

LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (LSIL)

Cytomorphology for Year 1-2 Registrars

Background

- LSIL includes the histologic entities of HPV effect as well as CIN1. The distinction is not accurate using cytology
- LSIL is a definite prediction of histologic LSIL whereas ASC-US means possible LSIL
- “Low-grade” includes both LSIL and ASC-US – both are managed in the same way

Overview of low-grade lesions in cytology samples

- Koilocytes are pathognomonic of HPV infection: the changes are so distinctive that one classic koilocyte is enough to make definite diagnosis of LSIL
- atypical parakeratotic cells are also a strong indicator of LSIL although are seen much less frequently than koilocytes in LBC samples
- LSIL can also be diagnosed on nuclear changes in low N:C ratio cells i.e. koilocytes or atypical parakeratotic cells are not always present
- ASC-US means possible LSIL: some features but not enough to be diagnostic of LSIL

LSIL: Koilocytes are diagnostic

- The term "koilocyte" is derived from the Greek "koiolos" meaning hollow or cavity, used to refer to the transparent balloon appearance of the cytoplasm.
- Koilocytes are squamous epithelial cells infected by HPV
- The perinuclear clear zone is due to cytoplasmic degeneration, a cytopathic effect of HPV. The sharp demarcation of the perinuclear zone is due to condensation of cytoplasmic fibrils peripheral to the area of degeneration.

Diagnostic koilocytes are intermediate or superficial squamous cells. Must have the characteristic cytoplasmic features and an abnormal nucleus.

(1) The **cytoplasm**

- is abundant (low N:C ratio)
- has a characteristic large perinuclear clearing with a sharp well-defined edge, and condensation of the more peripheral cytoplasm.
The appearance is distinctly different from the small soft-edged perinuclear halos often seen in reactive squamous cells.

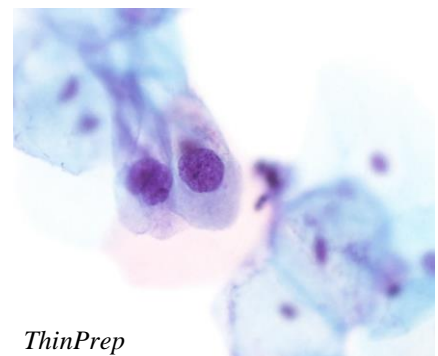
(2) The **nucleus** can be abnormal in a variety of ways. The degree of nuclear atypia varies.

- the cell may contain one, two or three nuclei which may be eccentrically located
- the chromatin is often poorly preserved, smudged and poorly defined or can be granular.
- the nuclear membrane can be irregular in outline, sometimes markedly so.

Cytomorphology of LSIL

- low N:C ratios, typically mature or superficial squamous cells.
- nucleus is enlarged, typically more than 3x the size of an intermediate cell nucleus.
- nuclei are hyperchromatic and the chromatin is granular.
- nuclear membranes usually irregular; quite marked angularity/cleaving may be observed.
- binucleation is common
- classic koilocytes may or may not be present

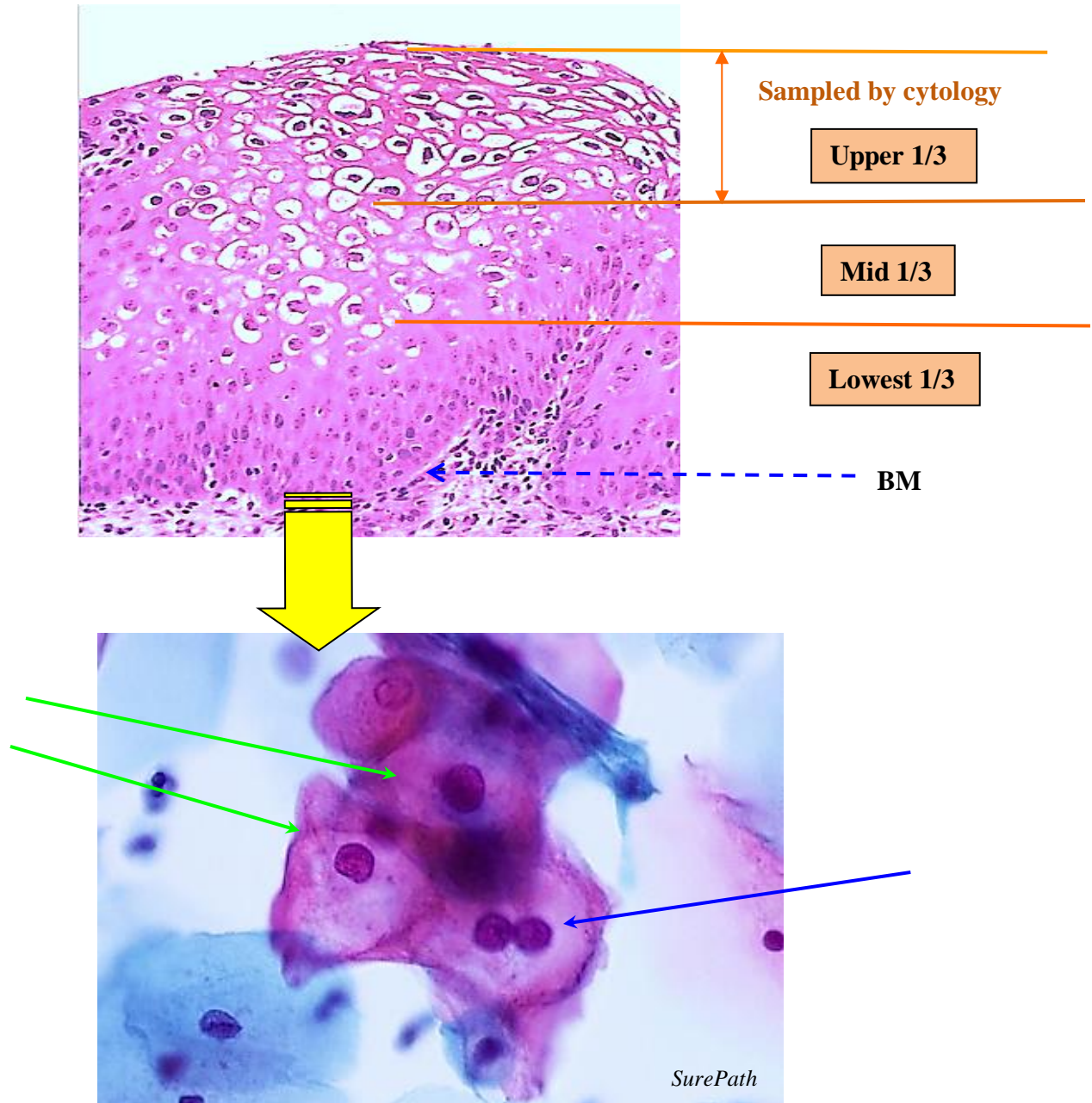
LSIL without koilocytosis



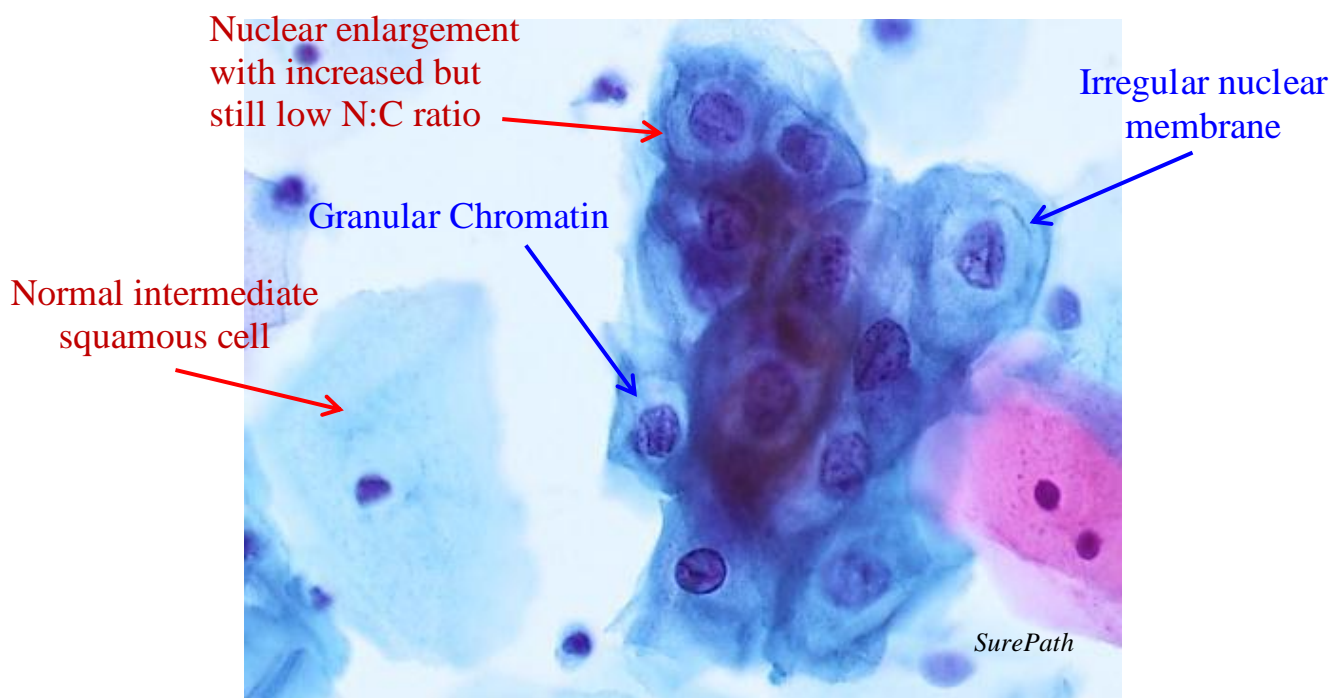
ThinPrep

Histologic evidence of HPV infection showing mature koilocytes at the epithelial surface. Cytoplasmic vacuolation is prominent and the nuclei are atypical showing hyperchromasia, size and shape variation, and occasionally binucleation.

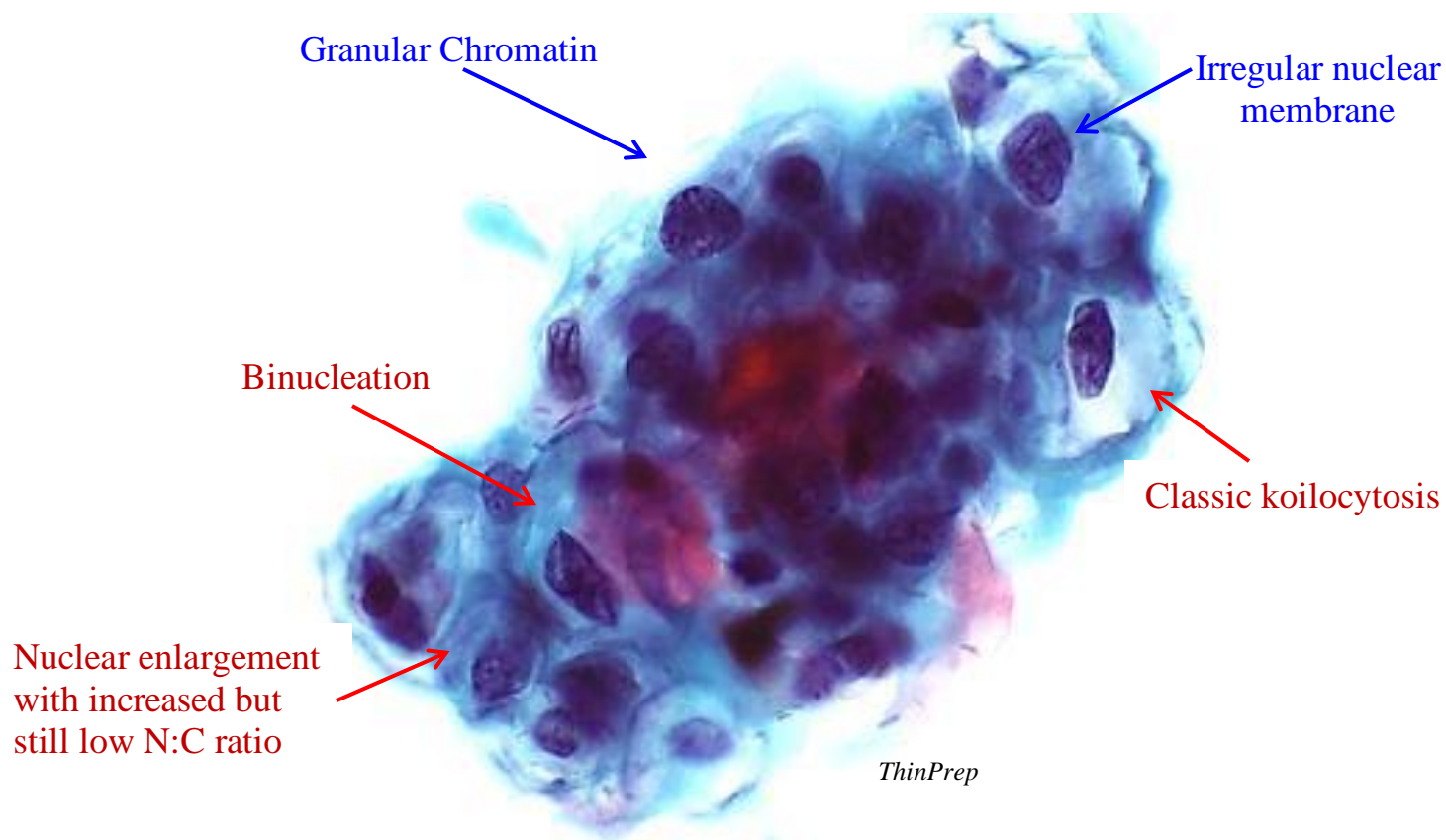
Koilocytosis is less evident and patchy in the more immature lower layers of the squamous epithelium and is not evident in the basal layer next to the basement membrane (BM).



Cytologic appearance of classic koilocytes showing a large cytoplasmic cavity that contains fragments of cytoplasm (bundles of tonofilaments). Note the well-defined outer rim of densely stained eosinophilic cytoplasm. Koilocytes are arrowed, with the blue arrow marking a binucleated koilocyte. The nuclei are atypical showing variable enlargement and hyperchromasia, some with a smudged appearance of the nuclear chromatin.



LSIL: CIN 1



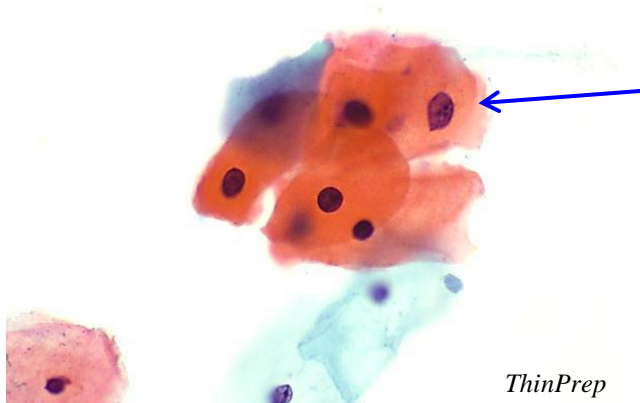
2. Atypical parakeratotic cells

- single cells are small, keratinised cells with an oval or spindle shape.
- groups form dense three-dimensional aggregates with several cell layers
- the cytoplasm is dense due to the presence of keratin and/or keratin precursors, usually staining an intense orange but can be cyanophilic (blue).
- the nuclei are usually condensed and pyknotic or hyperchromatic and may be irregular. Although small, the nuclei are larger than the nuclei of superficial cells. These are relatively small cells but the N:C ratio remains low with smudged chromatin. The nuclear atypia can vary from mild to marked with obvious changes of dysplasia.

Atypical parakeratotic cells are often present without koilocytes.

- Atypical parakeratotic cells are distinguished from normal parakeratotic cells by their atypical nuclei. As normal parakeratotic cells mature prior to exfoliation at the epithelial surface, the nucleus condenses and contracts, becoming smaller and darker. This is not true for atypical parakeratotic cells which may already be hyperchromatic. Large dark nuclei within a parakeratotic sheet are therefore a cause for concern, particularly if there are smaller nuclei within the sheet with lighter chromatin staining.
- Atypical parakeratotic cells can be seen with any grade of cervical intraepithelial neoplasia (CIN). Assessing the nuclear features is critical and the cytoplasm is often abundant so the N:C ratio is a poor predictor of CIN grade.

Cytology: Cluster of atypical parakeratotic cells with intense orange cytoplasm associated with koilocytes in adjacent squamous epithelium.



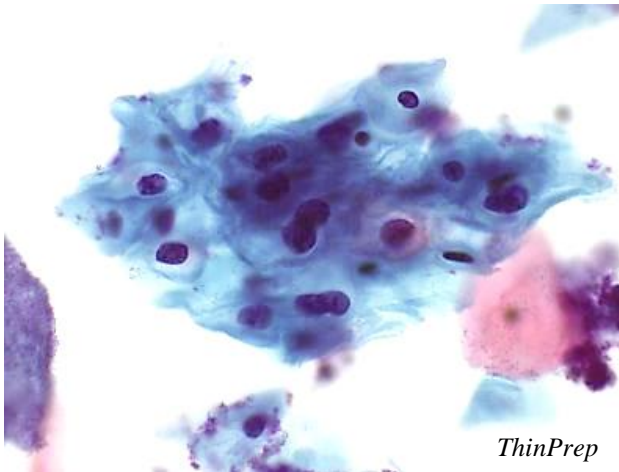
Single dissociated atypical parakeratotic cells in a case of LSIL. The cytoplasm is intensely orangeophilic because of the keratinisation. In comparison to normal parakeratotic cells, atypical parakeratotic cells have atypical nuclei.



A cluster of atypical parakeratotic cells where different focal planes illustrate a three-dimensional aggregate of atypical squamous cells. There is dense cyanophilic cytoplasm rather than the more common orangeophilia. This was reported as ASC-US.

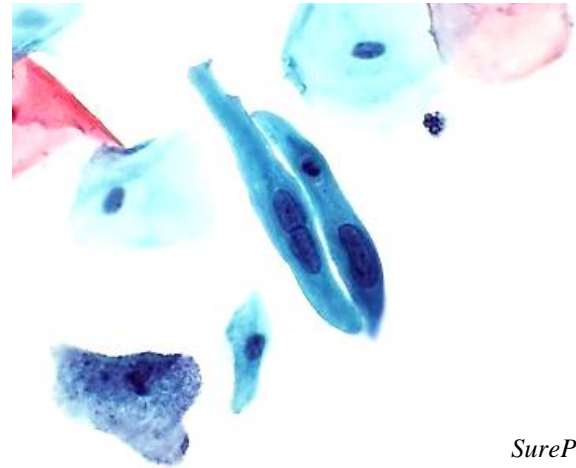
ATYPICAL SQAUMOUS CELLS OF UNDETERMINED SIGNIFICANCE (ASC-US)

- atypical cells with low N:C ratios but where the changes are not enough for definite LSIL
- Learning what is “enough atypia” to call a case definite LSIL rather than ASC-US is a skill acquired with experience. Experience is also required to determine what is enough atypia to justify calling ASC-US rather than negative/reactive. This is more critical because the distinction may result in more different patient management.



ThinPrep

ASC-US: Dark enlarged nuclei without nuclear membrane irregularity and small/moderate perinuclear halos are present. Follow-up cytology was negative.



SurePath

ASC-US: Elongated cells show nuclear enlargement and binucleation with granular chromatin but no nuclear membrane irregularity or perinuclear clearing.

DIAGNOSTIC DIFFICULTIES WITH LOWGRADE LESIONS

1. Cytologic mimics of HPV

The following are features and situations that may lead to a false positive diagnosis of HPV

- cytoplasmic polychromasia and mild perinuclear clearing associated with other infections such as candida or trichomonas.
- nuclear enlargement and multinucleation after radiation therapy or more rarely, with folic acid or vitamin B12 deficiency.
- heavily glycogenated squamous cells with a pseudokoilocytic appearance particularly when the glycogen has been washed out of the cell during processing.
- parakeratosis associated with other inflammatory/reactive changes causing confusion with atypical parakeratotic cells.
- cell degeneration resulting in nuclear pyknosis and large cytoplasmic vacuoles/cavities.
- mature keratinised cells associated with high-grade or invasive keratinising SCC

2. Florid HPV effect

- over-diagnosis can occur when HPV effect is pronounced, as may occur with acute HPV infection
- florid HPV effect can cause overdiagnoses, particularly keratinising high-grade squamous lesions or can result in a prediction of a higher grade of CIN than is confirmed

histologically (although sometimes this may occur because the acute infection has resolved by the time the person is seen at colposcopy).

3. Differentiating atypical parakeratosis due to HPV from HSIL/SCC

- HPV-affected cells have dark pyknotic nuclei with smudged chromatin whereas the chromatin in high-grade squamous lesions is usually crisp and granular.
- atypical parakeratotic cells are often small in appearance and particularly on low magnification, may give an impression of a high N:C ratio. High magnification examination will show that the nucleus usually occupies no more than half of the cell. This criterion also applies to spindle-shaped cells
- Well-preserved, sharp angles in the nuclear membrane contour are suspicious of a high-grade lesion.
- Atypical parakeratotic cells often have polygonal-shaped cell outlines whereas high-grade cells have a more rounded cell outline.
- Tumour diathesis is seen in invasive carcinoma but is not a feature of HPV effect.

Where there is diagnostic uncertainty and suspicion of SCC, this must be reported and investigated urgently at colposcopy. Suspicion of HSIL can be reported as Atypical Squamous Cells, cannot exclude HSIL (ASC-H).

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