HPV-Independent Lesions of the Vulva: A Practical Approach to Classification with Molecular Insights

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**No conflict of interest to disclose**

The Case

- Clinical history:
  - A 75 year old female presents with an 8.5 cm pedunculated vulvar mass. She has had a longstanding (>10 years) history of lichen sclerosus and vulvar masses which have been variably interpreted as condyloma, verruciform hyperplasia, or verruciform differentiated vulvar intraepithelial neoplasia (dVIN).

- A representative slide of the current mass was made available online.
Objectives

- Review of histologic features and pathobiology of HPV-negative vulvar lesions
  - Practical approach to diagnosis for proper clinical management
  - Recent molecular insights into HPV-negative vulvar lesions

Differential Diagnosis

- Invasive squamous cell carcinoma (SCC)
- Verrucous carcinoma (VC)
- Verruciform differentiated VIN (dVIN)
- Verruciform acanthosis with altered differentiated (VAAD)
- Verruciform lichen simplex chronicus (LSC)
- Atypical verruciform lesions/differentiated exophytic vulvar intraepithelial lesion (DE-VIL)
- Verruciform “hyperplasia”
**HPV in vulvar squamous carcinomas**

- **Verrucous carcinoma**
  - All are HPV negative

- **Keratinizing SCC**
  - HPV negative >> HPV positive

- **Moderate to poorly differentiated SCC**
  - HPV negative << HPV positive

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**Invasive Keratinizing SCC**

- Infiltrative invasion of stroma

- Keratinization/squamous pearls present in invasive carcinoma

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**Verrucous carcinoma**

- Extremely well differentiated form of SCC

- Broad based/bulbous rete ridges

- Lack of infiltrative invasion

- Pushing invasion/margin

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**Verrucous carcinoma**

- Minimal cytologic atypia

- Smooth epithelial-stromal interface

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**Pathways for vulvar cancer precursors**

T. Bosse, manuscript in preparation
Verrucous carcinoma

- Parakeratosis
- No koilocytosis
- Superficial epithelial pallor

**Practical pearl**

- ANY degree of conventional infiltrative or destructive invasion EXCLUDES the diagnosis of a (pure) verrucous carcinoma.
- Verrucous carcinoma may be associated with focal conventional keratinizing SCC- the biologic potential in this situation is that of an invasive keratinizing SCC and should be diagnosed as such.

Differentiated VIN (dVIN)

- Prominent basal atypia:
  - Hyperchromasia
  - Nuclear enlargement
  - Prominent nucleoli
  - +/- atypical mitoses
- Dyskeratosis/abnormal keratinization
- Prominent intercellular bridges in absence of inflammation
- Expansion of basal layer can mimic classic HSIL

Histologic mimic of dVIN

- Classic HSIL with superimposed LSC
- p16/p53 helpful in the distinction

Vulvar Acanthosis with Altered Differentiation (VAAD)

- Often seen adjacent to verrucous carcinoma
- Verruciform architecture (variable) without invasion

VAAD

- Minimal nuclear atypia
- Parakeratosis
- Hypogranulosis
- Surface epithelial cell pallor
Clinically worrisome, discrete lesions in older women should raise suspicion for neoplasia.

Careful examination for subtle features is critical to trigger proper clinical management.

Practical pearl

Verruciform LSC
- Acanthosis, hyperkeratosis
- Hypergranulosis/prominent keratohyaline granules
- Lacks koilocytosis at surface

Verruciform LSC
- Lacks cytologic atypia
- Lacks abnormal keratinocyte maturation

Practical pearl

Consider the possibility of Candida or other infection in vulvar lesions with inflammation and parakeratosis/hyperkeratosis.

Less likely in clinically isolated, discrete raised lesions, but should do a PAS-d to exclude.

Practical pearl

Verruciform LSC can be diagnosed in the setting of a discrete verrucous lesion, with the understanding that these lesions may be associated with concurrent or subsequent invasive SCC.

This information should be included in the pathology report.
Example report

Verruciform lichen simplex chronicus, see COMMENT.

COMMENT: The lesion displays a verruciform growth pattern with mild basal epithelial atypia and a lichenoid inflammatory infiltrate, most in keeping with verruciform lichen simplex chronicus. In my opinion, the lesion does not meet criteria for differentiated VIN due to a lack of abnormal keratinization/maturation and insufficient basal epithelial atypia. In our experience, verruciform lichen simplex chronicus can be associated with concurrent or subsequent invasive squamous cell carcinoma. For this reason, if a discrete visible lesion is present, I would recommend conservative excision, if clinically feasible. If excision is not an option, then clinical surveillance with repeat biopsy is recommended.

Verruciform Xanthoma

Case 2: Differential Diagnosis

• VAAD or verrucous carcinoma?
• Verruciform dVIN?
• Verruciform hyperplasia?

Atypical verruciform lesion.

• Atypia insufficient for dx of dVIN
• Wild type p53

Case 2 Diagnosis:

The case
“Atypical verruciform lesions”
• Verruciform lesions not fitting cleanly into dVIN, VC, VAAD, or LSC

“Atypical verruciform lesions”
• Verruciform lesions not fitting cleanly into dVIN, VC, VAAD, or LSC
  – Verruciform “hyperplasia”?  
  – Are these lesions neoplastic or reactive in nature?

Molecular alterations in atypical verruciform lesions (AVL) of the vulva
• Targeted NGS on 11 AVL; compared to a cohort of HPV-negative KSCC
  – 2 patients had both atypical verruciform lesion(s) and KSCC.

Molecular alterations in KSCC and AVL

Molecular alterations in atypical verruciform lesions of the vulva
• The presence of known pathogenic mutations = neoplastic lesions rather than reactive in nature
• The lack of TP53 mutations suggests a unique biology, distinct from that of dVIN.
• The term, “differentiated exophytic vulvar intraepithelial lesion (DE-VIL)” has been proposed to reflect the morphologic features and to distinguish these lesions from TP53-driven/dVIN-related pathway

Watkins et al Modern Pathology 2017
Practical pearl

- The term “hyperplasia” should be avoided for clinically discrete verrucous lesions with evidence of abnormal keratinocyte differentiation and/or any degree of cytologic atypia.

How to report atypical verruciform lesions?

- Atypical verruciform lesion, see Comment.
- Differentiated exophytic vulvar intraepithelial lesion, see Comment.

Comment: This is a verruciform squamous lesion with acanthosis, marked hyperkeratosis, and parakeratosis and some abnormalities in keratinocyte differentiation. There is no invasive carcinoma and the degree of nuclear atypia in the basal layer is mild; thus this is not a differentiated VIN. However, in our experience these lesions can recur and I believe they confer an increased risk for developing squamous cell carcinoma. Complete excision and re-biopsy of any new or growing lesion is recommended as clinically appropriate.

Encountering a verruciform lesion in the vulva:

- Is there evidence of invasion?
  - Infiltrative growth, irregular epithelial-external stromal interface → consider invasive SCC
  - Non-infiltrative growth but invasion in the form of blunt downward growth → consider VC
- Is there conspicuous basal cytologic atypia?
  - No → consider diagnosis of VC, LSC, atypical verruciform lesion/DE-VIL
  - Yes → is this a differentiated VIN? Is p53 abnormal?
  - Yes → consider this classic HSIL/VIN with superimposed LSC? Is p16 positive (block pattern)?
- Is there koliocytic change/ mild surface atypia?
  - No → consider condyloma (low-risk HPV)
  - Yes → consider condyloma (low-risk HPV)
- Is there abnormal keratinization/maturation?
  - No → consider keratinizing SCC (~65%), verrucous carcinoma (~5%)
  - Yes → consider subtyping HPV16, PIK3CA driven (DE-VIL/atypical verruciform lesion associated)

Summary/Conclusions

- In addition to the canonical HPV-driven vulvar SCC pathogenesis, there are likely at least two additional pathways to developing SCC:
  - TP53 driven (dVIN associated)
  - PIK3CA driven (DE-VIL/atypical verruciform lesion associated)
- Precise subtyping of pre-invasive lesions can be difficult as classification is determined in large part by the presence/absence of (and degree of) cytologic atypia, a highly subjective measure
- All atypical verruciform lesions should be conservatively excised if clinically feasible and patients monitored for regrowth/new lesion.
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“Giant” Condyloma

- Defined as a large non-invasive exophytic lesion with minimal atypia
- Koilocytosis is variable
- Recurrences common
- Some are HPV6 positive
  A subset are HPV negative**

Practical pearl

- Clinical correlation can be very helpful!
  - Is the lesion solitary, or is it rash-like?
  - Has the patient been treated with anything?
  - History of psoriasis?
  - History of other vulvar lesions/neoplasia?

Differentiated VIN (dVIN)

- Hyperkeratosis
- Elongation +/- anastomosis of rete ridges
- Often in the background of LSA

Thank you for your attention!

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My boss
Well-differentiated verruciform lesions of the vulva:

- Invasive (keratinizing) SCC
- Verrucous carcinoma (VC)
- Verruciform differentiated VIN (dVIN)
- Verruciform acanthosis with altered differentiated (VAAD)
- Other atypical verruciform lesions/differentiated exophytic vulvar intraepithelial lesion (DE-VIL)
- Verruciform lichen simplex chronicus (LSC)
- ("Giant") Condyloma
- Verruciform Xanthoma
- Psoriasiform or pseudopapillomatous hyperplasia
- *exclude Candida, psoriasis

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1990 vulva bx: Normal vulvar skin.

1995 vulva bx: Epidermal hyperplasia with focal severe atypia (VIN III) and lichenoid inflammation.

1993 partial vulvectomy: Non-specific chronic dermal inflammation and squamous reactive changes with pseudoepitheliomatous reaction and HK. No evidence of residual atypia.

2007 perianal bx: Verruciform squamous papilloma with HK. No evidence of residual atypia.

2007 perianal excision: Low grade squamous neoplasm with villiform architecture and minimal cytologic atypia. This lesion lacks the atypia required for the diagnosis of either a condyloma or VIN. There is no stromal invasion. The prominent fine villous architecture favors the classification of this squamous proliferation as a low grade neoplasm. Careful follow-up advised.

2012 perianal bx: Verruciform squamous hyperplasia with superimposed LSC.

2008 vulvar bx: Condyloma, associated with LSC.

2010 (PRE-MTG SLIDE): vulvar and perianal bx/excision: verruciform DVIN.

2012 vulva excision: INVASIVE KSCC, with positive margins (tumor 3.2 cm; invasive 1 cm).

2012 vulva excision: VERRUCOUS CARCINOMA.

2013 perianal bx: Verruciform squamous epithelial proliferation.

2014 vulva excision: verruciform VIN with focal INVASIVE SCC.

2014 vulva excision: VERRUCOUS CARCINOMA.
Non-invasive “precursor” lesions

- Differentiated VIN (dVIN)
- Verruciform acanthosis with altered differentiated (VAAD)
- Other atypical verruciform lesions, NOS

Verruciform Xanthoma

- Rare benign tumor first described in oral cavity
- Uncommon in vulva
- Usually associated with other vulvar conditions (LSA)
- Yellowish verrucoid plaques

Differential Diagnosis

- Diagnostic terminology and histologic features
- Molecular pathogenesis and biologic potential

Molecular alterations in atypical verruciform lesions of the vulva

- All non-invasive lesions lacked TP53 mutations
- 73% of atypical verruciform lesions had activating PIK3CA mutations
- In one patient with multiple atypical verruciform lesions who also developed KSCC, a TP53 mutation was identified in the KSCC in addition to the identical PIK3CA mutation

Psoriasis

- When involves vulva, most commonly “plaque-type”
- Symmetrically distributed erythematous circumscribed plaques
- Auspitz sign – removal of plaque leads to punctate bleeding
  – Disruption of superficial capillaries

Watkins et al. Modern Pathology 2017
Psoriasis

- Absence of basal atypia
- Lack of abnormal keratinization
- Parakeratotic plaque with neutrophils/ intraepidermal pustules (Munro’s microabscesses)

Psoriasis

In a nutshell... Past diagnoses

- Squamous hyperplasia
- Condyloma
- DVIN
- LSA
- LSC
- VC
- KSCC

Differentiated VIN (dVIN)

- Prominent basal atypia:
  - Hyperchromasia
  - Nuclear enlargement
  - Prominent nucleoli
  - Atypical mitoses
- Dyskeratosis/abnormal/a abrupt keratinization
- Prominent intercellular bridges
- Expansion of basal layer can mimic classic VIN

Verruciform Vulvar Lesions

- Complex papillary HSL
- Verrucopapillary squamous carcinoma (warty carcinoma)
- Keratinizing SCC
- Verrucous carcinoma
- Verruciform LSC
- Verrucous acanthosis with altered differentiation
- Verruciform differentiated VIN
- Verruciform xanthoma
- Acantholytic hyperkeratosis
- Psoriasiform or pseudopapillomatous hyperplasia
- Inverted keratosis
- Condyloma/Papilloma/Sakit

Differential Diagnosis

- Verruciform lesions of the vulva:
  - Invasive (keratinizing) SCC
  - Verrucous carcinoma (VC)
  - Verruciform differentiated VIN (dVIN)
  - Verruciform acanthosis with altered differentiated (VAAD)
  - Verruciform LSC
  - (Giant) Condyloma
  - Other atypical verruciform lesions/NOS
Acanthotic or verruciform vulvar lesions lacking cytologic atypia of classic VIN/HSIL—make into flow chart

- 1- exclude classic VIN with superimposed LSC [careful morphologic examination +/- HPV]
- 2-exclude condy/HPV related changes (surface) [careful morphologic examination, +/- HPV (low risk) testing]
- 3-is there basal cytologic atypia? Sufficient for DVIN? P53 IHC can support dx of dvin, but only in correct morphologic context
- 4- is there abnormal keratinization/maturation?
- 5- is there hypergranulosis or is there hypogranulosis?
- 6-is there blunt/bulbous invasion (rule out verrucous CA) 

Aging Vulvar Epithelium

[Diagram]

Atypical verruciform lesion, NOS
- Wastebasket term applied to verruciform lesions not fitting cleanly into one of the aforementioned diagnostic categories, but generally have abnormal maturation and may have some subtle cytologic atypia
- Can be associated with concurrent SCC or represent increased risk for subsequent invasive SCC

Non-neoplastic vulvar lesions
- LSA- not a precursor lesion per se but does indicate increased risk of developing DVIN/SCC
  - “hypertrophic LSA”
  - “atypical LSA”
- LSC
- LSA with superimposed LSC
- Maybe combine this with the next/with pics?

Past diagnoses (have pics pop up)

Past diagnoses (have pics pop up)
Aging Vulvar Epithelium

- Mixed patterns
  - Younger age
  - Smoking
  - Immunosuppression
  - Inflammatory Background
    - Longstanding LSA/LSC
  - Older age
  - Classic/Usual HSIL
  - Differentiated VIN
  - Keratinizing SCC (~65%)
  - Verrucous Carcinoma (~5%)
  - Moderate/Poorly Differentiated SCC (~30%)

HPV-negative squamous lesions

- Malignant
  - Keratinizing SCC
  - Verrucous Carcinoma
- Premalignant
  - Differentiated VIN
  - "VAAD"
  - "Other"
    - LSC
    - LSA
    - Atypical verruciform squamous hyperplasia

Premalignant vulvar neoplasia

- Classic VIN/HSIL
- dVIN

Classic VIN with superimposed LSC

- High risk HPV+
- May mimic dVIN
- p16/p53 IHC can be useful!