Gynaecological Pathology Reporting

What really matters: when and why?

Vulva and Vagina

Dr. A.Z. Faruqi, Barts Health NHS Trust

Common clinical questions

- Benign or malignant?
- Are the margins clear?
- Which is the minimum tumour-free margin?
- What is the distance this margin?
- Is pre-invasive disease excised?
- What is the tumour grade?
- Is there any non-neoplastic epithelial disease?

What we write/say matters

- **Prognostic factors**
  - Size
  - Site
  - Type and grade
  - Depth of invasion/thickness
  - LVSI
  - Perineurial (intraneural) invasion
  - VIN – dVIN vs. uVIN
- **Impacts on treatment**
  - Re-excision
  - Chemo/radiotherapy

VULVA

Specimen types

Incisional (diagnostic) biopsy

- For securing diagnosis only
- Ideally contain the interface between normal and abnormal epithelium
- Large enough to provide evidence of substage (in stage I cases)

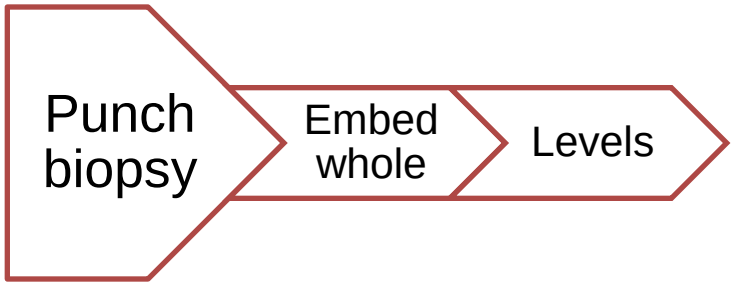
Excisional biopsy

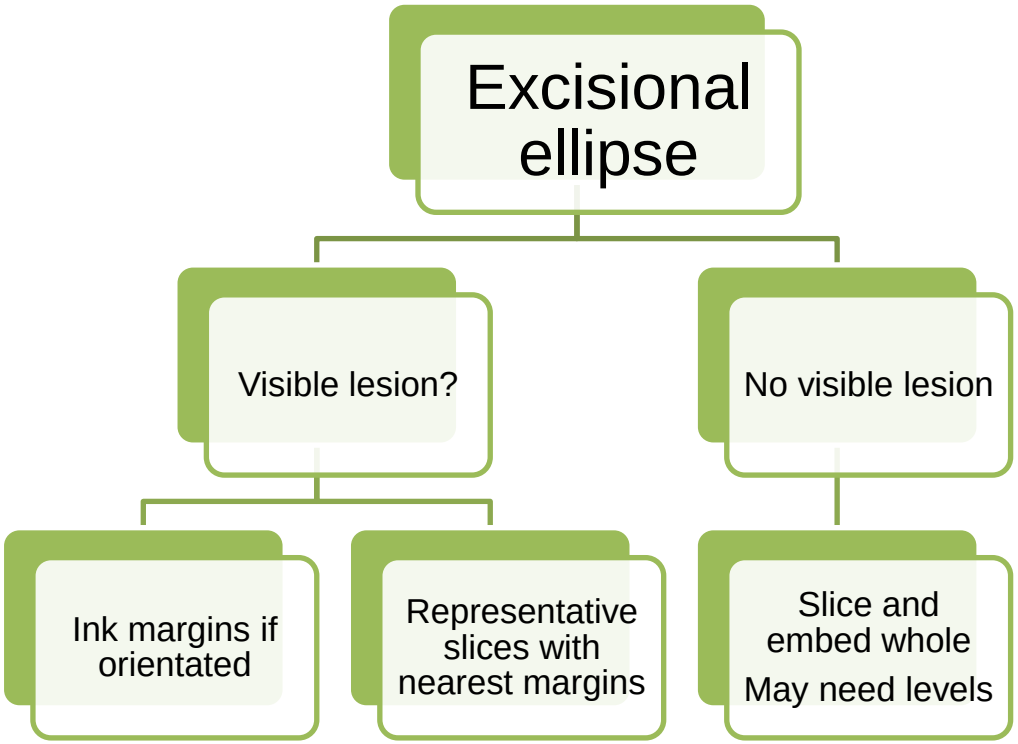
- Should include **all of the abnormal epithelium but does not provide a tumour-free zone of 1cm** (after fixation) on all dimensions
- Usually for cases of vulval intraepithelial neoplasia (VIN) or where there is low suspicion of invasive carcinoma

Radical excision

- Performed with the **intent of achieving clearance of at least 1cm** (after fixation) on all aspects of the tumour(s)
- Dependent on site and size of the tumour – could vary from wide local excision to a radical vulvectomy

Handling biopsies





Non-neoplastic biopsies

Lichenoid – lichen sclerosus, lichen simplex chronicus,

PAS – useful for basement membrane as well as fungi

Grams stain – unnecessary

DERMATOLOGY OPINION

Biopsies for precursor lesions and neoplasia

- Mapping biopsies
- Accurate documentation of site - diagram
- Presence or absence of dVIN or uVIN
- Presence or absence of non-neoplastic disease (esp. LS)
- Immunohistochemistry: p16, Ki67, p53

RCOG guidelines

1. The clinician should provide an accurate description of the site and appearance of the gross lesion

2. The request should also indicate whether the biopsy was excisional or diagnostic

3. Large radical resections should be pinned out on corkboard, kept moist with normal saline and sent as fresh tissue to the pathology department as rapidly as possible. If this is not possible, the specimen should be carefully oriented by means of marker sutures prior to fixation in the usual way

Vulvectomy

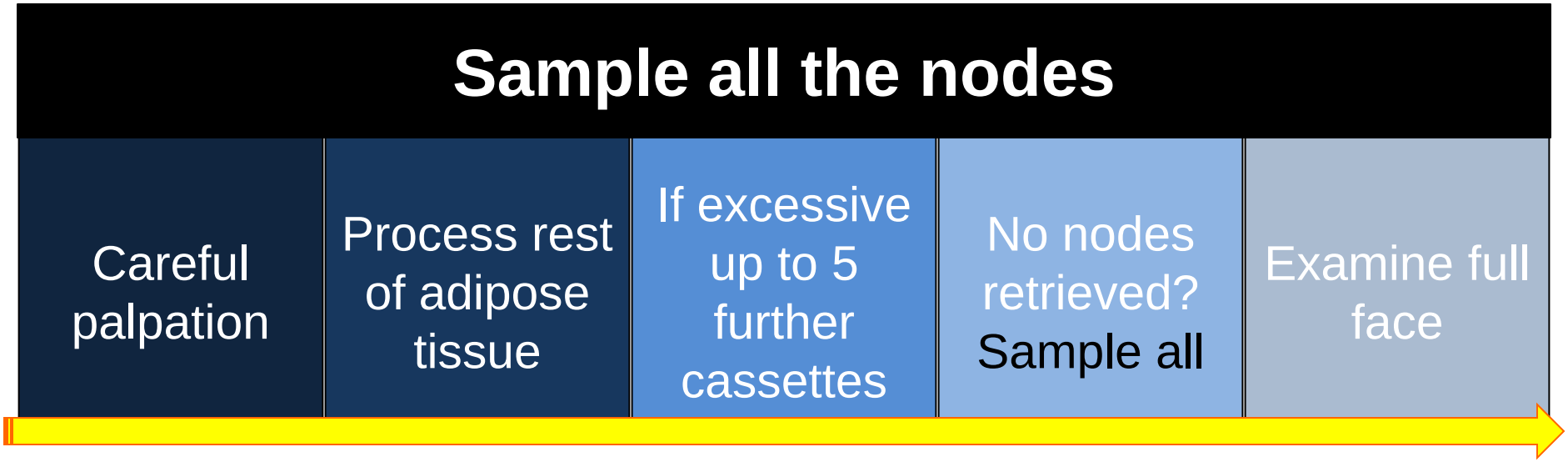
- **Diagram/photograph with blocks marked**
- **Type**
  - WLE, anterior, posterior, horseshoe, radical
- **Ink margins**
- **Macroscopic measurements**
- **Tumour**
  - Number (1 or more?), size in 3D, distance to margins
- **Background?, colour change?, other…?**

Lymph nodes

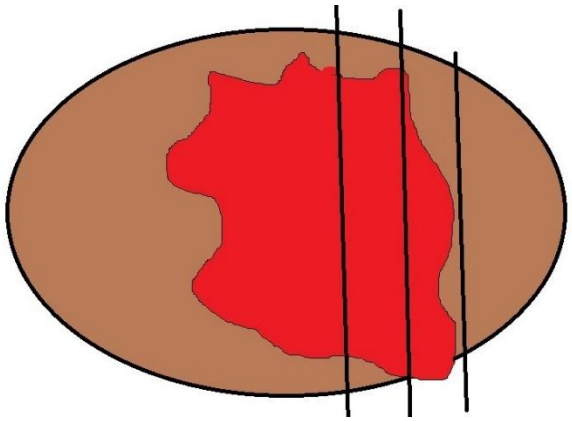
RCOG guidelines

- Surgery is the cornerstone of therapy for groin nodes
- Lateral tumours – only ipsilateral groin node surgery need initially be performed
- Contralateral lymphadenectomy - if ipsilateral nodes are positive
- Midline tumour – bilateral groin surgery

Lymph node sampling



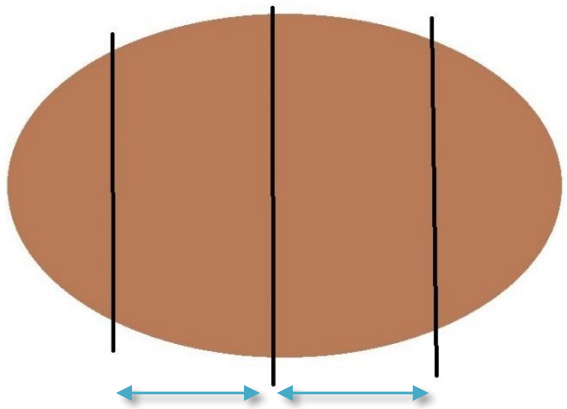
Visible tumour in LN?



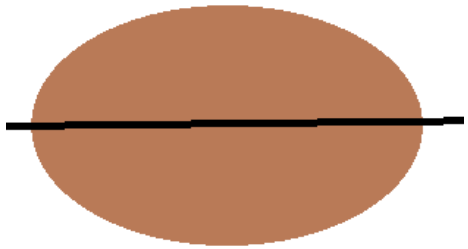
- No need to sample all
- Include capsule
- Measure the size of the metastasis
- Sample areas suspicious of extracapsular spread
- If in doubt embed whole

No visible tumour?

≥ 4mm
slice at 2-3mm intervals



≤ 4mm
embed whole OR bisect



Size of metastatic deposits

- 0.2 - 2mm = micrometastasis
- < 0.2mm = isolated tumour cells (ITC) regard as pN0 but mention in report

Sentinel Lymph Node (SLN) examination
Why?

- Rationale: first draining nodes – if negative, full inguinofemoral lymphadenectomy may not be necessary in selected cases
- Reduces length of hospital stay (cost implication)
- Reduces morbidity (lymphoedema, cellulitis, wound infection)
- Fewer LN to examine

Gynecologic Oncology 138 (2015) 472–477

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Review Article

Update on sentinel lymph node biopsy for early-stage vulvar cancer

Brian M. Slomovitz [a,*], Robert L. Coleman [b], Maaike H.M. Oonk [c], Ate van der Zee [c], Charles Levenback [b]

[a] Division of Gynecologic Oncology, Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL 33136, United States
[b] Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States
[c] Department of Gynecologic Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

HIGHLIGHTS

- SLN is the standard of care for management of early stage vulvar cancer
- preoperative lymphocscintigraphy may be useful
- ultrastaging increases ability to identify nodal metastasis

Gynecologic Oncology 114 (2009) 151–156

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Meeting Report

Sentinel lymph node biopsy in patients with gynecologic cancers
Expert panel statement from the International Sentinel Node Society Meeting, February 21, 2008

Charles F. Levenback [a,*], Ate G.J. van der Zee [b], Lukas Rob [c], Marie Plante [d], Al Covens [e], Achim Schneider [f], Robert Coleman [a], Eugenio Solima [g], Hermann Hertel [h], Emmanuel Barranger [i], Andreas Obermair [j], Michel Roy [d]

[a] Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA
[b] Department of Obstetrics and Gynaecology, University Medical Center Groningen, Groningen, The Netherlands
[c] Department of Obstetrics and Gynecology, Charles University Prague, 2nd Faculty and University Hospital Motol, Prague, Czech Republic
[d] Department of Obstetrics and Gynecology, Laval University, Quebec City, Quebec, Canada
[e] Obstetrics and Gynecology, Toronto Sunnybrook Cancer Centre, University of Toronto, Toronto, Ontario, Canada

Gynecologic Oncology 137 (2015) 351–361

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Review

Sentinel lymph node biopsy in vulvar cancer: Systematic review, meta-analysis and guideline recommendations

Al Covens [a], Emily T. Vella [b], Erin B. Kennedy [b,*], Clare J. Reade [c], Waldo Jimenez [d], Tien Le [e]

[a] University of Toronto, Odette Cancer Centre, Division of Gynecologic Oncology, Canada
[b] McMaster University, Cancer Care Ontario, Hamilton, Ontario, Canada
[c] Juravinski Cancer Centre, Hamilton, Ontario, Canada
[d] McMaster University, Juravinski Cancer Centre, Division of Gynecologic Oncology, Hamilton, Ontario, Canada
[e] Department of Obstetrics and Gynecology University of Ottawa, Ottawa Regional Cancer Centre, Canada

HIGHLIGHTS

Clearly, there is complexity to understanding the gene mutations involved in cancer. Stepping beyond initial BRCA testing may be problematic for the primary clinician. A practical algorithm incorporating recommendations for testing of a second set of mutations is needed for clinical management.—LVL)

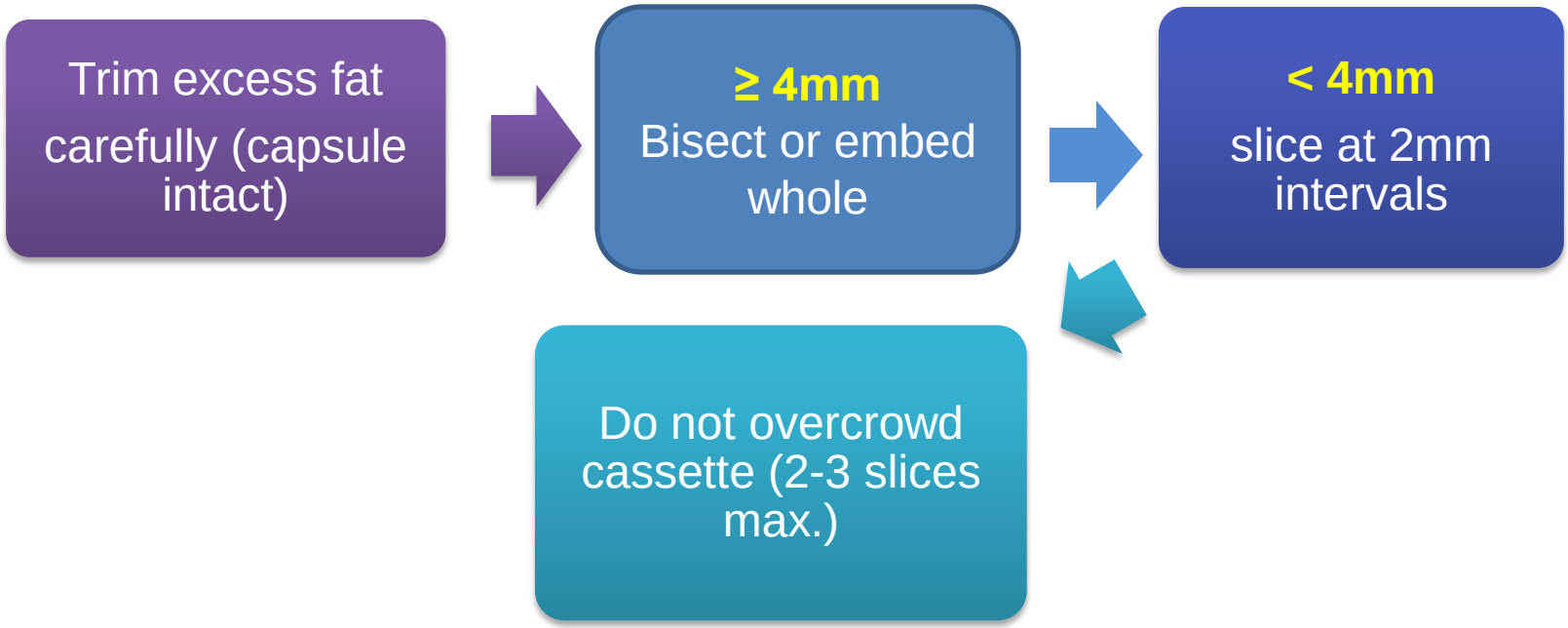
Sentinel Nodes in Vulvar Cancer: Long-Term Follow-up of the Groningen International Study on Sentinel Nodes in Vulvar Cancer (GROINSS-V) I

N. G. te Grootenhuis, A. G. J. van der Zee, H. C. van Doorn, J. van der Velden, I. Vergote, V. Zanagnolo, P. J. Baldwin, K. N. Gaarenstroom, E. B. van Dorst, J. W. Trum, B. F. M. Slangen, I. B. Rumenbaum, K. Tanussino, R. H. Beemans, D. M. Provencher, G. H. de Bock, J. A. de Hullu, and M. H. M. Oonk

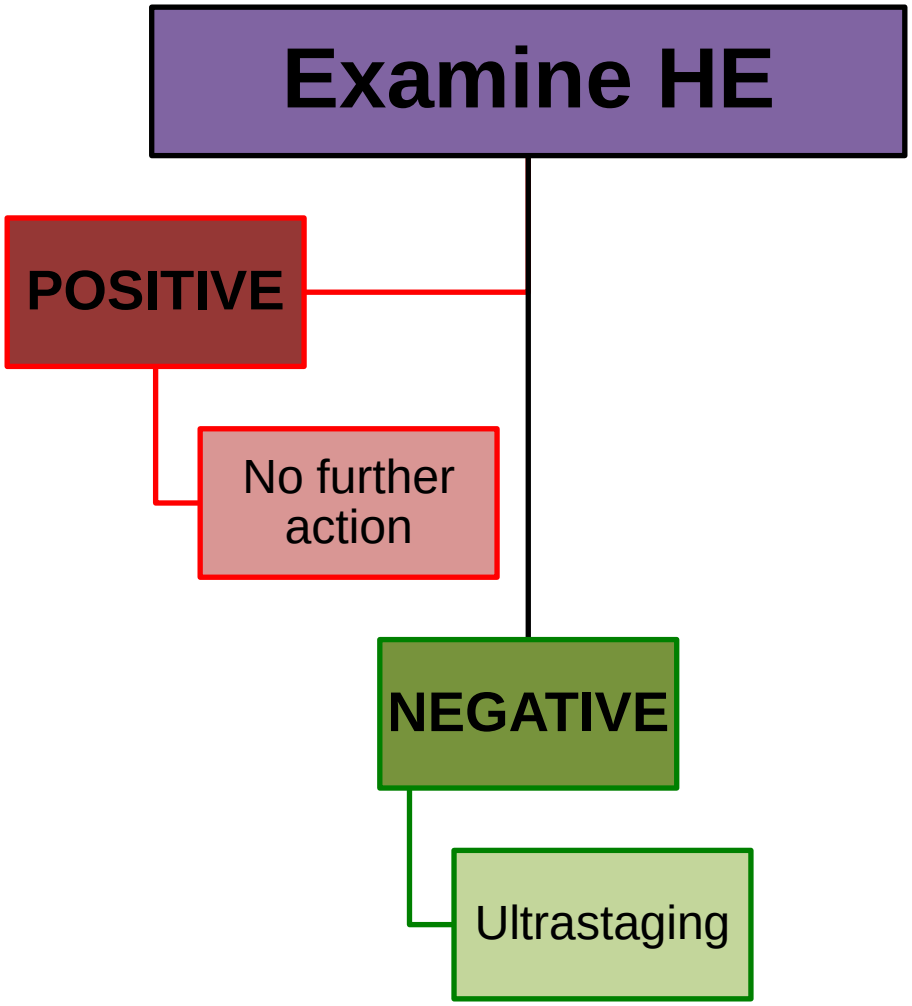
SLN: selection criteria

- Primary squamous vulval cancers
- Cancers measuring less than 4 cm in maximum dimension
- Macroscopic unifocal cancers
- No clinical or radiological evidence to suspect lymph node metastasis
- No known safety issues for the use of Patent Blue dye and/or technetium-99
- Informed patient consent and acceptance of close follow-up (recommended 2-monthly in the first year)
- If a sentinel lymph node cannot be identified following peritumoural injection of technetium-99 and/or Patent Blue dye then the patient should be considered for a complete inguinofemoral lymphadenectomy and (counselled time of consenting)

SLN method


Trim excess fat carefully (capsule intact)
≥ 4mm
Bisect or embed whole
< 4mm
slice at 2mm intervals
Do not overcrowd cassette (2-3 slices max.)

SLN method *(cont'd)*



Ultrastaging

Why?

- Negative SLN ➡ no inguinofemoral lymphadenectomy
- Important to make reasonable efforts to identify metastases

BUT

- Labour intensive and expensive
- Requires support - oncology team, managers

Ultrastaging *(cont'd)*

Why? Evidence that it identifies micrometastases

	Number	Routine HE	Ultrastaging
GOG Study 2012	200	Negative	46
GROINS-V	403	80	55
Knopf et al	421	Negative	25
Terada et al	9	1	2

Ultrastaging - methods described by different groups How far should we go?

Study	Number of pts	Protocol	False negative
Vidal-Sicart, 2007	70	1HE+1CK 400µm into block	0
Hampl, 2008	127	Cut through block at 200µm, HE+US for CK	3/127
Van der Zee, 2008	457	3 HE/mm +CK if neg	N/A
Achimas-Cadariou, 2009	59	Cut through block at 200µm, for a maximum of 6 HE	0
Devaja, 2011	60	1HE+CK at 400µm (max of 7 pairs)	0
Levenbeck, 2012	418	HE + CK at 40µm from HE	11/418

Adapted from Euscher, USCAP presentation

How far should we go? (*contd)*

Cut through block?

Too many slides

Too costly (time and labour)

No tissue left

Leave some tissue in the block

?miss micrometastases

Risk of non-sentinel-node metastases by largest tumour burden in the sentinel node

	Number of SN+ groins	Number of SN+ groins with IFLA (inguinofemoral lymphadenectomy)	Number of groins with non-SN metastases	Non-SN metastases percentage
ITC	51	24	1	4.2
≤ 1mm	13	10	1	10
> 1-2mm	12	9	1	11.1
> 2-5mm	15	15	2	13.3
> 5-10mm	16	13	5	38.5
> 10mm	9	8	5	62.5
Total	116	79	15	19

Oonk 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, Lancet Oncol. 2010 Jul;11(7):646-52.

How far should we go? (*contd)*

Sentinel Lymph Node Biopsy: Strategies for Pathologic Examination of the Specimen
Meyer et al, Journal of Surgical Oncology 1998;69:212–218

Methods: Sentinel node tissue is sliced at 2-mm intervals for fixation and paraffin embedding. Probabilities of finding spherical micrometastases of specific sizes randomly distributed in lymph nodes were calculated geometrically for several microsectioning plans.
Results and Conclusions: Sentinel node tissue can be studied by systematic serial sectioning technique designed to find metastases of given diameters with specific probabilities. A procedure whereby three microsections are prepared repeatedly at intervals of 250 mm appears to be practical. Two sections from each level can be examined by routine staining and the third by immunohistochemical stain; the latter is recommended particularly for infiltrating lobular carcinoma. This method will find metastases of 0.25-mm diameter with theoretical probability of 1, and metastases of 0.10-mm diameter with probability of 0.46, with reasonable costs. Metastases of these sizes are consequential and worth finding on biological and clinical

How far should we go? (*contd)*

Ultrastaging Improves Detection of Metastases in Sentinel Lymph Nodes of Uterine Cervix Squamous Cell Carcinoma

Elizabeth D. Euscher, MD, Anais Malpica, MD,* Edward Neely Atkinson, PhD,† Charles F. Levenback, MD,‡ Michael Frumovitz, MD,‡ and Michael T. Deavers, MD**

10 SLN with less than 2 mm foci.
Greater than 95% probability (5 microsections at 250µm intervals.

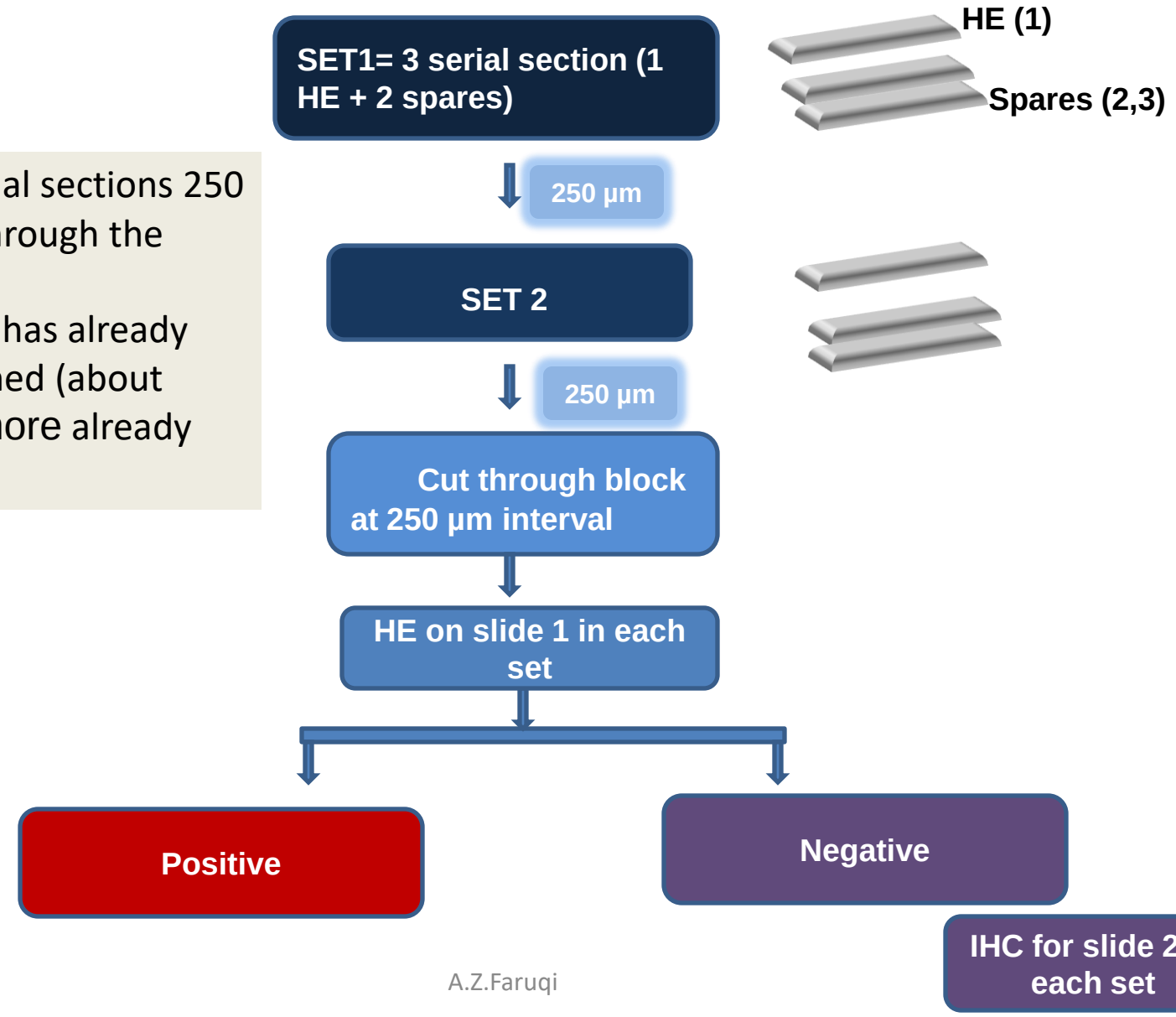
Significance of micrometastasis

- Davaja – 3 pts with one positive LN each recurred. 2 pts had metastases less than 2.0mm
- Terada – after groin recurrence, the SLN was sectioned at 400 and a micrometastasis discoverd
- Tamussino – 1.0mm metastasis, no groin surgery, pt recurred

Gynecologic Oncology Volume 76, Issue 1, January 2000
Gynecologic Oncology Volume 86, Issue 1, July 2002
portal.uscap.org/system/storage/serve/55903/Euscher-final.pdf

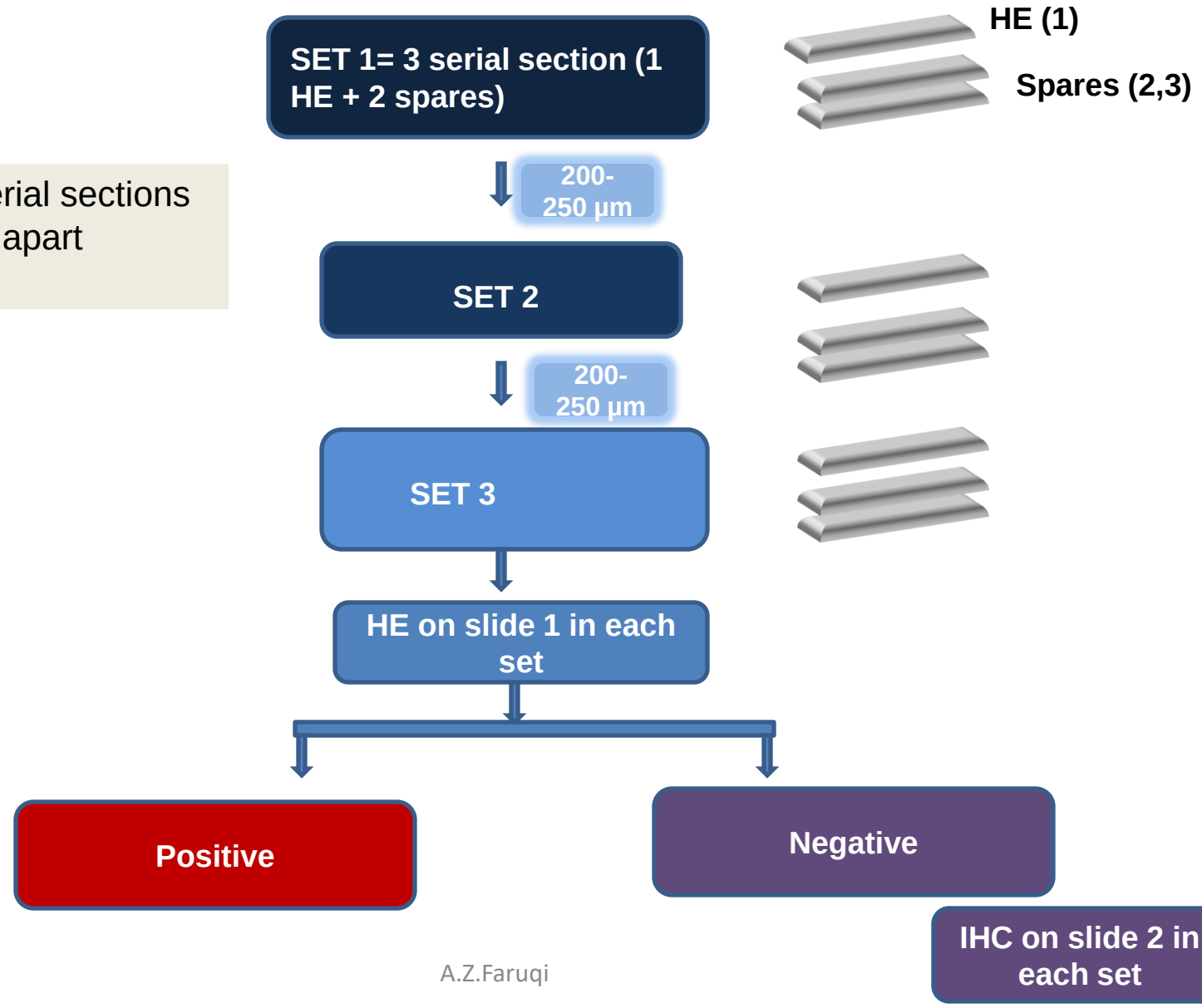
Ultrastaging (Suggestion 1)

Sets of 3 serial sections 250 µm apart, through the block.
An initial HE has already been examined (about 500µm or more already removed)



Ultrastaging (Suggestion 2)

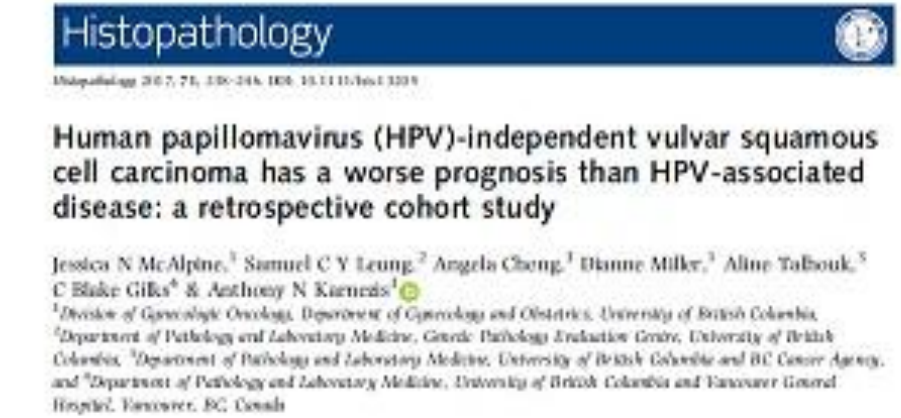
3 sets of 3 serial sections 200-250 µm apart



HPV mediated vs. HPV independent neoplasia

- **Should try and determine this**

- ***Why?***
  - Accumulating evidence that HPV mediated tumours have better prognosis
  - Precursor lesions in HPV-mediated can be treated non-surgically

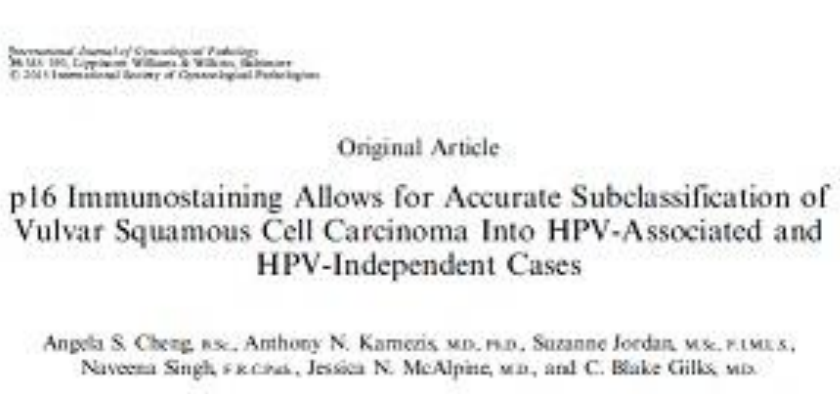


Histopathology

Human papillomavirus (HPV)-independent vulvar squamous cell carcinoma has a worse prognosis than HPV-associated disease: a retrospective cohort study

Jessica N McAlpine,[1] Samuel C Y Leung,[2] Angela Cheng,[1] Dianne Miller,[3] Aline Talhouk,[3] C Blake Gilks[4] & Anthony N Karnezis[1]

[1]Division of Gynecologic Oncology, Department of Gynecology and Obstetrics, University of British Columbia, [2]Department of Pathology and Laboratory Medicine, Genetic Pathology Evaluation Centre, University of British Columbia, [3]Department of Pathology and Laboratory Medicine, University of British Columbia and BC Cancer Agency, and [4]Department of Pathology and Laboratory Medicine, University of British Columbia and Vancouver General Hospital, Vancouver, BC, Canada



Original Article

p16 Immunostaining Allows for Accurate Subclassification of Vulvar Squamous Cell Carcinoma Into HPV-Associated and HPV-Independent Cases

Angela S. Cheng, B.Sc., Anthony N. Karnezis, M.D., Ph.D., Suzanne Jordan, M.Sc., F.I.M.L.S., Naveena Singh, F.R.C.P.(C), Jessica N. McAlpine, M.D., and C. Blake Gilks, M.D.

Gynecologic Oncology 142 (2016) 293–298

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated with radiotherapy

Larissa J. Lee [a,f,*], Brooke Howitt [b,f], Paul Catalano [d,f], Cynthia Tanaka [a], Rita Murphy [a], Nicole Cimbak Rebecca DeMaria [a], Paula Bu [a], Christopher Crum [b,f], Neil Horowitz [c,f], Ursula Matulonis [e,f], Akila N. Viswanathan [a,f]

IJC

International Journal of Cancer

HPV status and favourable outcome in vulvar squamous cancer

Kate Wakeham[1,*], Kim Kavanagh[2], Kate Cuschieri[3], David Millan[4], Kevin G. Pollock[4], Sarah Bell[5], Kevin Burton[1], Nicholas S Reed[6] and Sheila V Graham[7]

	HPV mediated	HPV independent
Precursor lesion	Usual/classical VIN (uVIN)	Differentiated VIN (dVIN)
Associated with:	Koilocytosis	Dermatoses - Lichen sclerosus
Tumour morphology	Often basaloid or warty	Often well diff keratinising SCC
Immunohistochemistry	Often p16 overexpression	*p53 overexpression or null*

LAST project

- **Lower Anogenital Squamous Terminology standardisation**

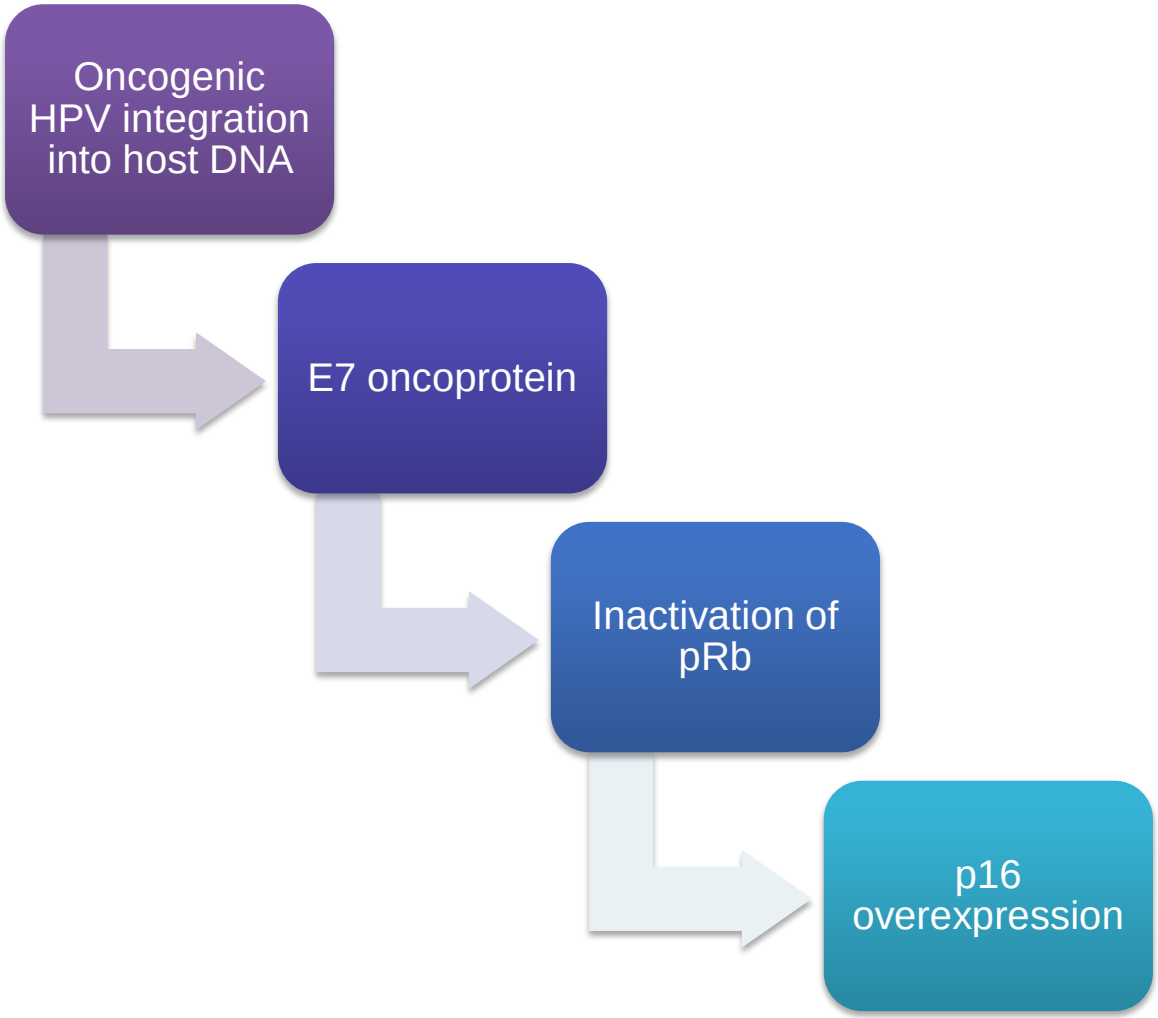
- **Project (LAST) *for HPV-associated lesions***
  - Single set of diagnostic terms for all lower anogenital sites
  - Two-tiered nomenclature for intraepithelial lesions
  - Low grade SIL (LSIL), generally self-limited lesions
  - High grade SIL (HSIL), can progress
  - In UK, recommend use together with VIN terminology [i.e. VIN1 (LSIL); VIN2 or VIN 3 (HSIL)]

Usual/classical VIN

- **Young women: increasing incidence in 30s and 40s**
- **History of condylomas, herpes infection, HIV disease, smoking**
- **Linked to HPV, usually HPV 16**
- **Varied appearance**
  - Papules, plaques, polyps
  - White, red, or pigmented
- **Multicentric disease in vagina or cervix**

p16

Surrogate marker for oncogenic HPV



p16 overexpression

- Continuous, linear moderate to strong nuclear and cytoplasmic staining
- Affects basal 1/3 to 2/3 of epithelium
- Often fades towards the surface

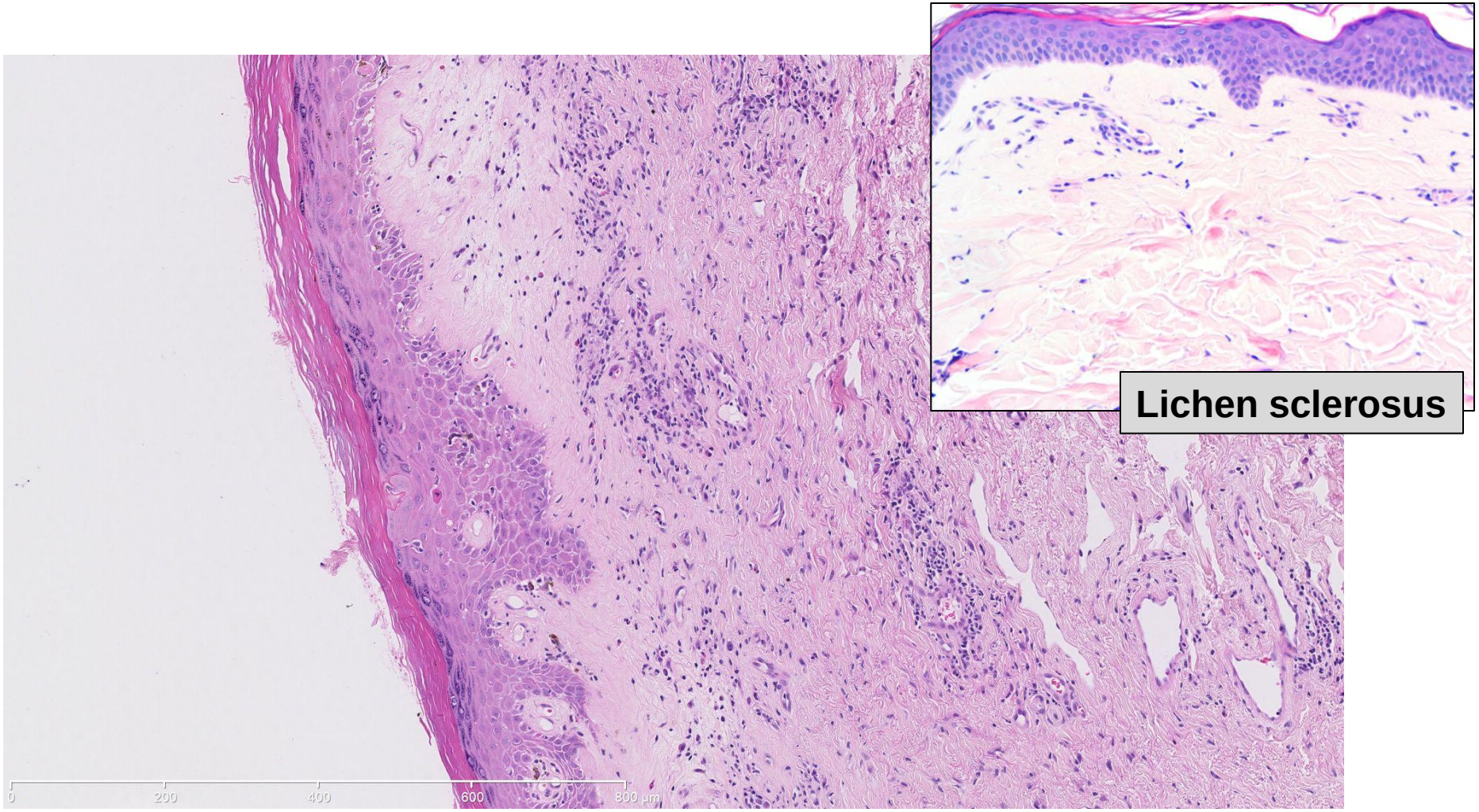
Differentiated/simplex VIN

- 2-10% of VIN
- Older age group: over 60s
- Grey or white plaques, may be multifocal
- Association with lichen sclerosis
- Not associated with HPV
- ? Greater potential for progression to invasive carcinoma; invasive carcinoma is keratinising squamous cell type

Histology

- Acanthosis
- Hyper/parakeratosis
- Elongation and anastomosis of the epithelial rete
- Intercellular oedema
- Basal atypia
- Dyskeratosis - keratinised
  cells whorls in the lower or mid epithelium
- Loss of keratohyaline granules
- Increased eosinophilia
- Abrupt change from normal to abnormal

dVIN



dVIN *(cont'd)*

- **TP53 mutation:**

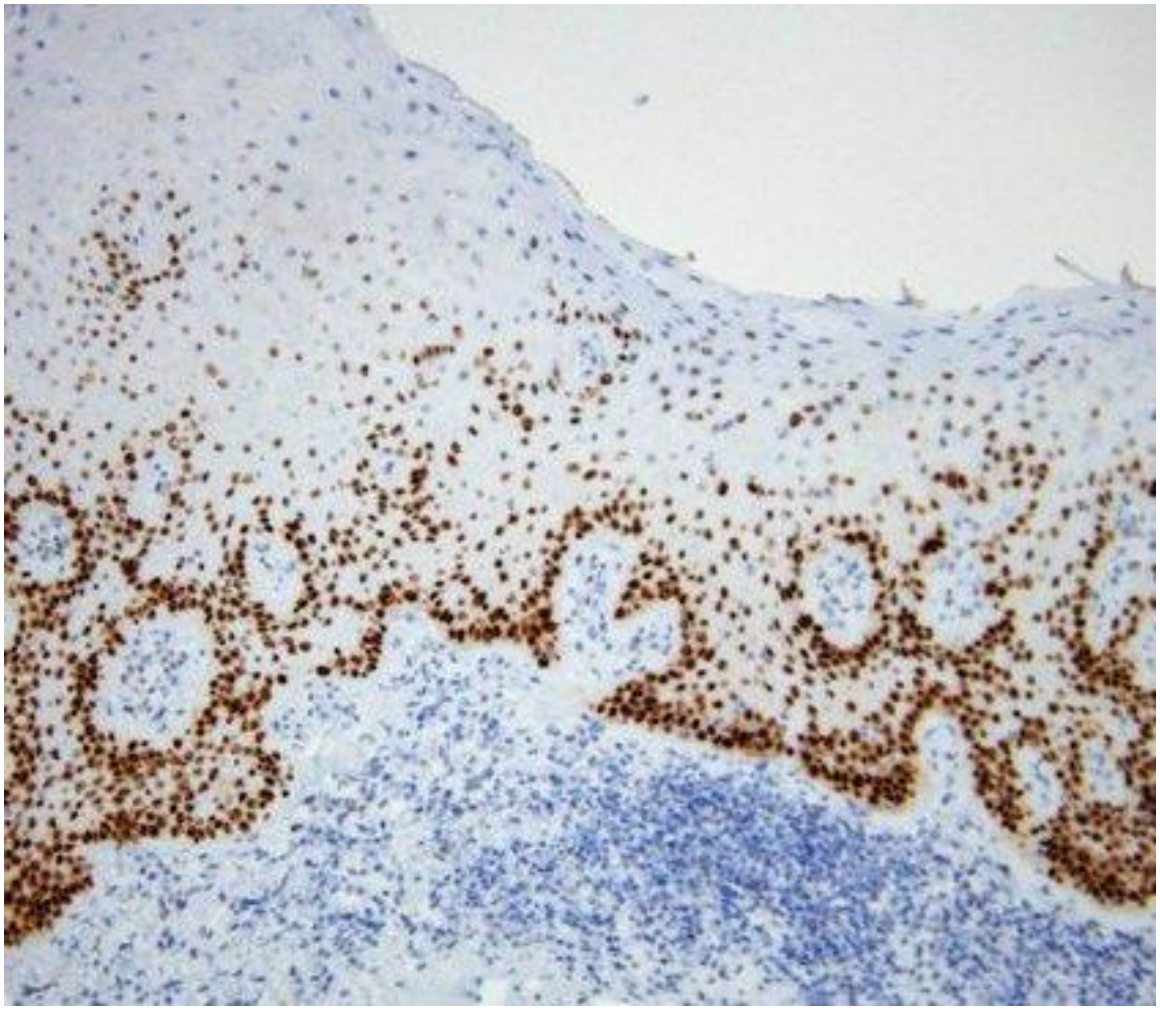
Missense (overexpression)

OR

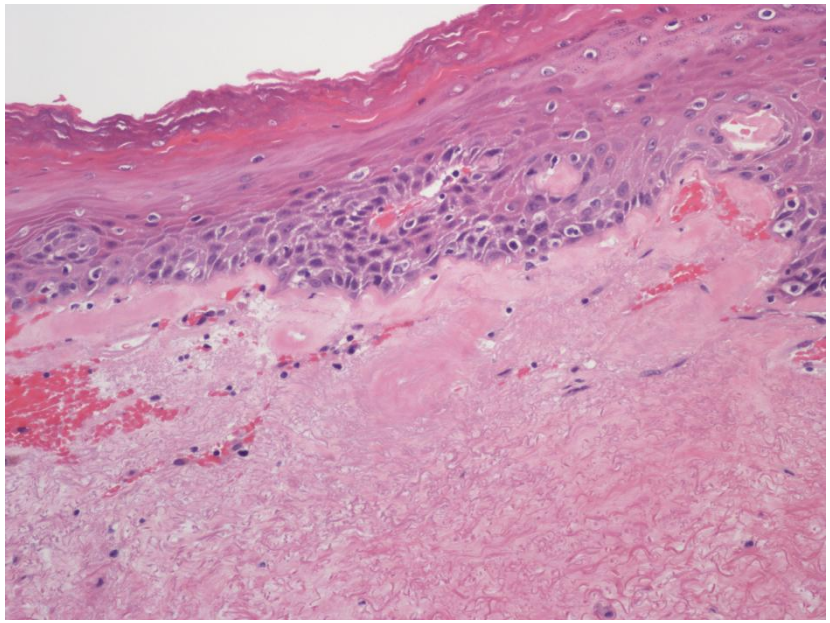
Nonsense (null)

- **p53 immunohistochemistry not always helpful and can be difficult to interpret**
- **Normal basal cells are positive, overexpression if suprabasal cells are positive**
- **Null if no expression throughout affected epithelium**

p53 overexpression



Null p53



dVIN

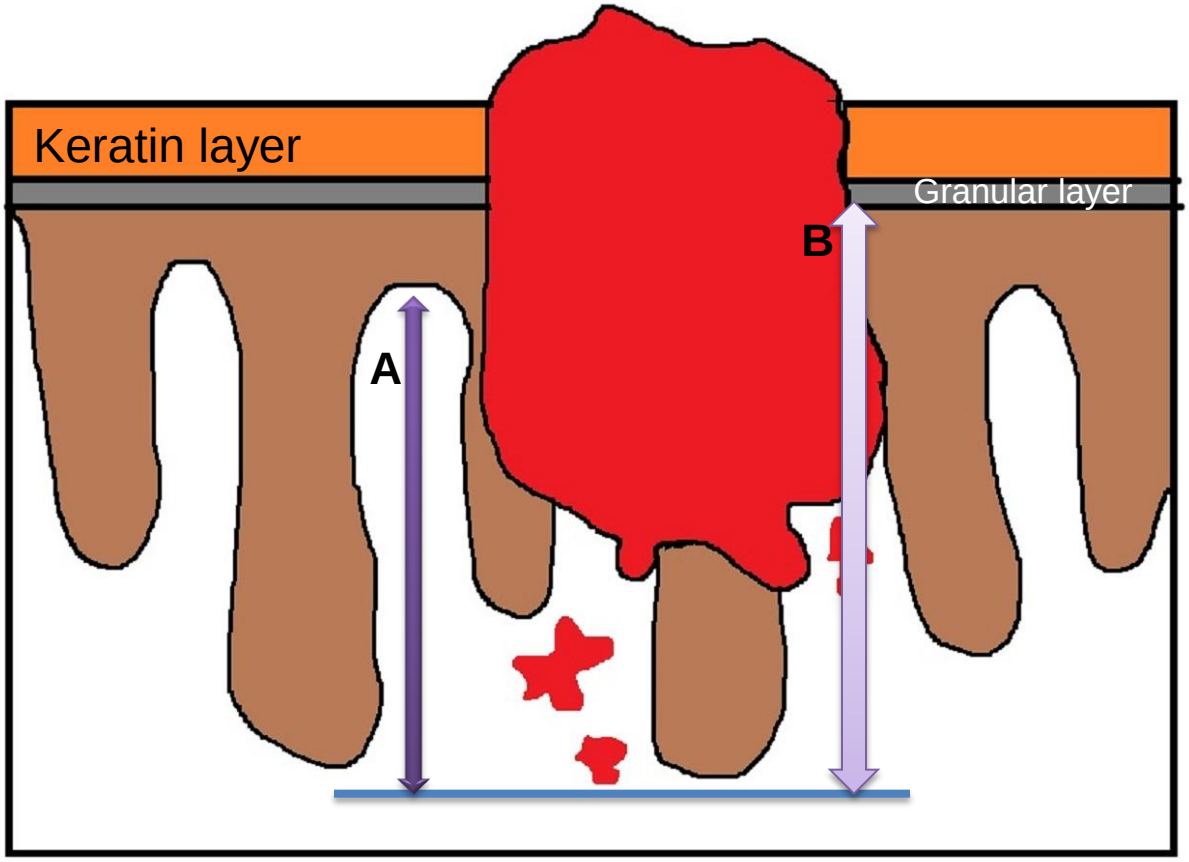
Problems in diagnosis

- Subtle
- Can be mistaken for VIN1
- Can be under or overdiagnosed in the presence of LS
- Can be masked or mistaken for reactive changes, especially if there is itching and lichenoid changes

Measuring vulval SCC

A. Depth of invasion
From the adjacent most superficial dermal papilla to the deepest point of invasion.

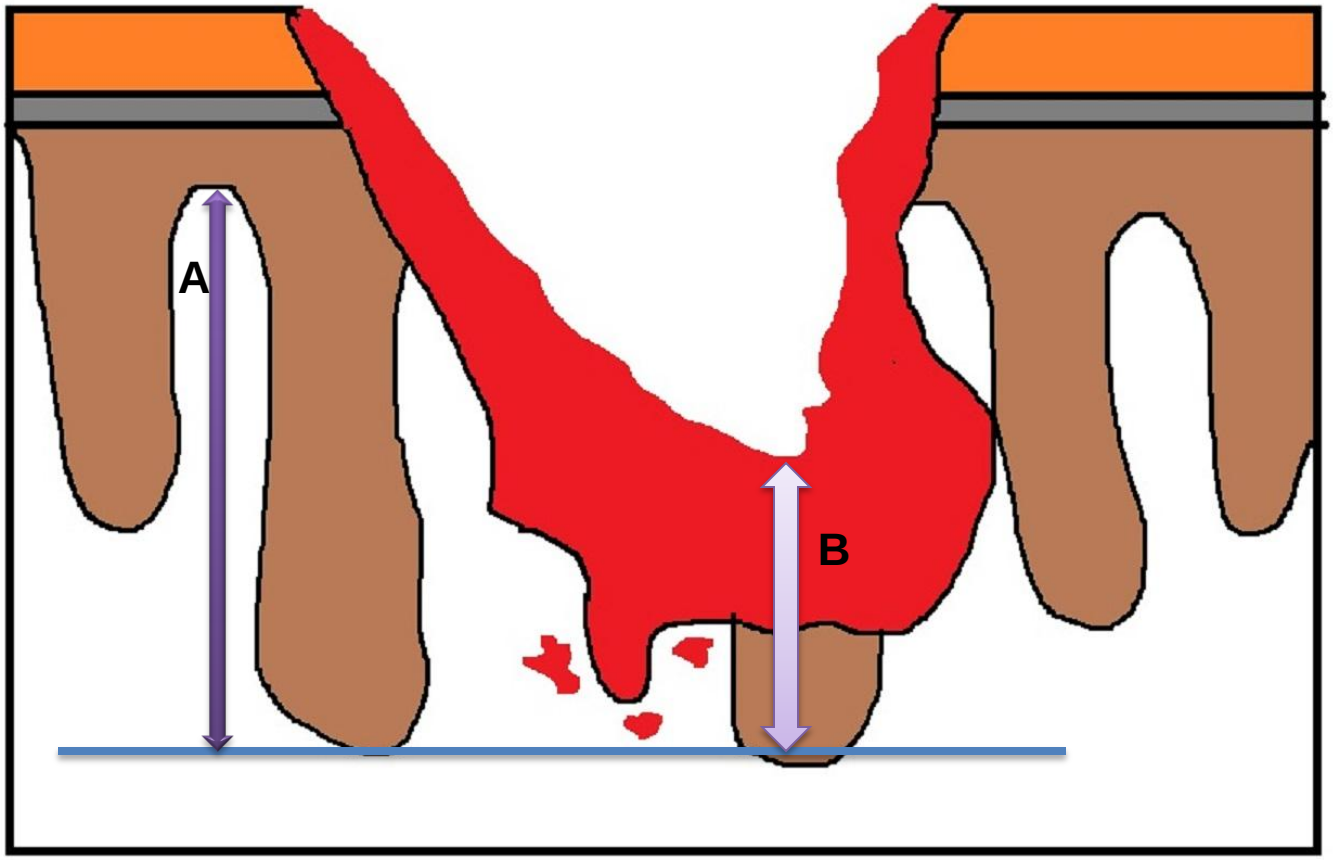
B. Tumour thickness
Keratinised tumours – from the base of the granular layer to the deepest point of invasion



Measuring ulcerated tumours

A. Depth of invasion
From the adjacent most superficial dermal papilla to the deepest point of invasion.

B. Tumour thickness
From the surface of the ulcer to the deepest point of invasion



Other malignant epithelial vulval tumours

1. Extramammary Paget's disease
2. Bartholin gland tumours
3. Melanoma

	CK7	CK20	CEA	S100
Extramammary Paget's	Positive	Negative	Positive	Negative
Associated with lower GI adenocarcinoma	Usu negative	Positive	Positive	Negative
Melanoma	Negative	Negative	Negative	Positive
Pagetoid VIN	Positive	Negative	Negative	Negative

Cystic lesions of vulva and vagina

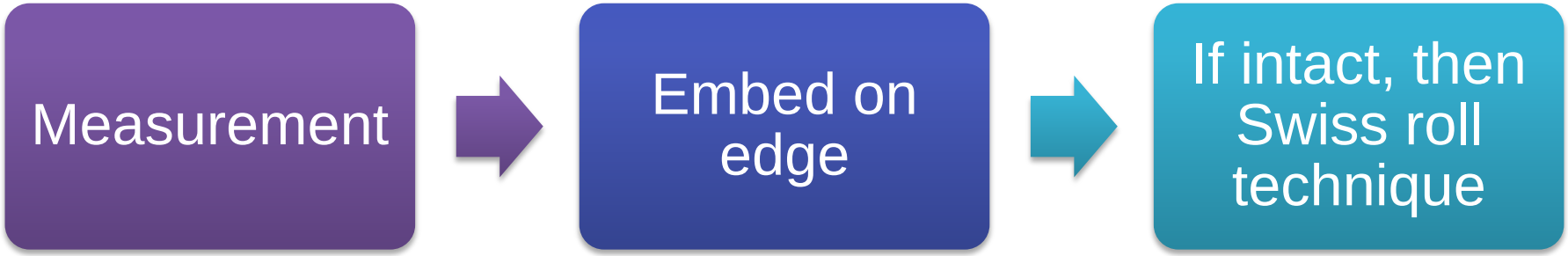
Clinical queries

- Benign or malignant?

BUT

- Usu minimal clinical information (“vulval lesion”, “vulval biopsy”, “cyst”)

Macroscopy

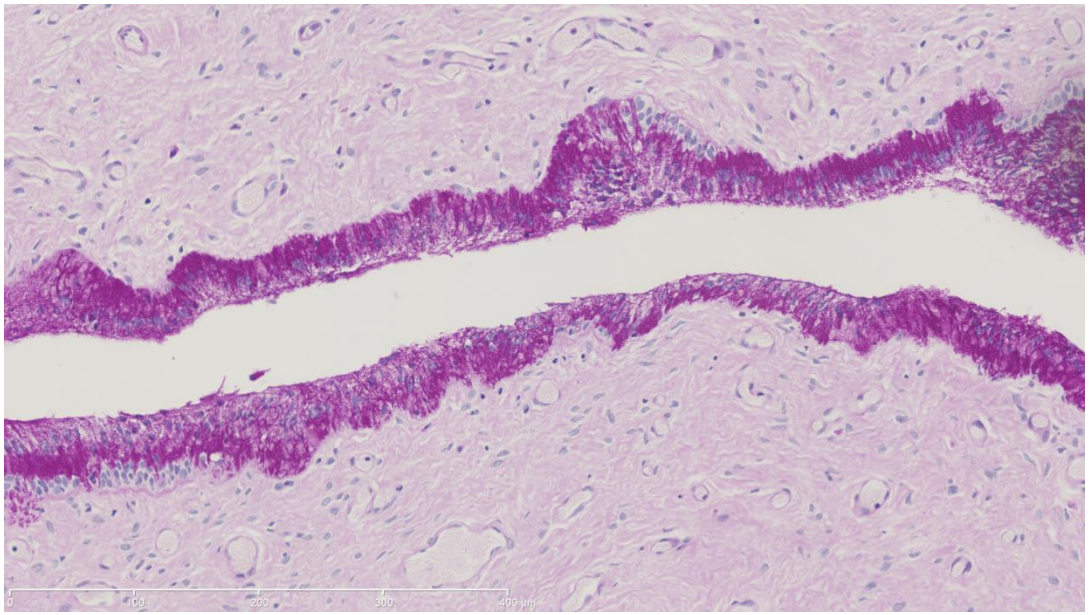


Usually fragmented so aggregate measurement is good enough

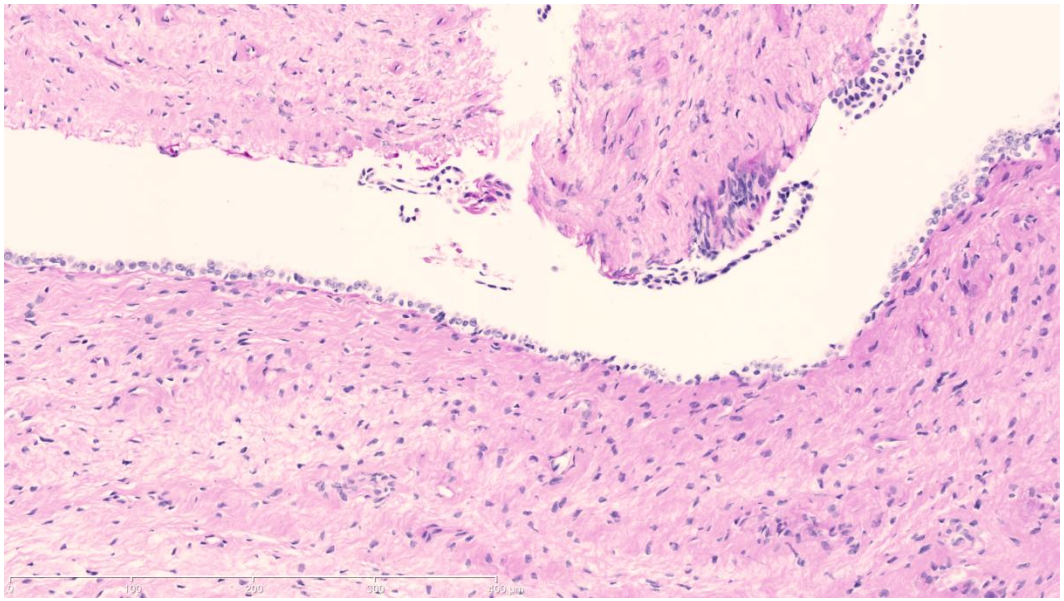
- Looks benign?
- Don’t worry about exact terminology (“benign mucinous, non-mucinous, squamous, transitional cyst”)
- If squamous, remember FGM

Benign vulvo-vaginal cysts

LINING		SITE	ORIGIN
Mucinous	Mullerian cyst	Upper vagina, usually anterio-lateral wall	Mullerian duct
	Mucous cyst of vestibule	Usually vestibule	Minor vestibular glands
	Bartholin's cyst	Posterio-lateral vagina	Bartholin's gland/duct
Non-mucinous, non-ciliated, cuboidal	Gartner's cyst	Lateral wall of vagina	Wolffian remnants
Columnar endometrial type (may be ciliated)	Mullerian cyst	Upper vagina, usually anterio-lateral wall	Mullerian duct
	Endometriotic cyst	Vulvovaginal endometriosis	Site of trauma/scars. Vault or posterior fornix
Transitional	Skene's cyst	Close to urethral meatus	Skene's duct
Squamous	Any	Any	Metaplasia in the above, trauma including female genital mutilation



Mullerian cyst - mucinous

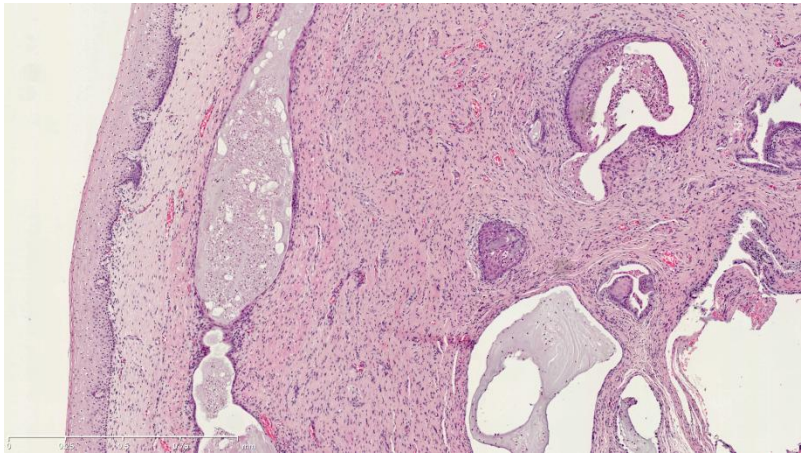


Non-mucinous lining – Gartner’s cyst

Vagina

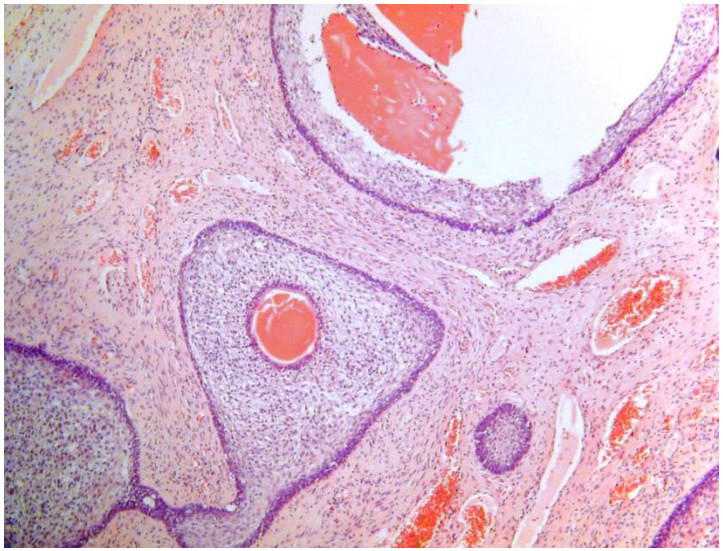
- **Normally no glands in the vagina**

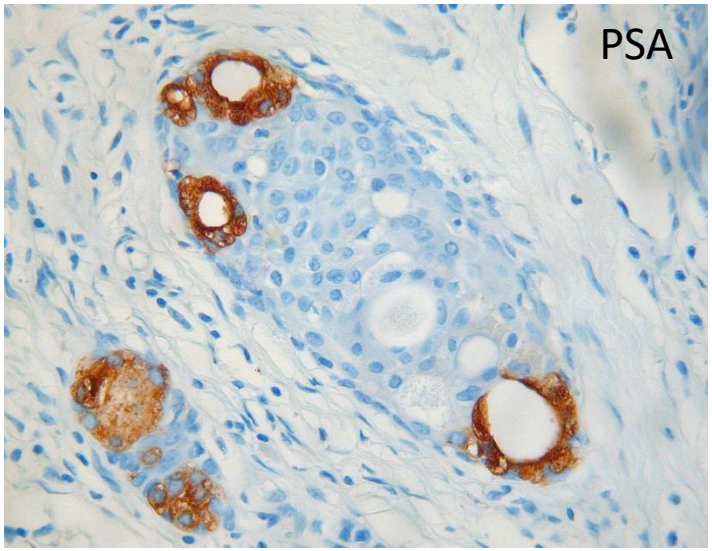
- **If glands or glandular epithelium present:**
  - Endometriosis
  - Endocervicosis
  - Adenosis – DES, OC pill, Tamoxifen
  - Prolapsed fallopian tubes (history of hysterectomy)
  - Polyp arising from prostatic type glands (tubulosquamous vaginal polyp)
  - Lobular hyperplasia of Bartholin's gland (v. adenoma - rare)



Vaginal adenosis

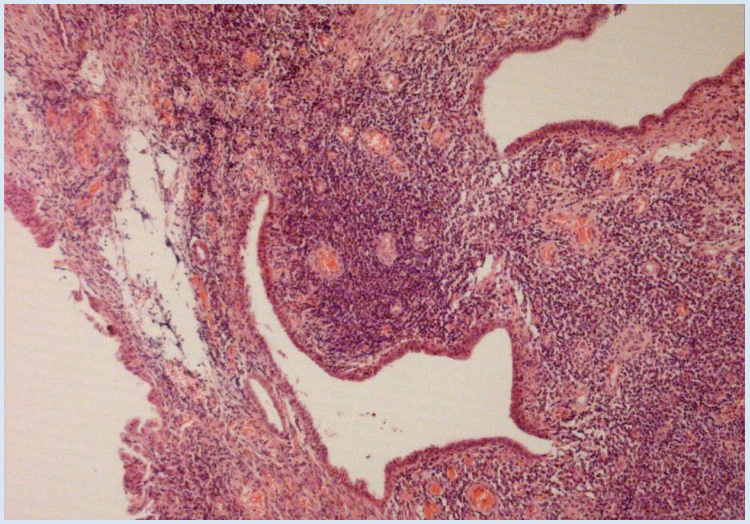
Tubulosquamous vaginal polyp





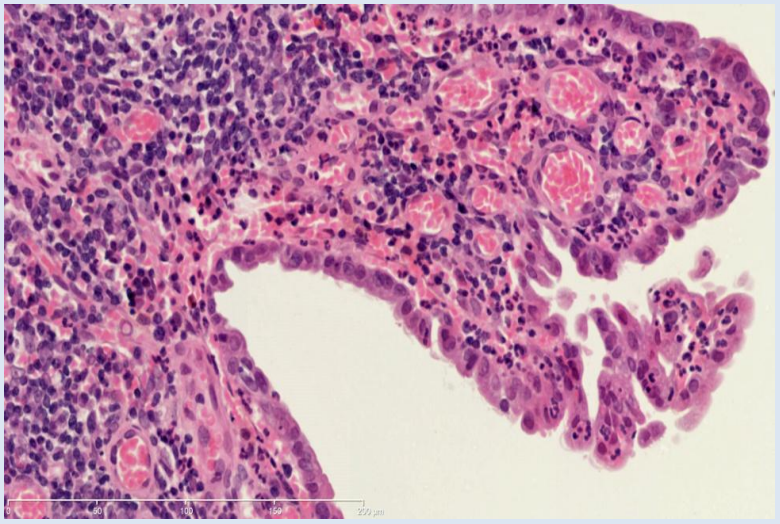
Courtesy Prof G.McCluggage

Endometriosis

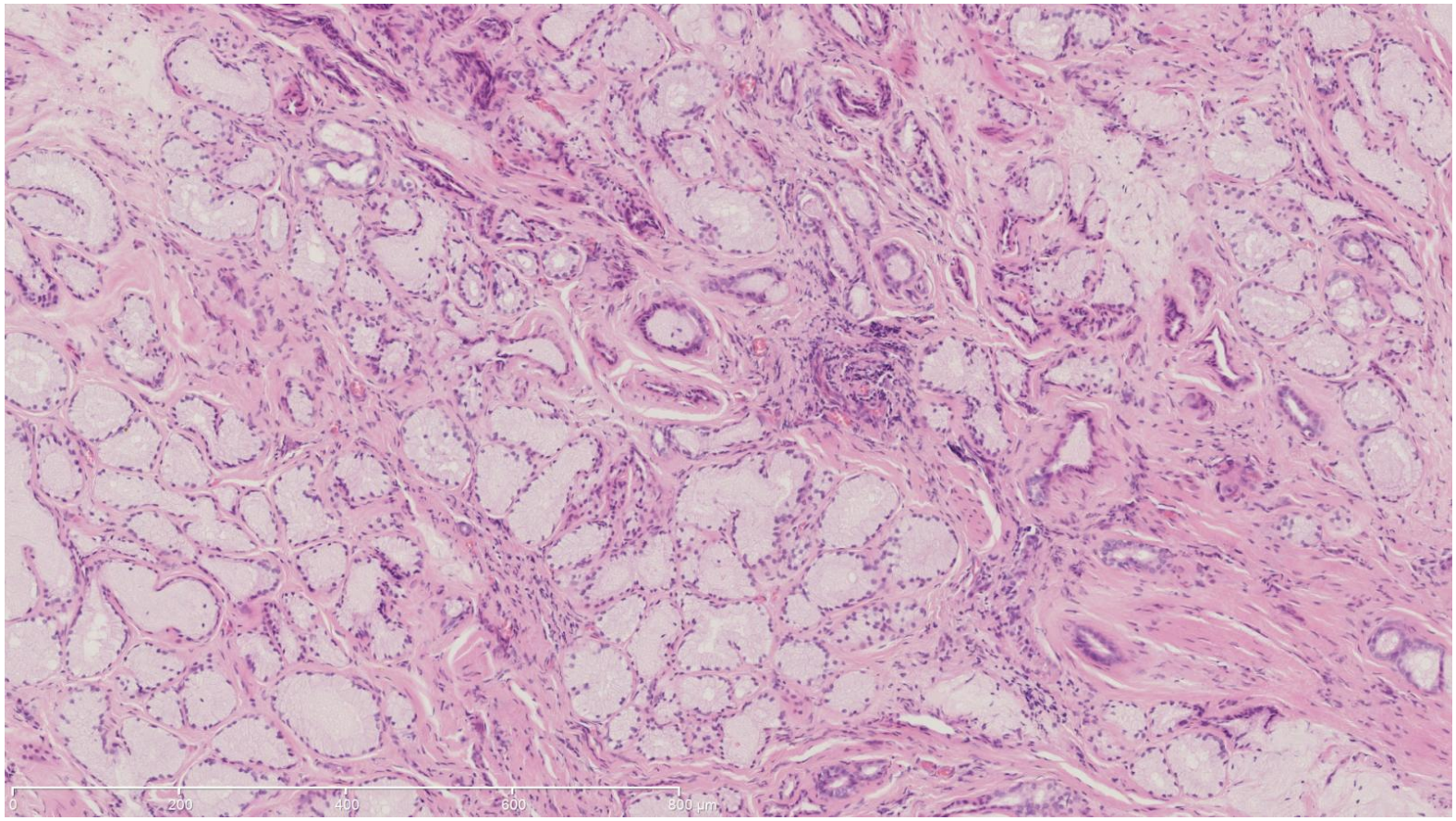


- Superficial lesions - sites of trauma due to implantation therefore not usu. associated with pelvic disease
- Deep lesions associated with pelvic endometriosis

Prolapsed Fallopian Tube



- Presents as polyp or mass, may be clinical suspicion of adenocarcinoma.
- History of hysterectomy



Lobular hyperplasia of Bartholin's gland (DDX adenoma - rare)

Malignant vaginal lesions

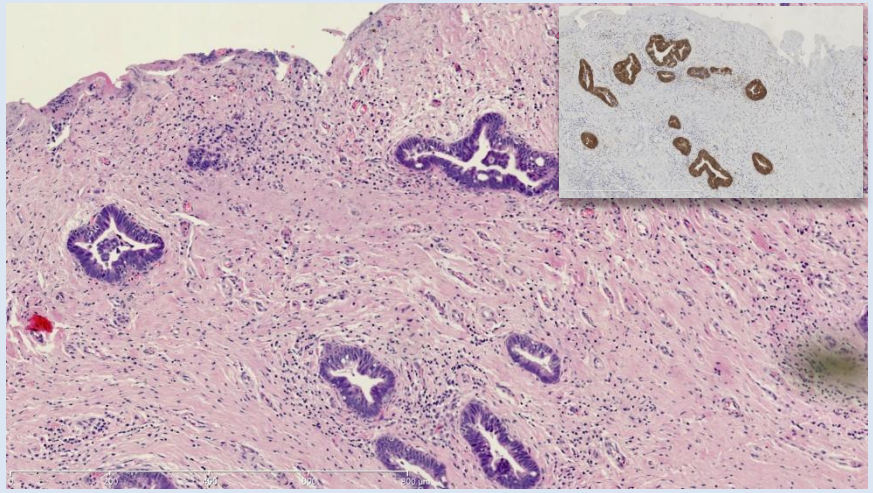
- **Squamous cell carcinoma**

- **Adenocarcinoma (rare):**
  - Endometrioid (from endometriosis)
  - Clear cell carcinoma, sporadic or DES associated
  - Enteric type (cloacogenic)

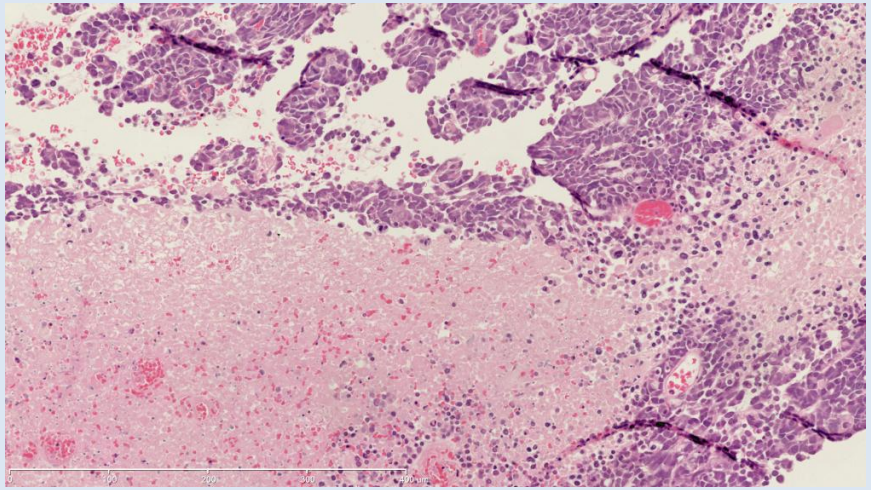
- **Bartholin gland tumours**

Don’t forget metastases!

Vaginal thickening- previous history of cervical carcinoma (inset p16)



Vaginal nodule – spread from HG ovarian serous carcinoma



Mesenchymal lesions

- **Angst!!!**

- **Clinical queries:**
  - What is it? (many come with clinical diagnosis of “cyst”)
  - Benign or malignant?
  - Excised?
  - Further management?

- **Important to correlate with imaging**

- **Site specific lesions but don’t forget non-site specific**

Site-specific vulvo-vaginal lesion

- **Arise from the specialised sub-epithelial stroma:**
  - Fibroepithelial stromal polyp
  - Angiomyofibroblastoma
  - Cellular angiofibroma
  - Superficial myoblastoma
  - Aggressive angiomyxoma
  - Others – massive vulval oedema, “cyclists nodule”

Macroscopy

- Size (important)
- Intact – ink
- Fragmented – measure in aggregate
- Consistency – soft, firm, hard, myxoid
- Circumscription

- Most lesions are circumscribed apart from aggressive angiomyxoma
- Most stain with ER, PR, CD34 and desmin
- Most are cytologically bland
- Overlapping morphology
- IMPORTANT: recognise aggressive angiomyxoma as it tends to recur

Aggressive angiomyxoma

- Deep location, often large
- Myxoid
- Poorly circumscribed, infiltrative
- Cellularity – low
- Morphology – bland
- Blood vessels - many, variable calibre, capillary like large and muscular with smooth muscle fibres spinning out
- Myxoid stroma
- Extravasated red cells

- HMGA2 – nuclear stain
- Transcription factor Ch 12 – rearranged overexpression

→

- Other mesenchymal lesion generally more cellular, less myxoid and circumscribed
- Radiological correlation

Angiomyofibroblastoma

- Circumscribed
- Polypoid
- Hypo and hypercellular areas
- Delicate capillary sized vessels
- Apart from spindle cells also epithelioid / plasmacytoid cells often around blood vessels (desmin positive)

Fibroepithelial polyp

- Common
- No Grenz zone – useful for diagnosis
- Can have mitoses, atypical cells
- Stroma – all sorts
- Large lesion with myxoid stroma ?FEP ?Aggressive angiomyxoma
- FEP is polypoid, has multinucleate stromal cells and no Grenz zone

Smooth muscle tumours of vulva and vagina

- The criteria for malignancy are not the same as for uterine tumours
- Nuclear atypia
- Mitoses – any!
- Infiltrative edge
- ***Regard all smooth muscle tumours with a suspicious eye – even banal ones can recur***