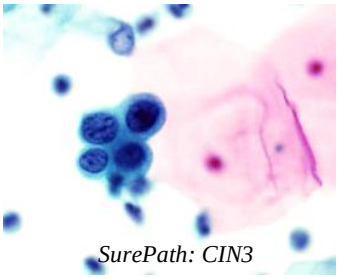
NCPTS
National Cervical Pathology Training Service

HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL)
Cytomorphology for year 1-2 Registrars

DEFINITION
- HSIL is a true pre-cancerous change in squamous cells occurring as a result of HPV infection
- HSIL encompasses histologic CIN2 (moderate dysplasia) and CIN3/CIS (severe dysplasia, carcinoma in situ) and is reported as HSIL under The Bethesda System for reporting cervical cytology. Laboratories may further sub-classify cytology samples as predictive of CIN2 or CIN3 in cytology reports if they wish but the distinction is not held on the NCSP-Register.

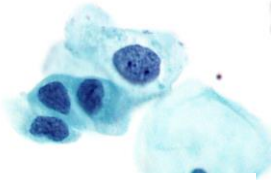
OVERVIEW
- multiple abnormal criteria must be present in nuclei and in cell groups to diagnose HSIL as different features are seen in different cases and no single feature is diagnostic or invariably present
- **unpredictable variation** within the abnormal cell population is more reliable than the presence of any single abnormal criterion alone
- different appearances have no clinical relevance and often co-exist but identifying these differences assists with diagnostic criteria and differential diagnoses.

HSIL: CIN3
- key features: cells with markedly increased N:C ratios (>65%) and abnormal variable nuclei. Usually small cells but this is can vary.
- cells can be single, clustered, in crowded groups or in syncytia
- **nuclear variability is central to the diagnosis**: size varies, nuclear membranes are often irregular with indentations, hyperchromasia is usually seen – chromatin is variably fine or coarsely granular and is evenly distributed within the nucleus
- nucleoli are uncommon but can occur
- the cytoplasm can be squamoid, delicate, densely metaplastic or keratinised



SurePath: CIN3

HSIL: CIN2
- shows similar nuclear features to CIN3 but N:C ratios are lower (50-65% of the cell occupied is by the nucleus)
- When abnormal HSIL cells are keratinised, the distinction between CIN2 and CIN3 is not reliable



SurePath: CIN2

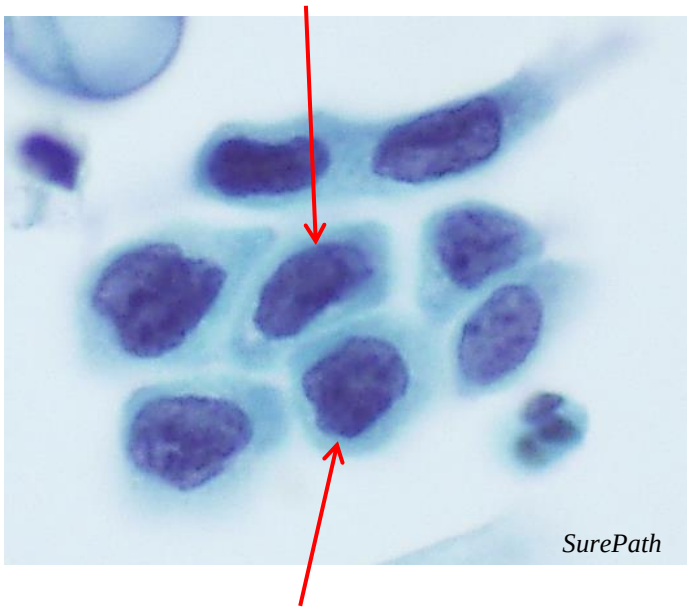
HSIL: Cytomorphology predictive of CIN3.

Cells can be single, clustered, in crowded groups or in syncytia (sheets). The loss of cell cohesion which occurs in neoplasia usually results in the presence of at least some single HSIL cells, but this is not always the case and crowded sheets of HSIL only may be present.

Nuclear variability is central to the diagnosis of HSIL. Within the abnormal cell population in any one sample, nuclei may vary in size, shape, chromatin pattern and/or nuclear membrane characteristics. Not all features will be present in every case. Comparison of different cells and cell groups is essential to confirm that nuclear variability is present.

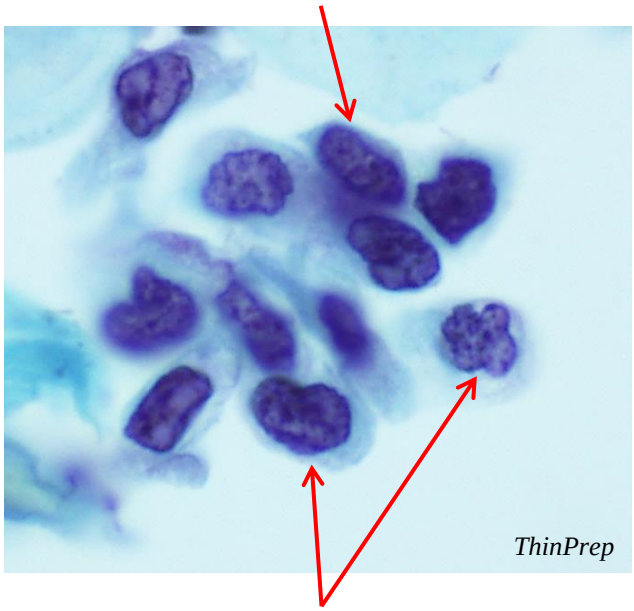
- There is a marked increase in N:C ratio with the nucleus occupying >65% of the diameter of the cell.
- Nuclei are usually hyperchromatic.
- The nuclear membranes are usually thickened. Irregularity or angularity of the nuclear membrane may be marked or subtle, particularly in metaplastic cells.
- The chromatin is finely or coarsely granular and evenly dispersed within the nucleus.
- Macronucleoli are not a feature, but small nucleoli may be observed (2-5% of cases).
- The cytoplasm can be squamoid, delicate, densely metaplastic or keratinised.

Granular evenly distributed chromatin



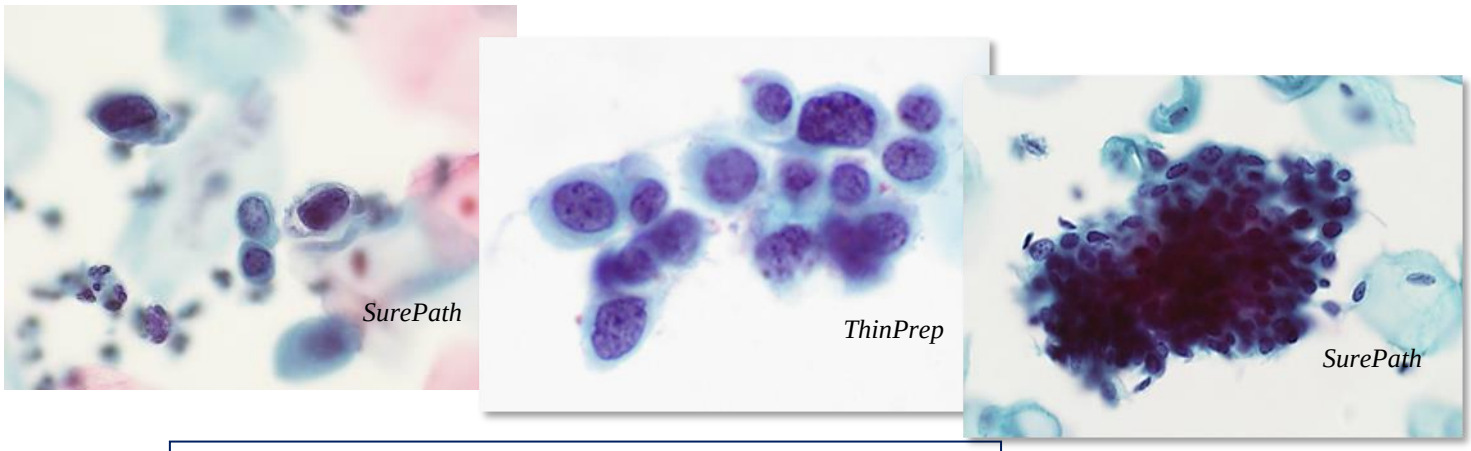
Irregular nuclear membranes

Very high N:C ratios



Variation in nuclear size, shape and chromatin pattern

High-grade squamous intraepithelial lesions consistent with CIN 3



HSIL can present as single cells, in small clusters or in large thick groups

PATTERNS OF PRESENTATION OF CIN3

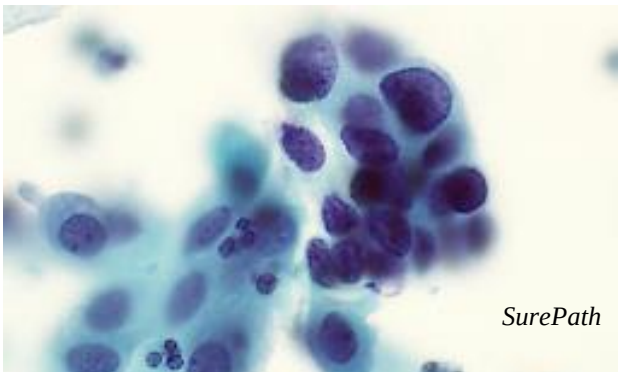
Ron Bowditch (Australian cytoscientist) produced some ground-breaking work during the 1990's identifying the different ways that HSIL presents in cytology samples. He is particularly acknowledged for his work defining the diagnosis of HSIL in crowded sheets. His approach to the diagnosis of CIN3 emphasises the three main ways that HSIL presents in cytology samples:

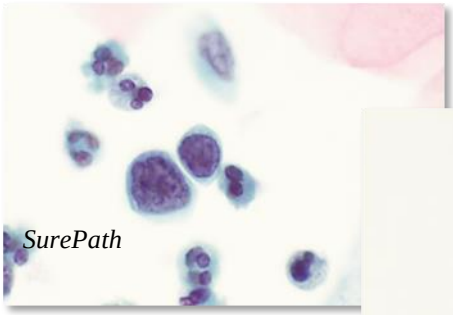
1. Metaplastic CIN3
2. Crowded sheets of CIN3
3. Parakeratotic CIN3

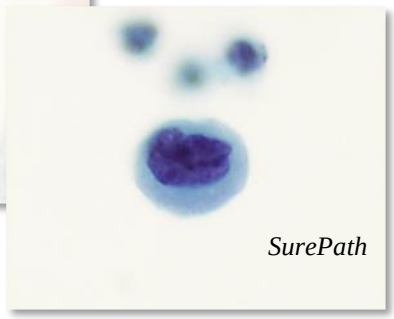
1. Metaplastic CIN3

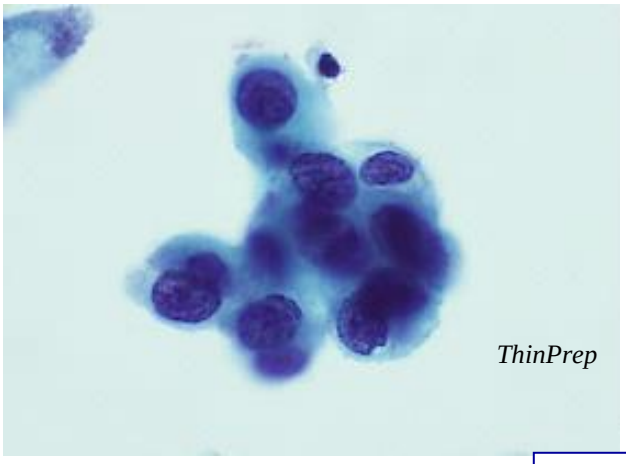
HSIL presents as single cells and small groups with cytoplasmic features resembling the cells of immature squamous metaplasia.

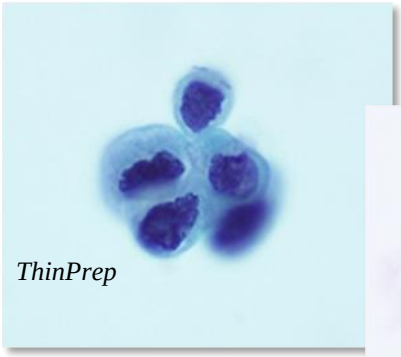
- cells often single or in small groups of 2-5 cells, often dispersed across the slide and therefore individually inconspicuous. Cell comparison is difficult particularly when the total number of abnormal cells present is small, because the cells don't lie together.
- Nuclear variation is key:
  - vary in size, chromatin, structure, chromasia, border thickness and shape
  - Abnormal nuclear shapes are usually present.
  - Cytoplasmic density and texture varies from fine and delicate to thick and squamoid, reflecting the transformation zone origin. May see keratohyaline granules.

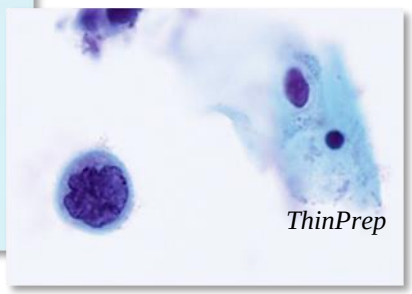










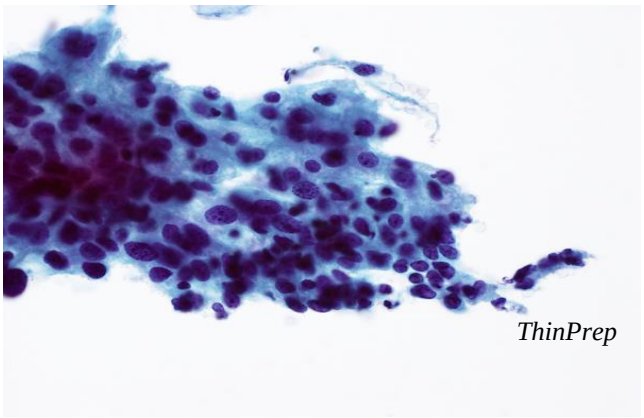


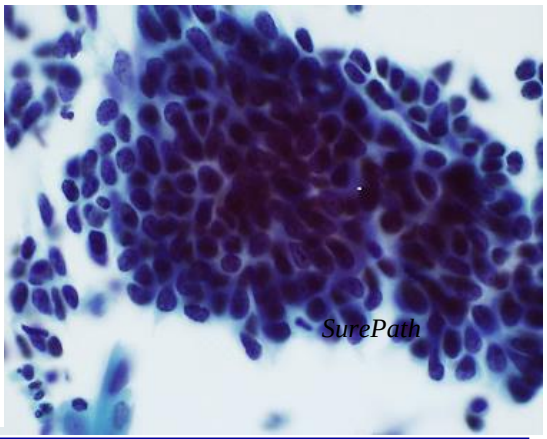
HSIL: Metaplastic CIN 3

2. Crowded Sheets of HSIL

HSIL may present in crowded sheets, often referred to as hyperchromatic crowded sheets/groups (HCG's).

- Sheets or tissue fragments are removed by brushing or scraping the lesion when the cytology sample is taken, so these fragments are in effect, microbiopsies.
- High power examination is essential for all crowded groups especially those that are hyperchromatic with overlapping nuclei.
- There may not be any single HSIL cells in the sample.





CIN3 presenting in hyperchromatic crowded groups. Focusing through the group is essential to assess the morphology.

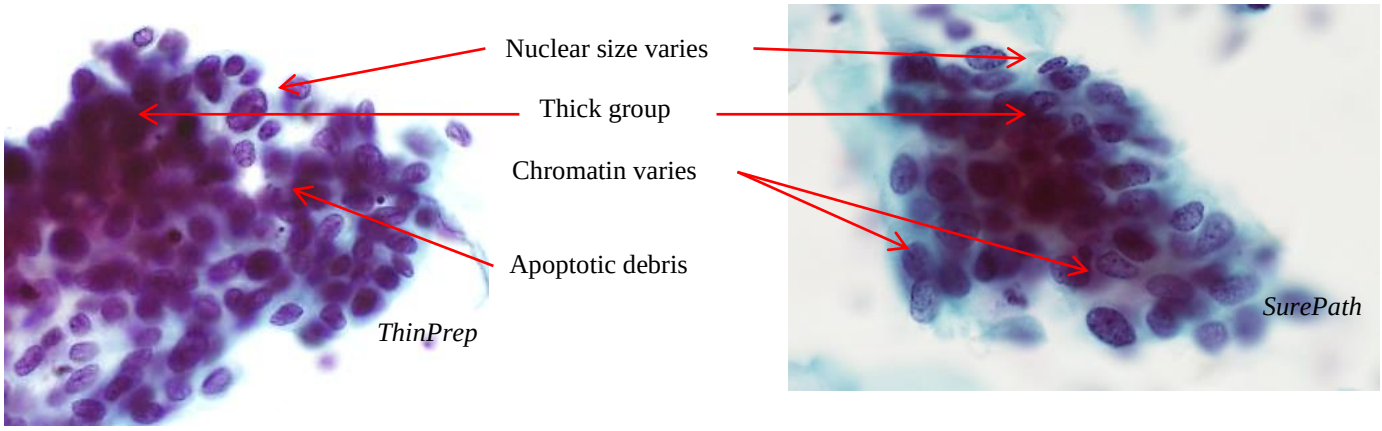
- Crowded sheets are easy to detect at screening as they contain 20 to thousands of nuclei. Nuclei are very crowded and overlapped. Sheets are usually at least 4 nuclei thick in HSIL. To assess how thick a group is, carefully focus through the group using high magnification and count through each plane of focus to determine the depth of the sheet.

- Every crowded cell sheet or thick cell group should be assessed as potentially high-grade. Individual cells can often be seen best at the edge of cell sheets, but much valuable information can also be obtained by focusing up and down through sheets.

The key cell parameters within cell groups that should be carefully examined to ascertain if there is a **loss of predictability from cell to cell associated with neoplasia** are:

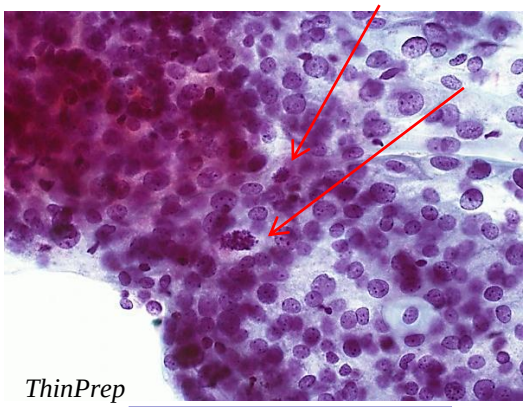
- Cell-to-cell spacing and polarity
- Nuclear chromasia
- Nuclear structure
- Nuclear membranes
- Nuclear size
- Nucleolar variation and enlargement
- Embedded mitoses i.e. mitoses in the middle layers of the group as focus through it
- Apoptosis

The thickness of the sheet is also a very useful parameter to assess (4 or more cell layers is high-risk) and should be combined with the features above. A consistent approach is essential to minimise false negative samples.



HSIL in hyperchromatic crowded groups

- Mitoses are very significant, especially if embedded in a sheet. Assess by focusing up and down through the sheet to determine if the mitosis is in the middle.

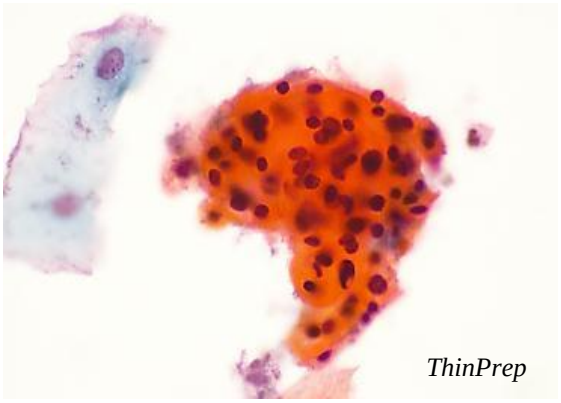


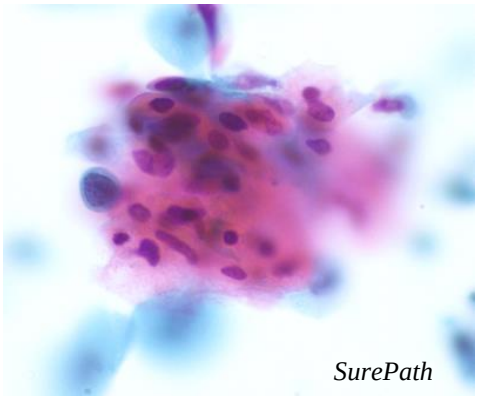
Mitoses in a sheet of HSIL

3. Parakeratotic CIN 3

- Maturation at the surface of CIN2 or CIN3 can result in shedding of a variety of small mature and keratinised squamous cells
- are small cells but have relatively more cytoplasm than is usual with HSIL.
- Nuclei may be small, condensed or pyknotic: parakeratosis or miniature squamous cells are suspicious of HSIL if the nucleus is retained. Look for nuclear variability. Nuclear size variation may be the only abnormal nuclear feature, especially if pyknotic.
- A smaller nucleus will be darker than a larger nucleus in the process of normal nuclear pyknosis, so larger darker nuclei are a cause for concern.
- HSIL is often missed when this is the dominant pattern of HSIL

Remember: Parakeratosis and hyperkeratosis may occur in every degree of abnormality in the squamous spectrum, from minimal reactive atypia to HPV infection through to squamous cell carcinoma. **Any parakeratotic change must be scrutinised with great care.**



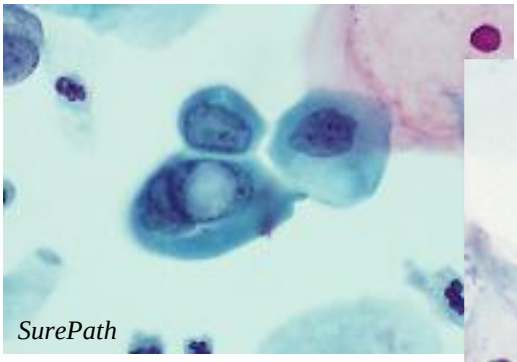


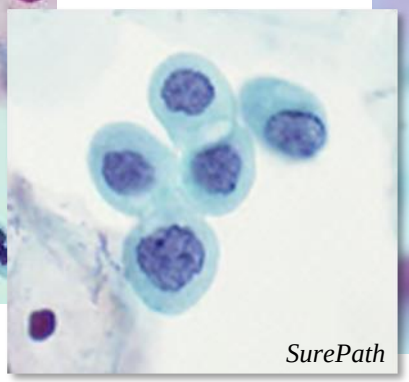
Parakeratotic HSIL: The keratinised cytoplasm may be more abundant than is usually seen in HSIL. Look for nuclei that are larger and darker than other nuclei in the group.

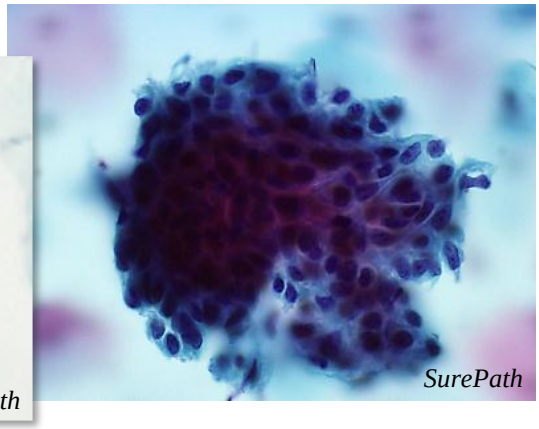
HSIL: Cytomorphology predictive of CIN2.

Increased N:C ratio but less so than with CIN 3. The nucleus occupies 50-65% of the cell.
The nuclear features otherwise show the same features as CIN3.

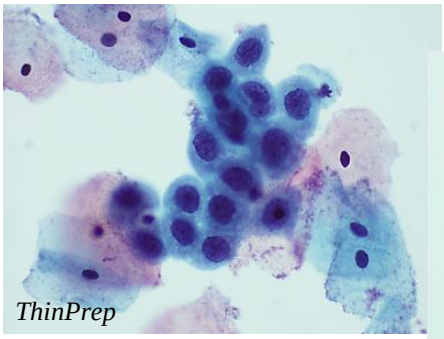
- CIN 2 often accompanies CIN1 so a careful search for HSIL cells is always warranted
- When the abnormal cells are keratinised, the distinction between CIN2 and CIN3 is not reliable.

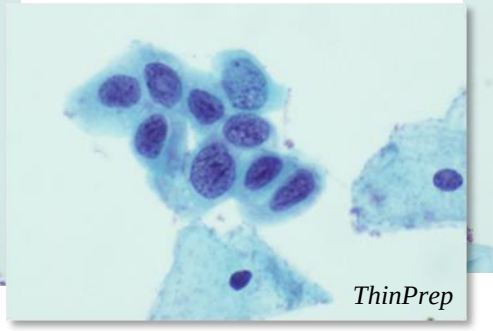


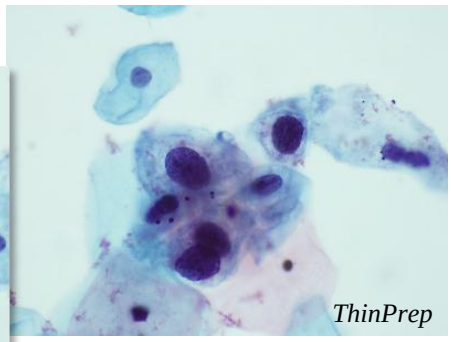




HSIL: CIN2







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