-Empty Node

- May be related with experience
- 10 additional procedures may decrease EN by 40.1% (95% CI 12.4% - 58.6%)*
- 8% (24/300 p) of SLN were EN in early stage End Ca**
 - Frozen detected 100%
 - May prevent inappropriate staging due to EN

Conclusion

- SLN in Gyn Tm has a low FN, acceptable
- Patient selection
- Intraoperative evaluation
 - One step surgical procedure
 - Low accuracy, high FN rates
 - Molecular methods, R-IHC
- Ultrastaging
- SLN
 - Long term results of randomised prospective trials
 - SENTIX, SENTICOL3, ENDO-OSNA, etc...

