

Cervical smear preparations of postmenopausal women: Application of p16 immunostaining

Senile (atrophic) colpitis is microscopically characterized by the predominance of parabasal squamous cells and the paucity of superficial cells. The activated parabasal cells or dyskeratotic superficial cells may be confused with squamous intraepithelial lesion (SIL) in the routine cytology practice. Immunostaining for p16-INK4a can be performed after cell transfer to silane-coated glass slides. Only one tenth of the cytology specimens judged as HSIL cytologically revealed p16 positivity in clustered atypical parabasal cells. The overcytodiagnosis is avoidable with the aid of p16 immunostaining.

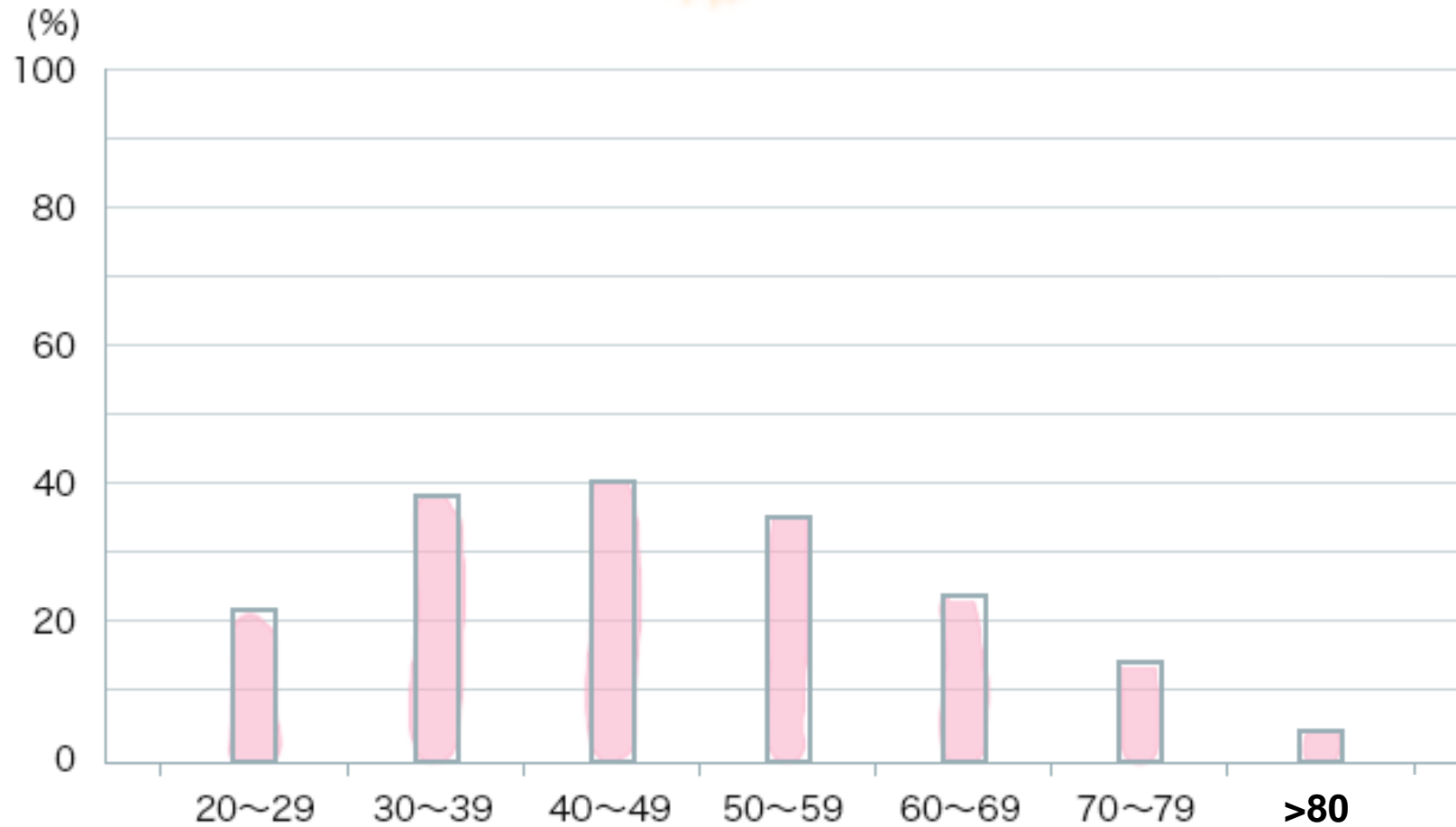
Ref. Tsutsumi Y, et al. p16 immunostaining can avoid overdiagnosis in postmenopausal cervical cytology. *Int Res Med Med Sci* 2021; 9(1): 1-8.
doi: 10.30918/IRJMMS.91.20.056

Cytology evaluation and HPV-DNA analysis in Japan

- 1) When the cytodiagnosis is categorized as ASC-US, HPV-DNA analysis is strongly recommended in Japan.
- 2) When the cytodiagnosis is ASC-H, biopsy will be performed. When the histodiagnosis is CIN1/CIN2, HPV-DNA analysis is strongly recommended in Japan.

- **Cytological diagnosis of ASC-US indicates a message for the need of HPV-DNA analysis.**
- **In post-menopausal patients, parabasal cells often have enlarged nuclei due to secondary irritation. The judgment of ASC-US should be avoided as much as possible.**

In Japan, 1/4 - 1/3 of gynecological cytology specimens are taken from postmenopausal women.



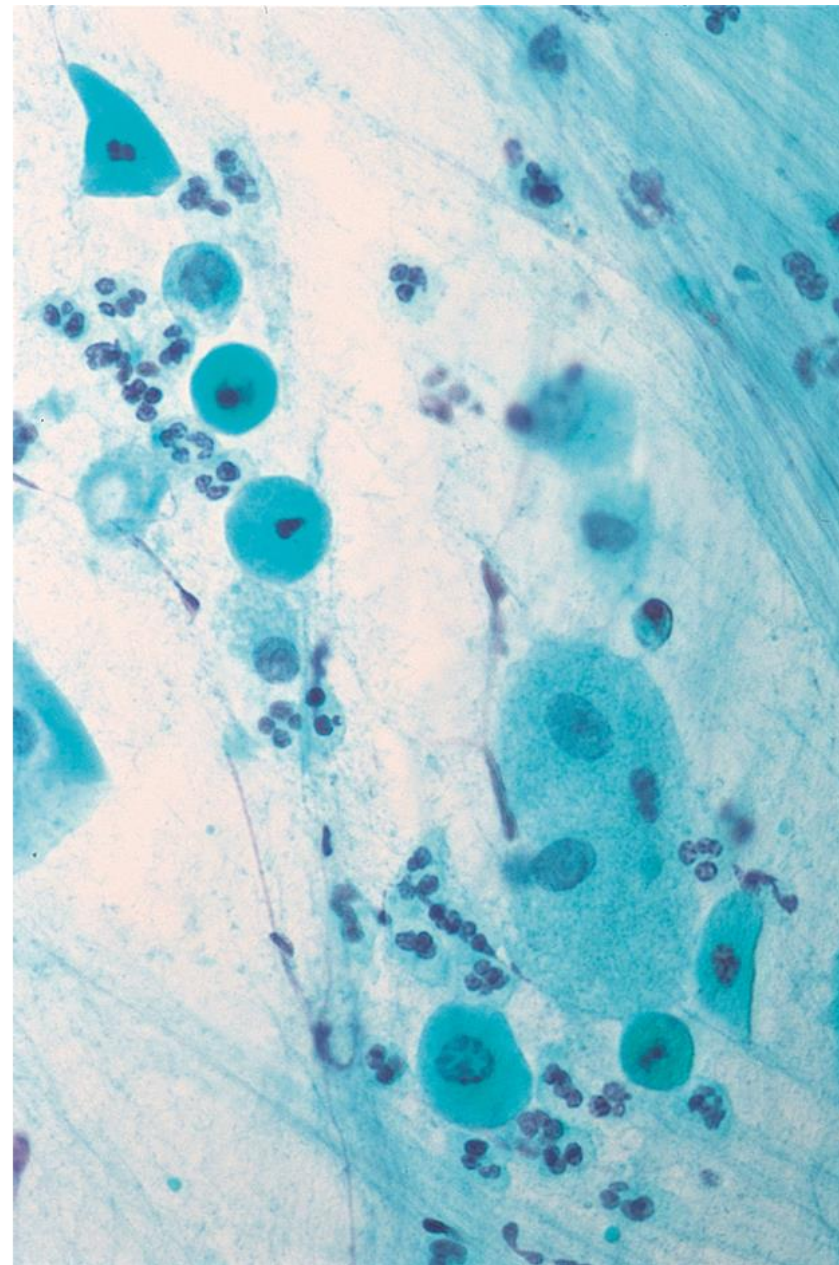
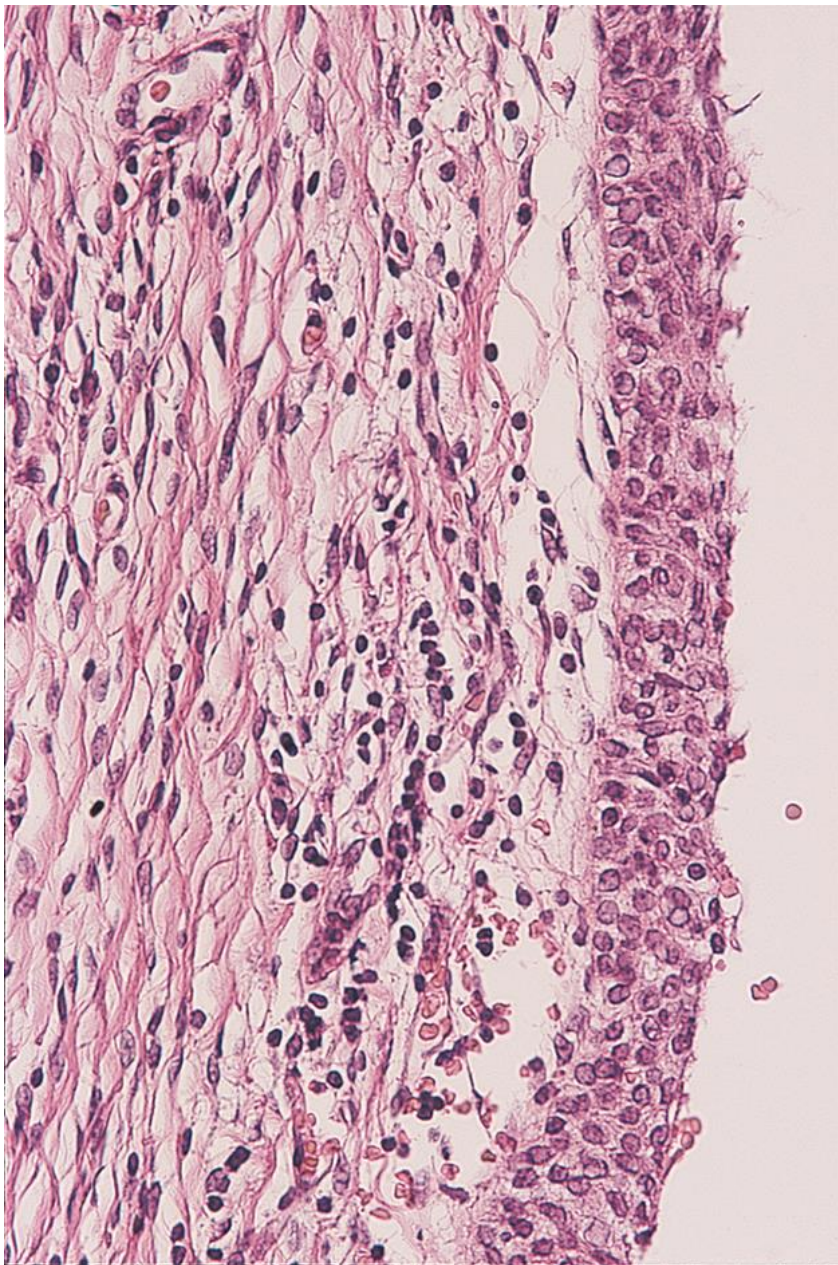
Rates of gynecological check-up by age group (from HP of the Japan Medical Association)

Senile (atrophic) colpitis

In the post-menopausal state, serum estrogen levels are decreased to provoke atrophy of the vaginal squamous mucosa and secondary bacterial infection. The senile (atrophic) colpitis is featured, as follows.

- 1) Superficial squamous cells disappear.
- 2) Parabasal squamous cells become the main epithelial component.
- 3) Pyknosis is often seen in the remaining superficial-type cells.
- 4) No Döderlein bacilli or *Gardnerella vaginalis* are seen.
- 5) Secondary infection of *E. coli* or *Pseudomonas aeruginosa* provokes neutrophilic infiltration.

→ **The diagnostic criteria in post-menopausal state should not be the same as those in the pre-menopausal state. Do not easily use ASC for the post-menopausal specimens.**



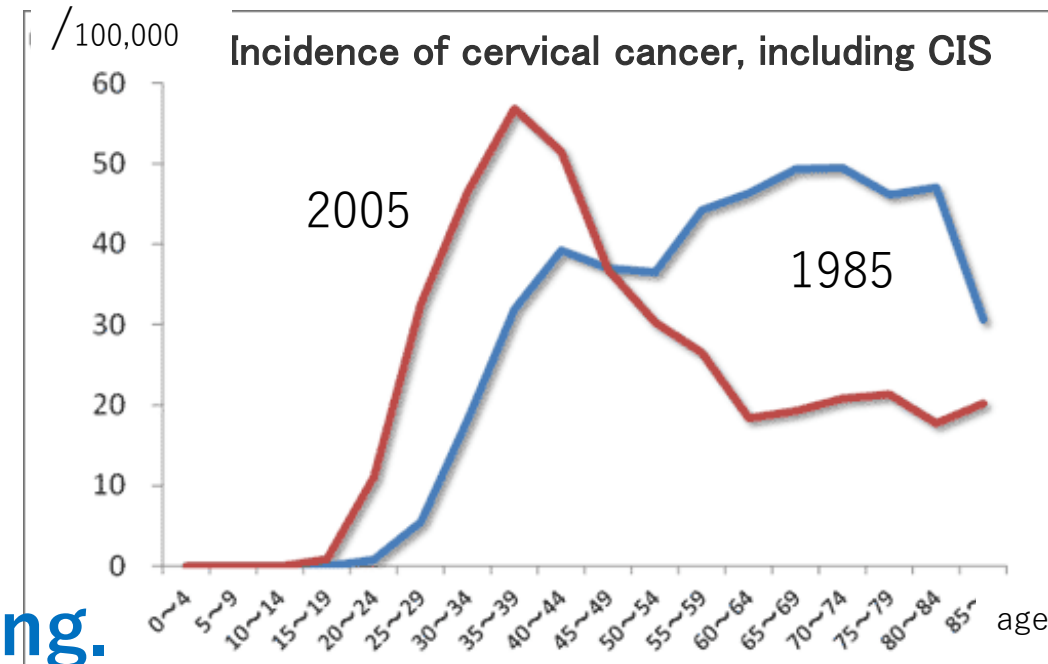
Histology (left) and cytology (right) of senile (atrophic) colpitis.

Cytodiagnosis of senile (atrophic) colpitis

- 1) Parabasal-type cells in post-menopausal specimens often show nuclear enlargement due to inflammation or vaginal dryness.
- 2) Clusters of parabasal-type cells are often obtained.
- 3) The change is physiological and reactive in nature, so that the diagnosis should be NILM theoretically. However, the nuclear change tends to be judged as ASC, in order to exclude the possibility of dysplasia.
- 4) The diagnosis of ASC-US should be avoided as much as possible, because ASC-US suggests the need for HPV-DNA analysis.

→ Squamous cell carcinoma may occur in post-menopausal women.

→ This makes a dilemma in cytoscreening.



When the superficial squamous cells are predominant in cytological specimens from post-menopausal women,

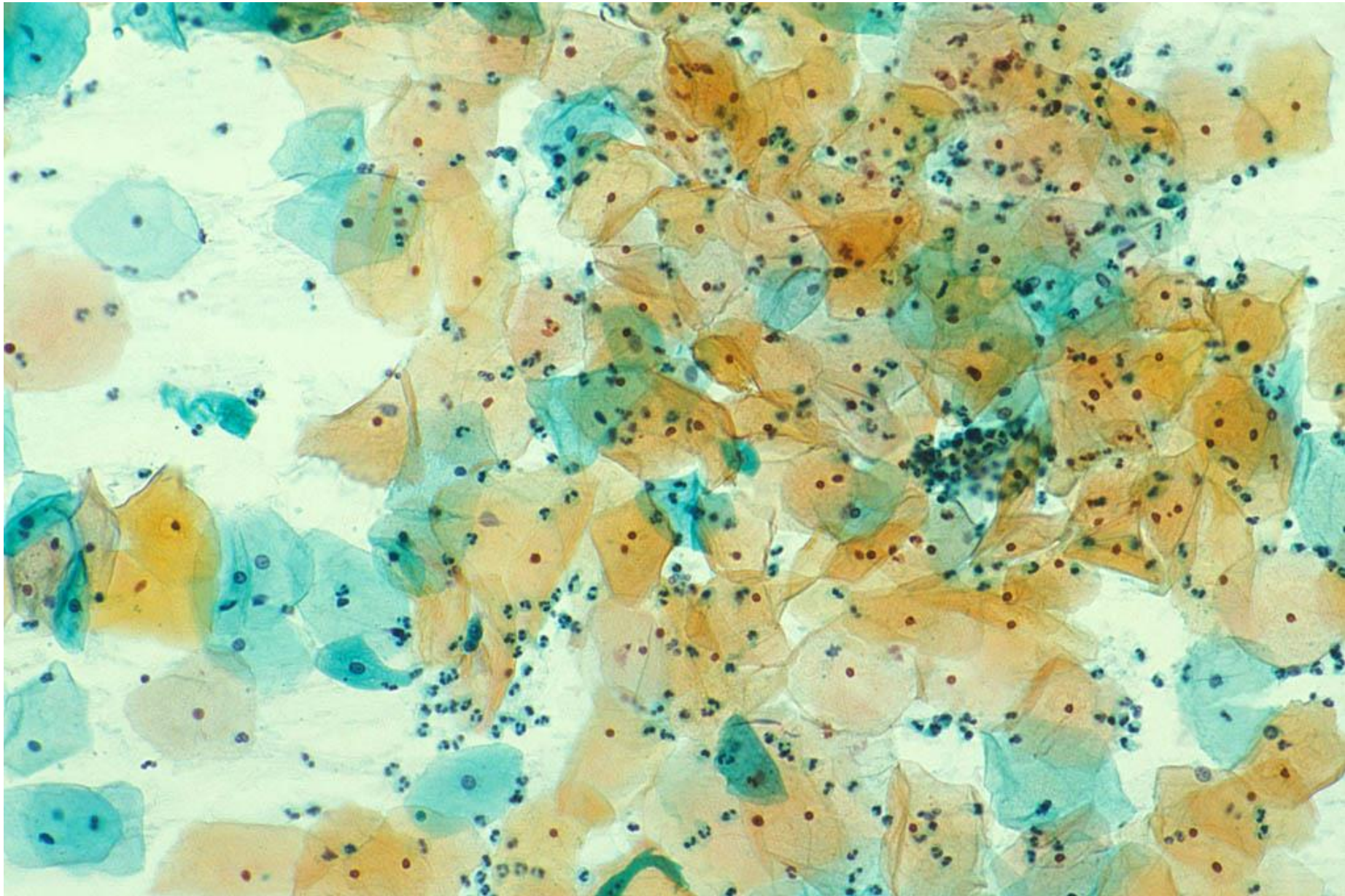
The possibility of the use of hormonal drugs or herbal medicine containing phytoestrogen activity should be considered. Infrequently, estrogen-producing ovarian tumor may be identified.

→ **Superficial squamous cell predominance should be reported as an abnormal finding.**

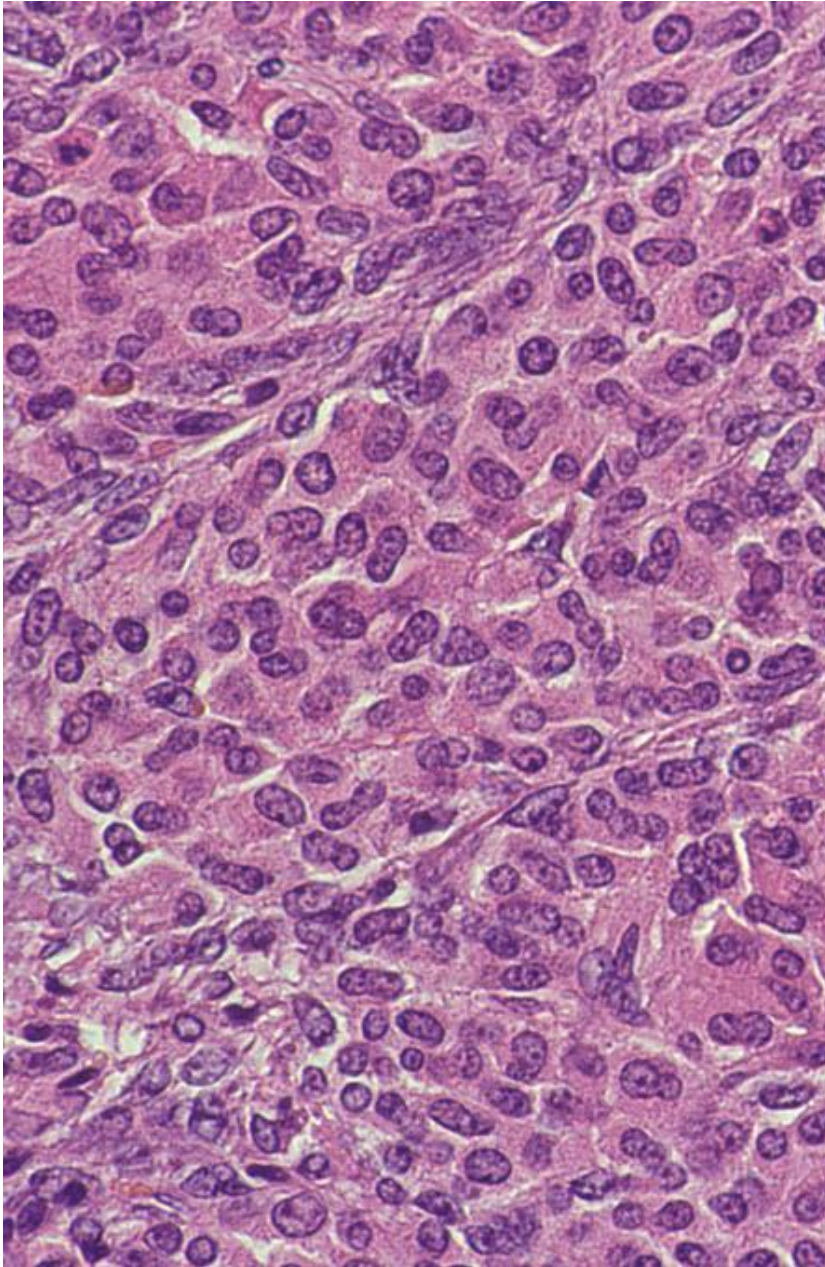
A 70 y-o lady

She visited a hospital because of metrorrhagia. Cervical smear contained numbers of superficial-type cells.

There is no history of taking hormonal medicine. The left ovary contained 10 mm-sized thecoma. She had been proud of her young and glowing skin, but the surgery deprived her daily happiness.



Cervical smear from a 70-year-old female patient (Pap). Note young-looking cells under the microscope.



The facts caused by the ovarian thecoma

- 1) Young and glowing skin of her secret of youth
- 2) Occurrence of metrorrhagia
- 3) No risk for her life because of benignity of the tumor
- 4) The diagnosis of thecoma confirmed after surgical removal.

Uncertain fact

- 1) Hyperestrogenemia will increase the risk of cancer of the breast and endometrium.

Functioning ovarian thecoma secreting estrogen (H&E). This tumor was the secret of youth in this 70-y-o lady.

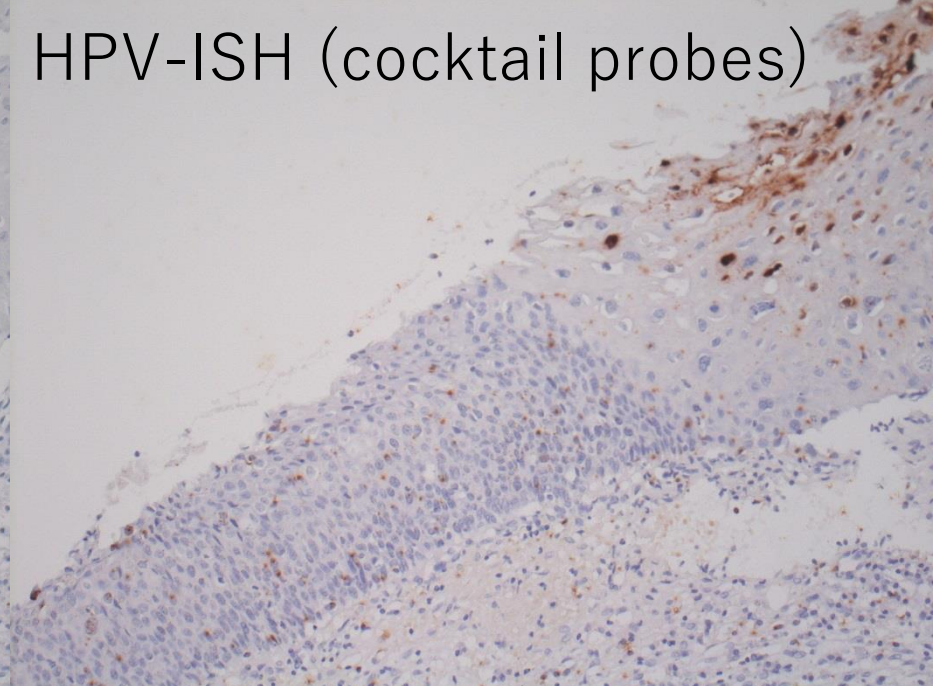
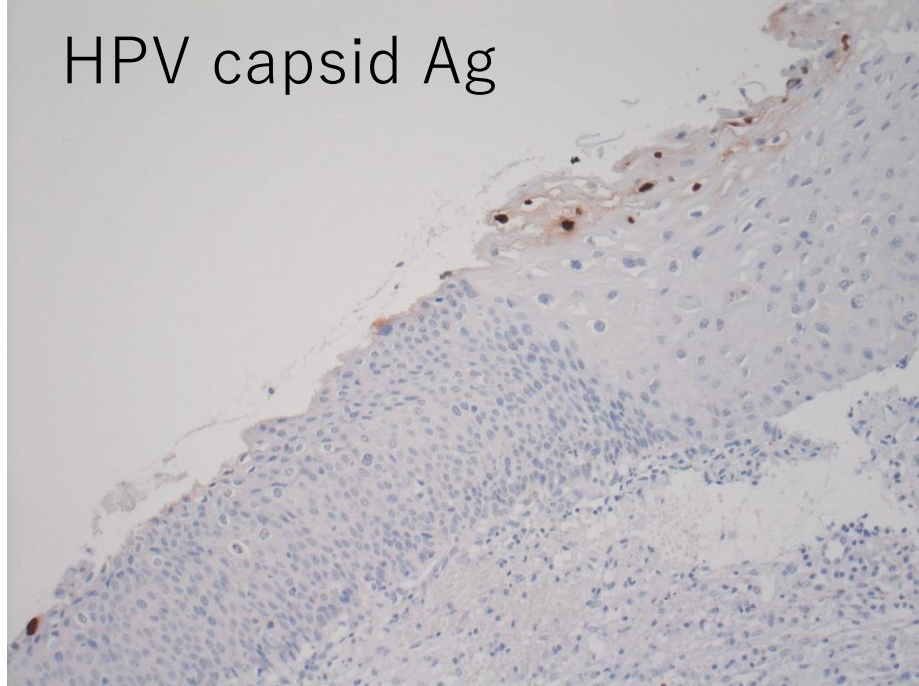
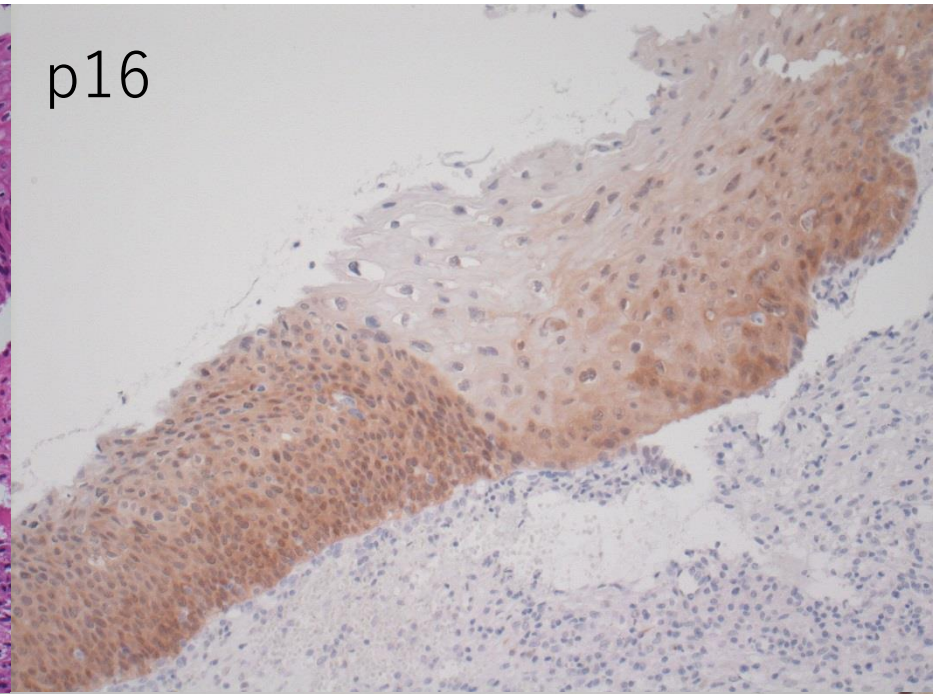
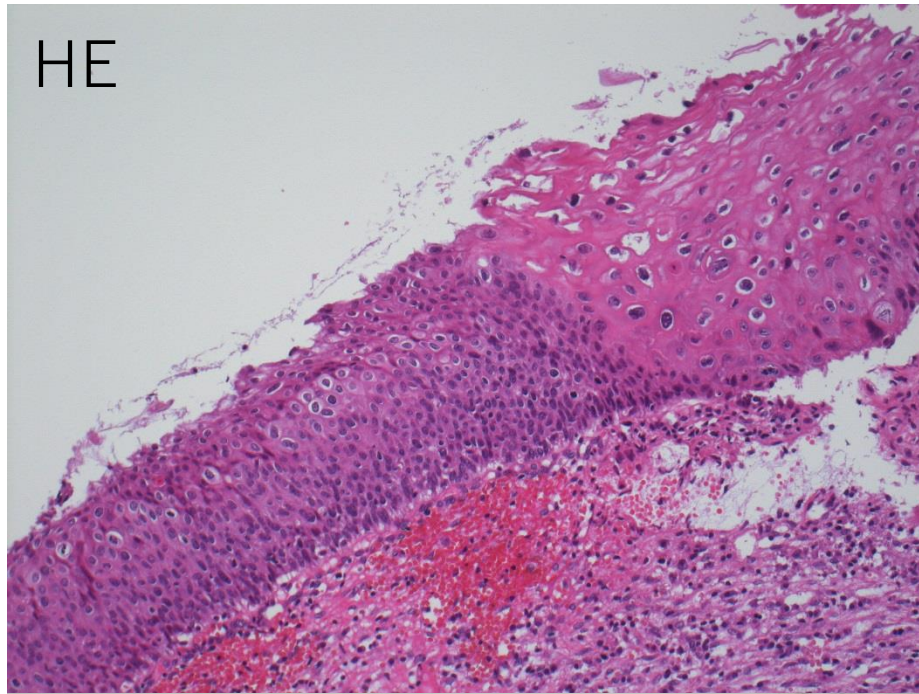
Differential diagnosis between senile colpitis and dysplasia: Application of p16 immunostaining

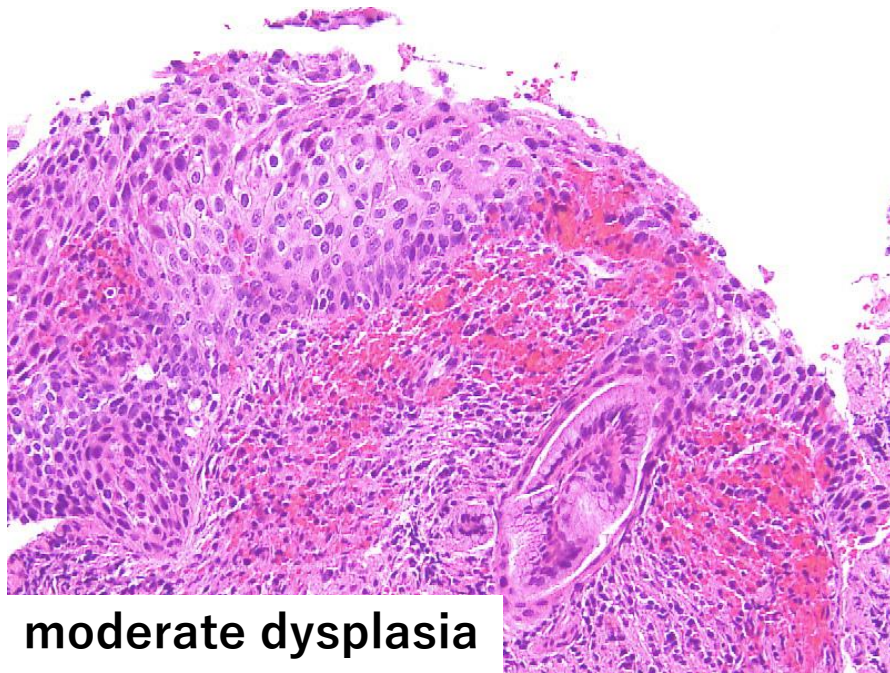
In infection of carcinogenic HPV, the lesion is consistently immunoreactive for p16-INK4a (p16), while senile colpitis is negative for p16. For immunostaining, cell transfer technique should be utilized in case of routine cytology specimens. In case of liquid-based cytology specimens, additional immunostaining can easily be performed.

p16-INK4a: Carcinogenic HPVs contain oncogene E7, inactivating Rb gene in the infected cell. In lesions with carcinogenic HPV infection (dysplasia and carcinoma), the infected cells grow with highly expressed p16 protein. p16 overexpression means the state with high expression of E7. In the uterine cervix, immunohistochemical overexpression of p16 indicates infection of carcinogenic HPV.

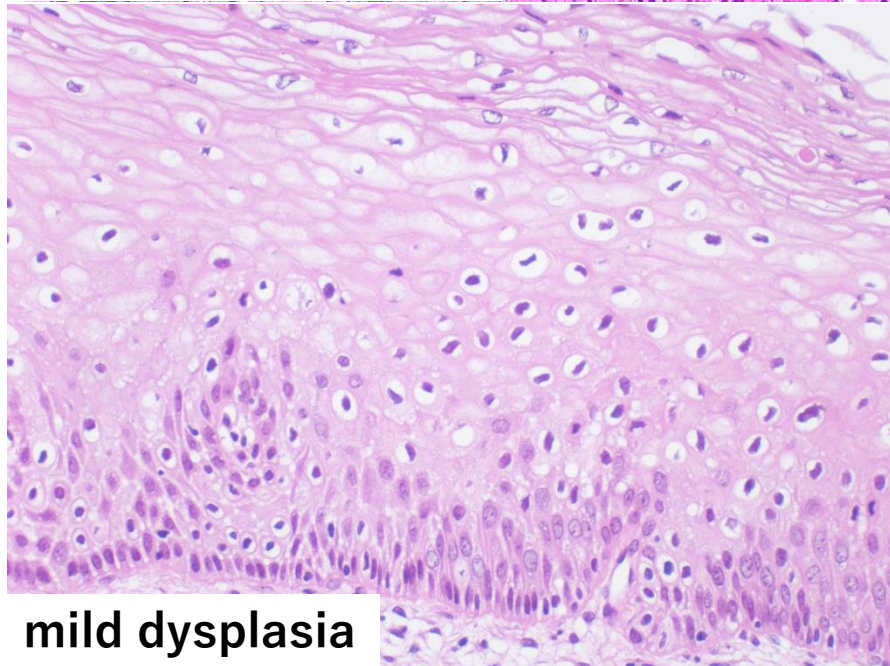
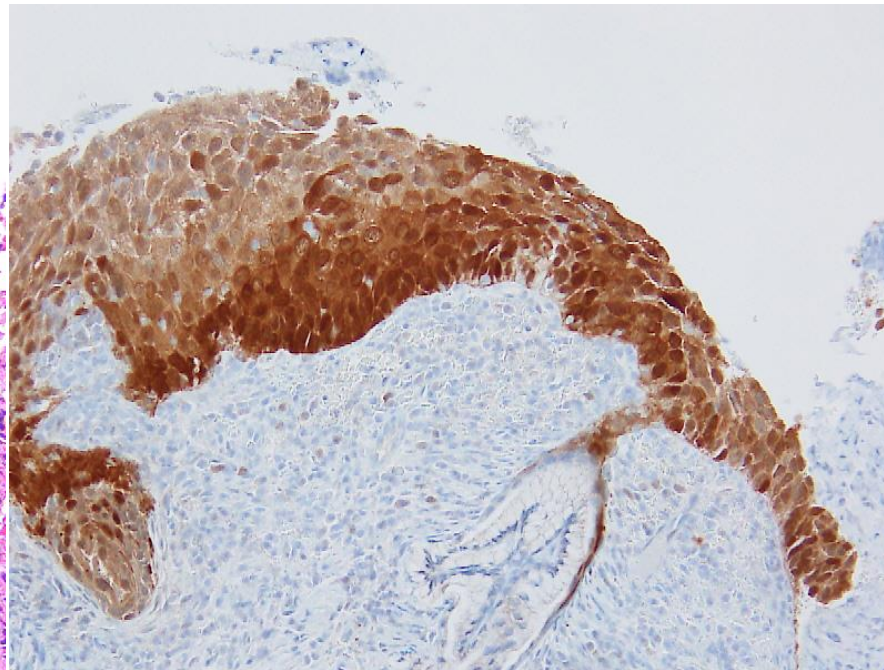
42F
CIN1
+
CIN2

Immunostaining
for p16 is highly
sensitive for
detecting
carcinogenic
HPV infection.

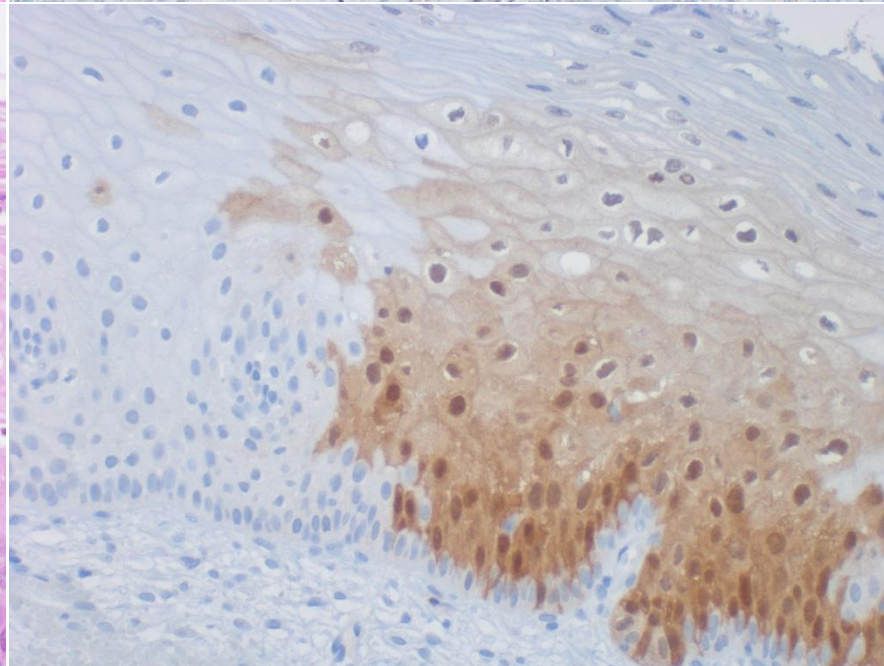




moderate dysplasia



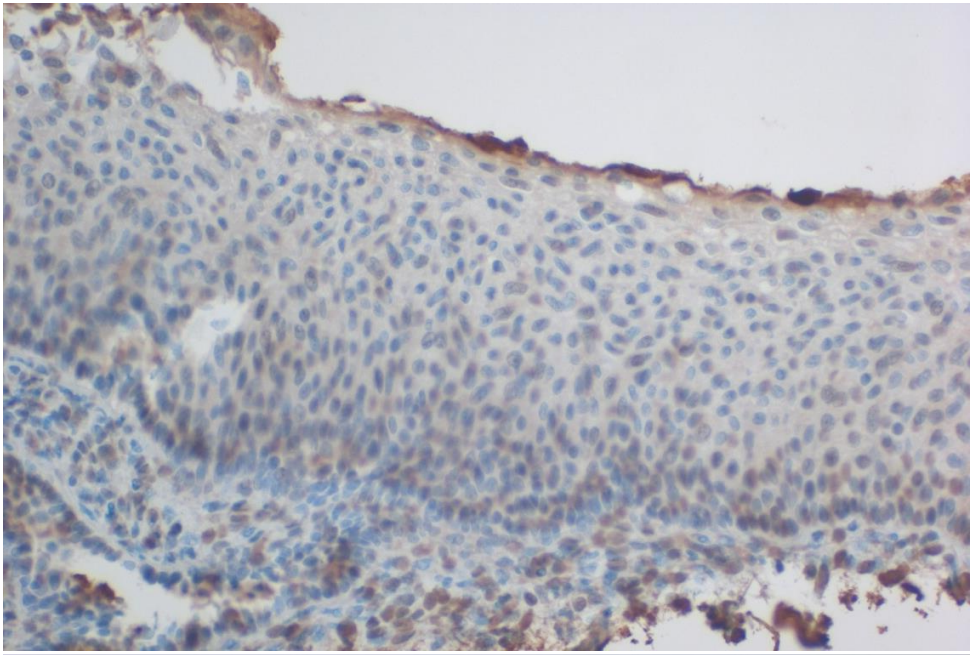
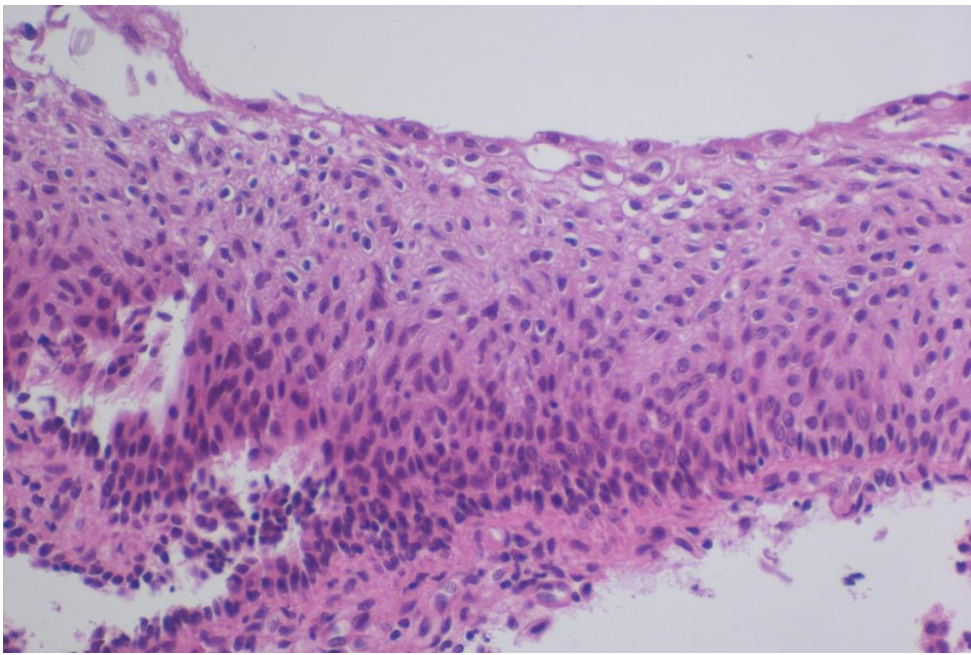
mild dysplasia



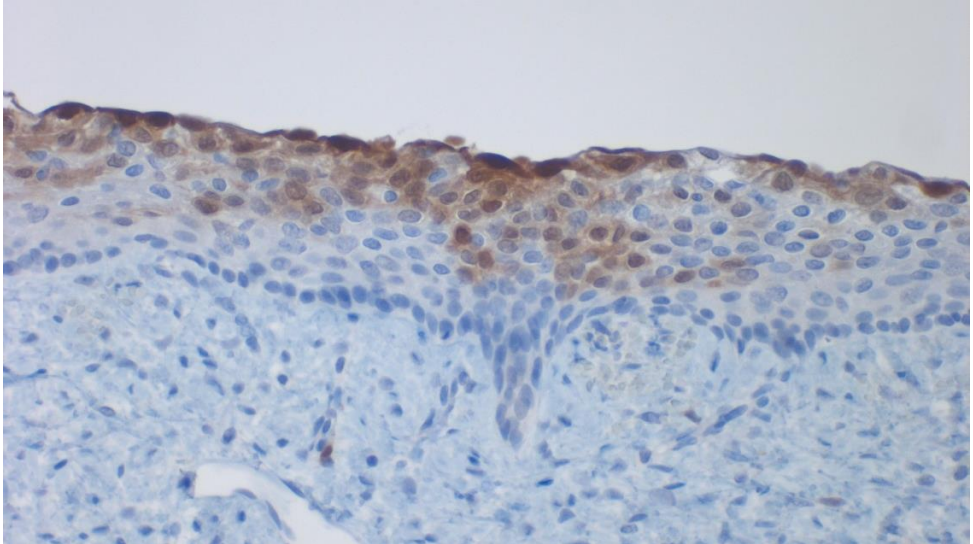
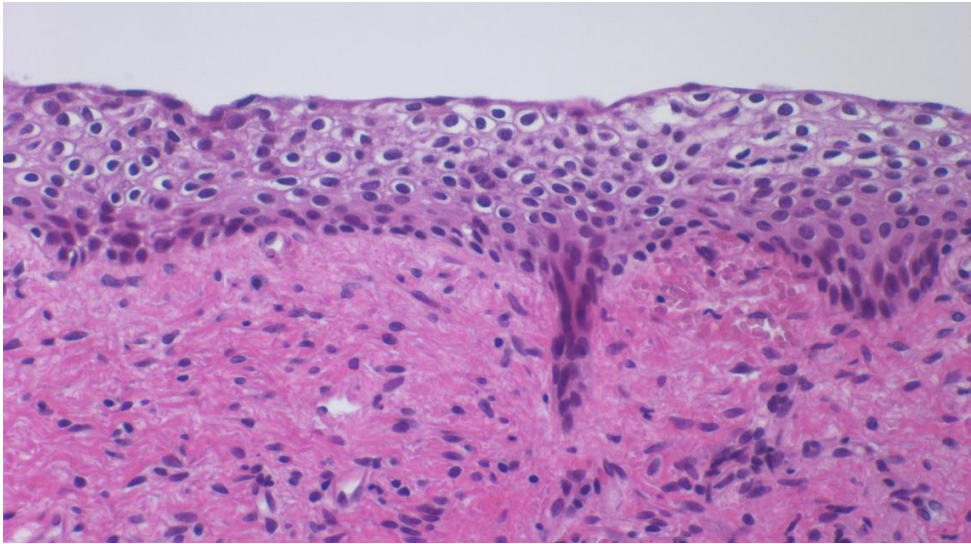
p16 immuno- staining

(punch biopsy,
formalin-fixed,
paraffin-
embedded
sections)

Mild dysplasia is
recognized by
p16
immunostaining.



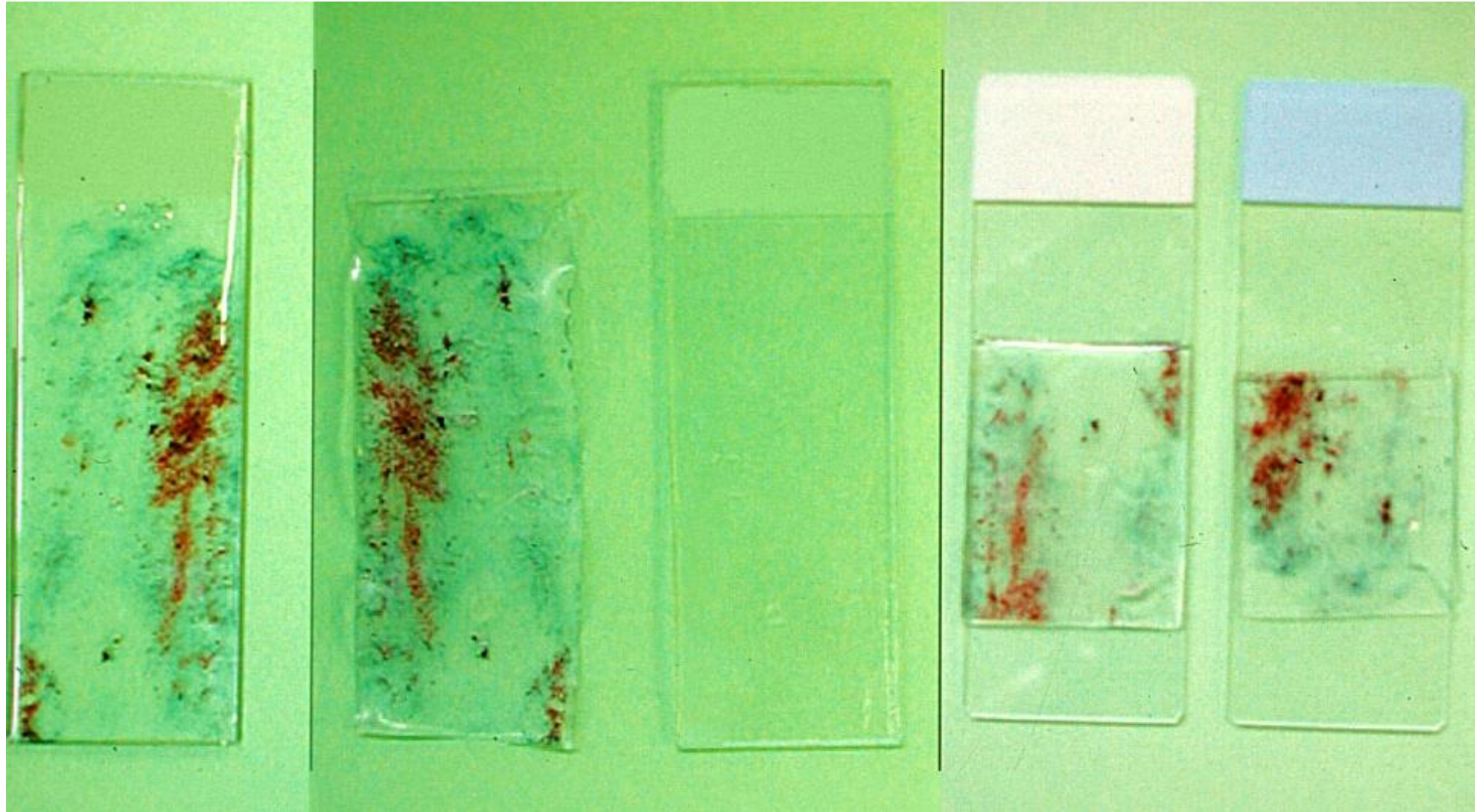
Case 1



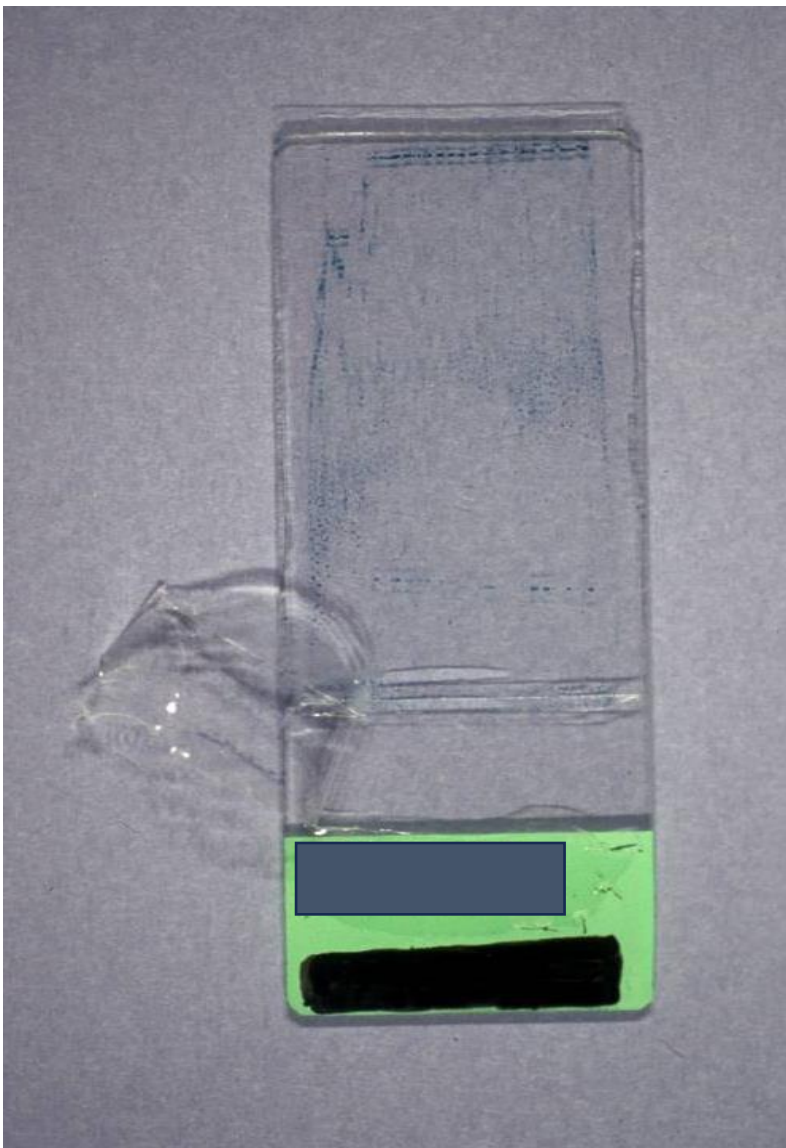
Case 2

p16 immunostaining in senile colpitic (the staining is negative in the parabasal layer)

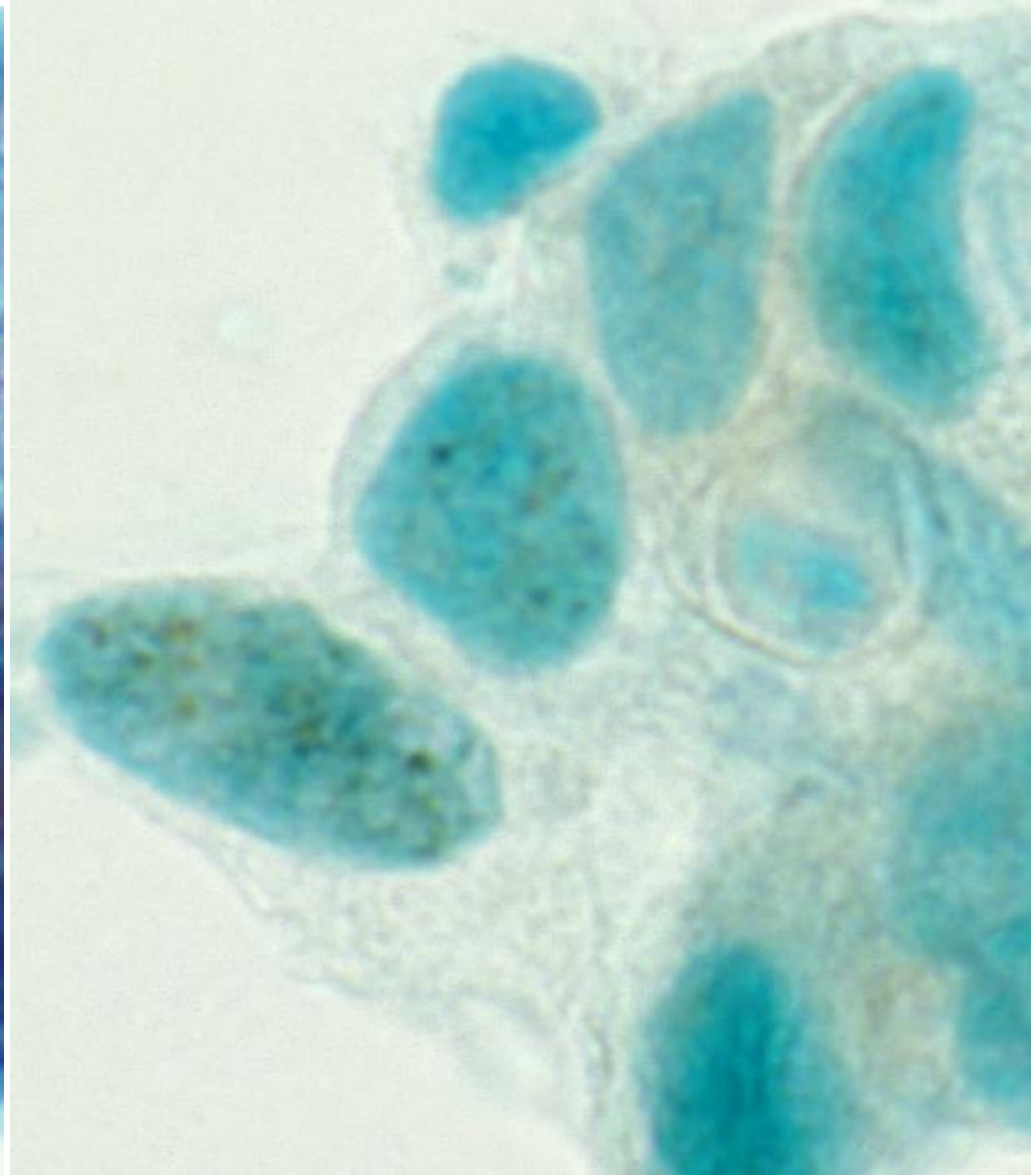
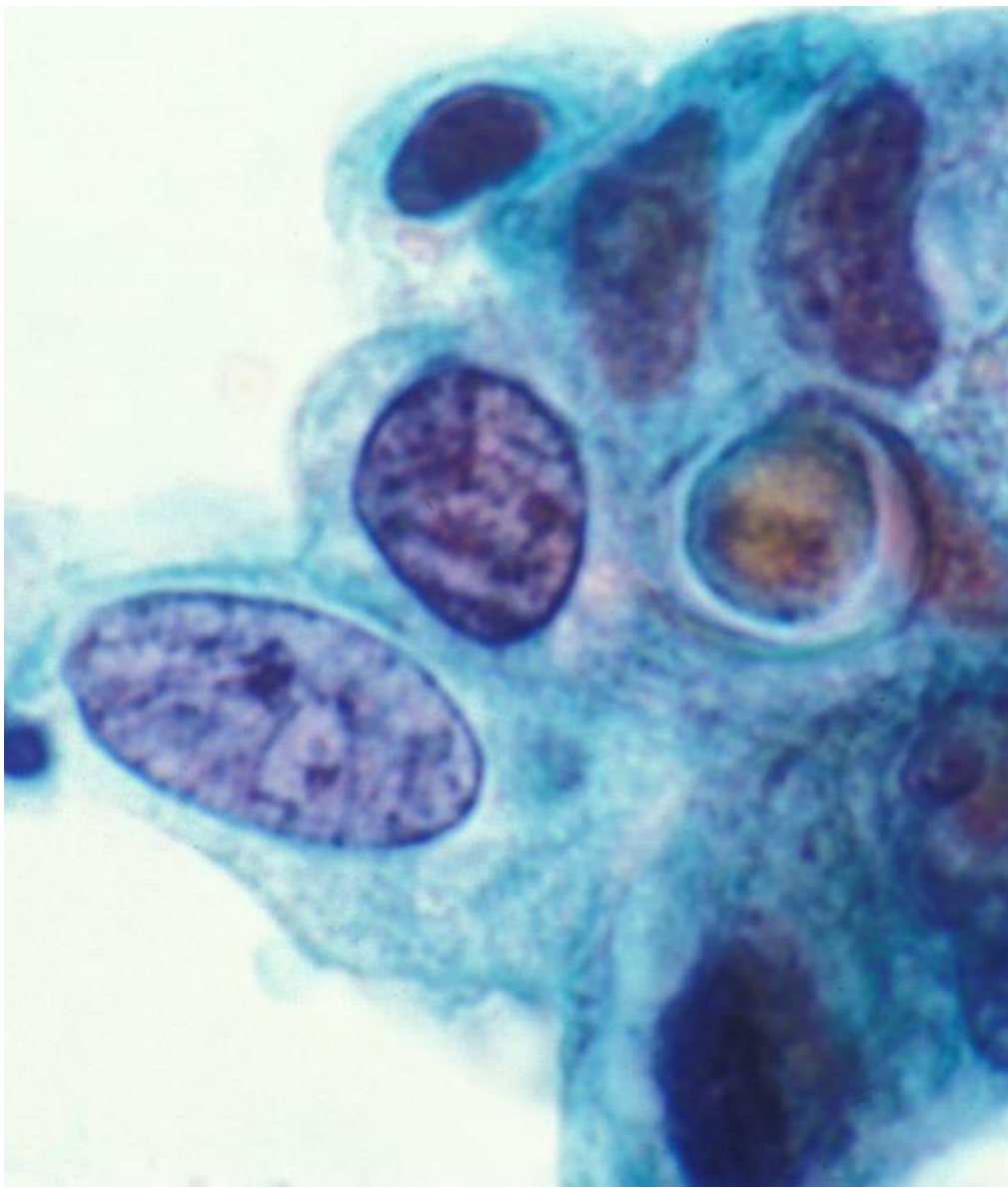
Cell transfer technique for applying immunostaining



After detaching Malinol membrane from the original glass slide, the membrane was cut into 2 pieces, which were then mounted on Silane-coated glass slides.



In gynecological cytology specimens, we can transfer the cells in the area protruded from the cover slip onto the Silane-coated glass slide. We do not need to remove the cover slip.



ISH for HPV-16 in the cervical cytology preparation of severe dysplasia (CIN-3). Left: Papanicolaou, right: ISH for HPV-16). The cells were transferred to a Silane-coated glass slide to avoid cell detachment during heating treatment for ISH. Dotted brown-colored signals are seen in the nuclei of the dysplastic cells.

Cytology preparations prepared from post-menopausal women (n=13)

Age: 56~82 years (average: 64.7, median: 64)

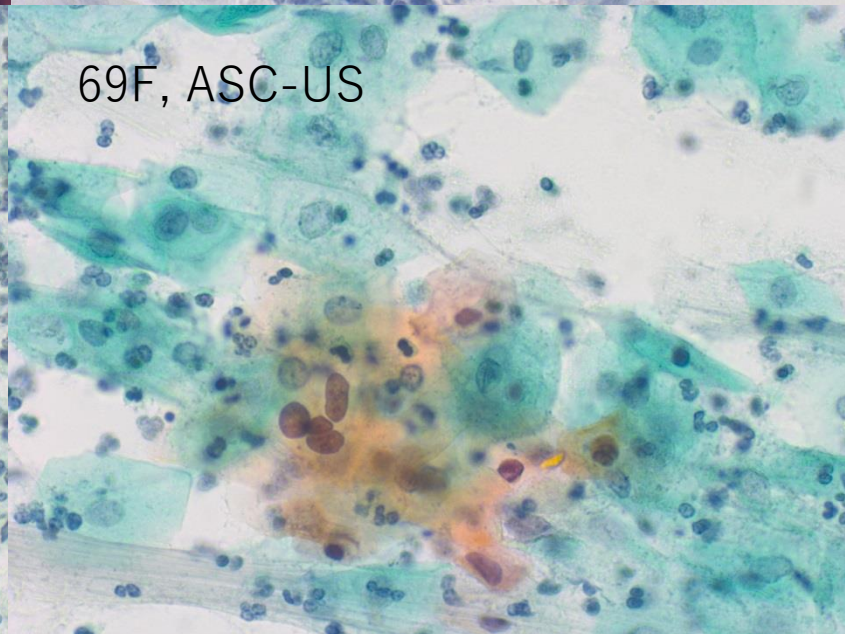
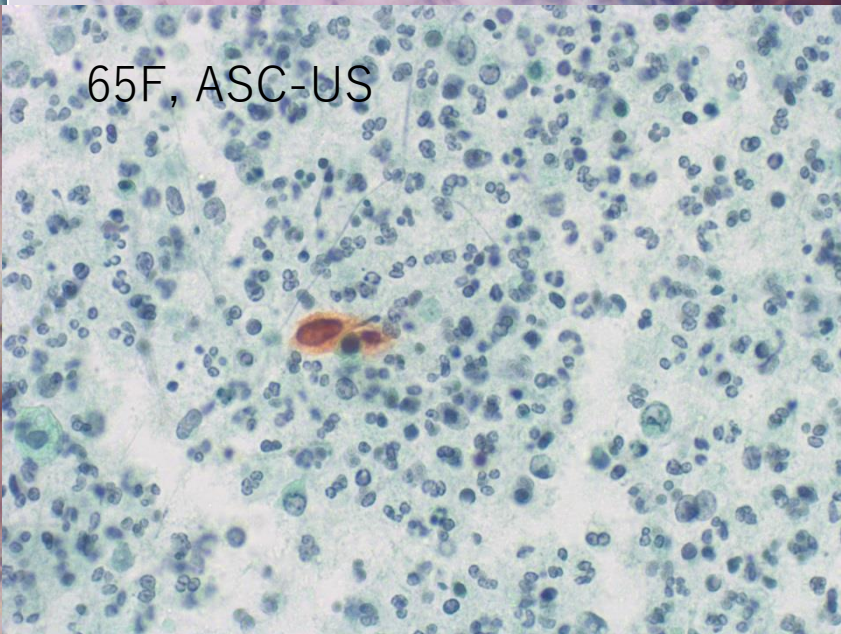
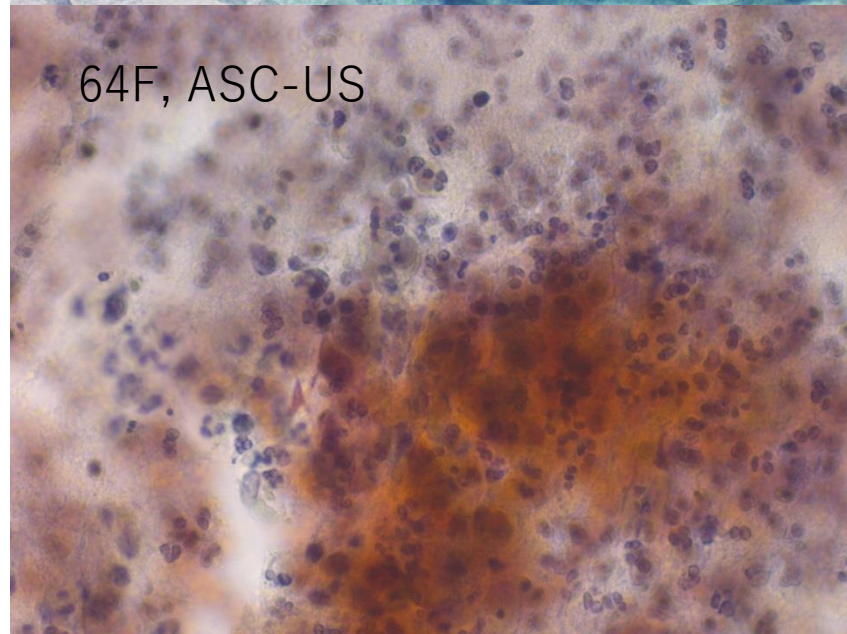
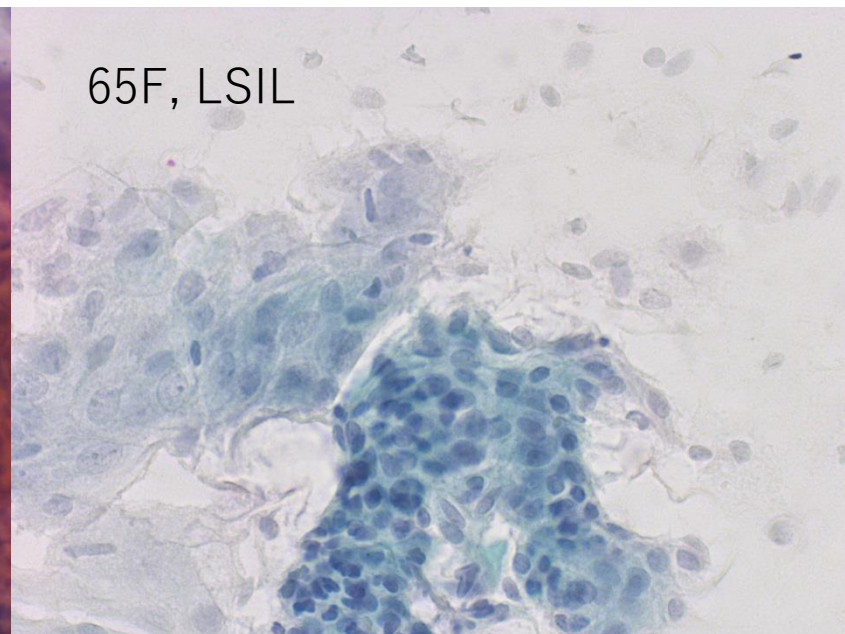
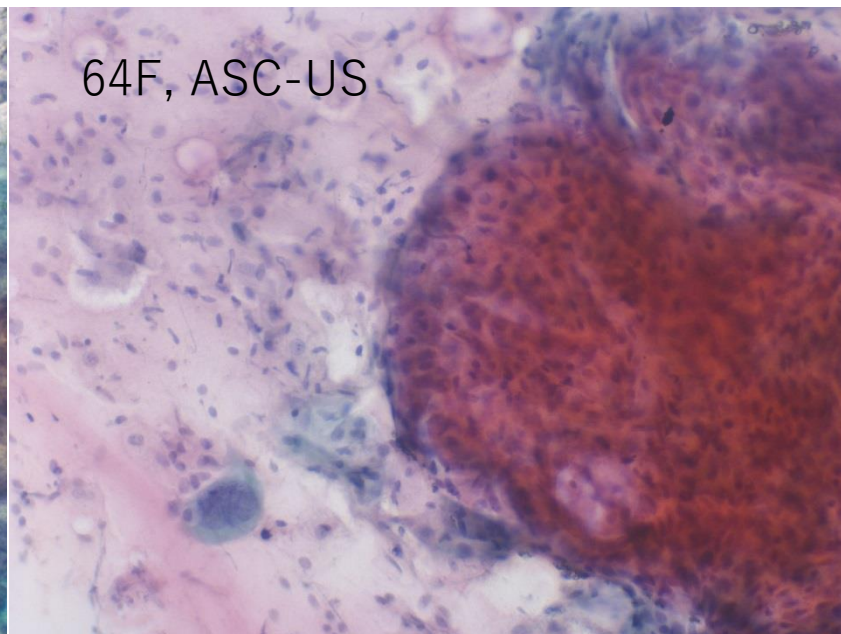
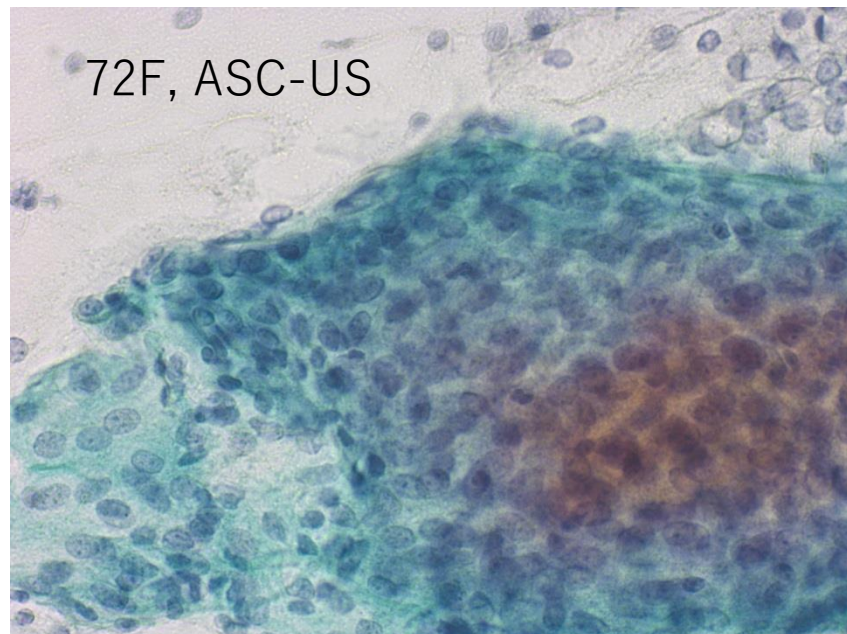
Total number of specimens: n=29

Class II: 6 lesions, class IIIa: 16 lesions, class III: 7 lesions

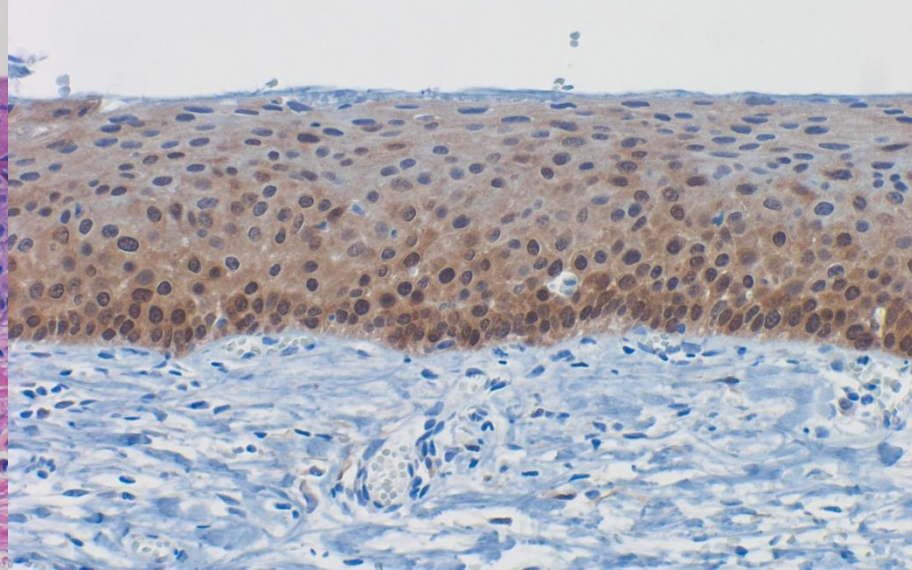
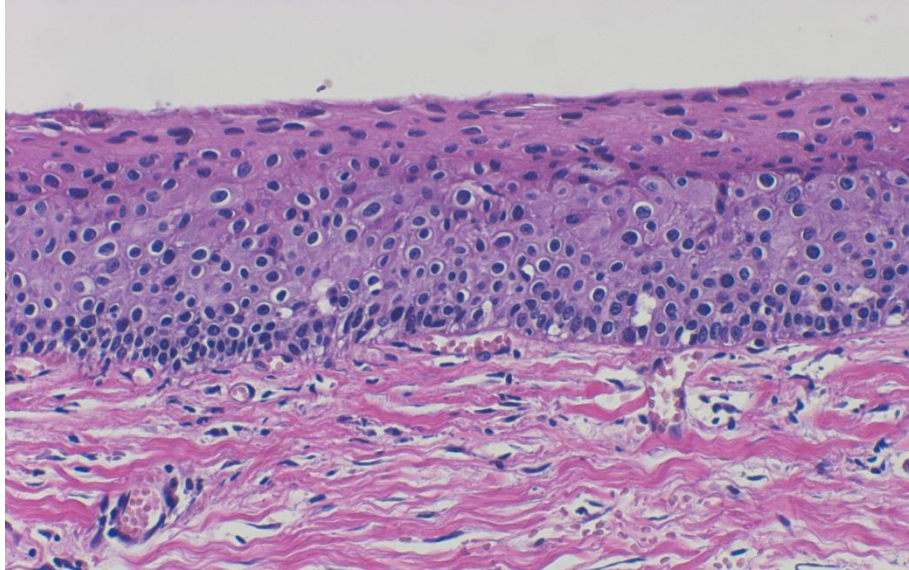
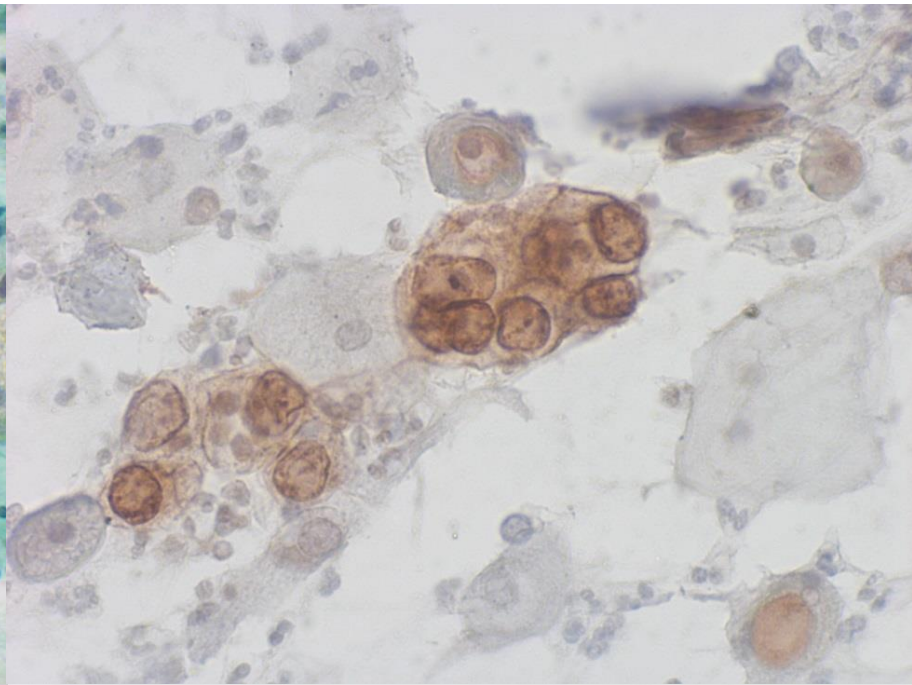
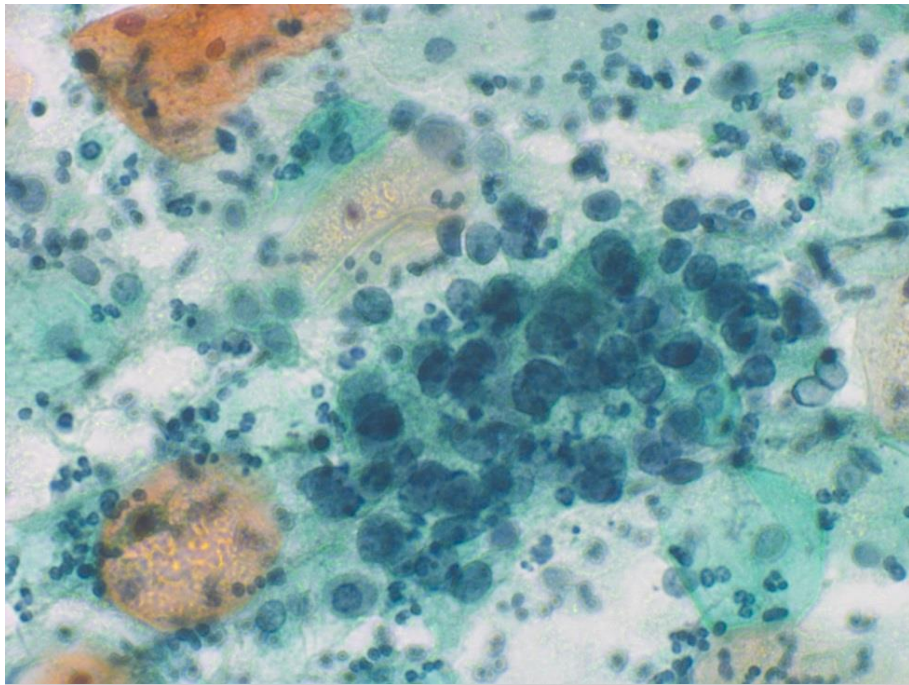
Bethesda classification: ASC-US 20 lesions, ASC-H 2 lesions,
LSIL 4 lesions, HSIL 3 lesions

p16-positive: 3 lesions in 2 cases (both diagnosed as HSIL)

Biopsy (13 lesions): CIN1 1 case (ASC-US), CIN2 1 case (HSIL)

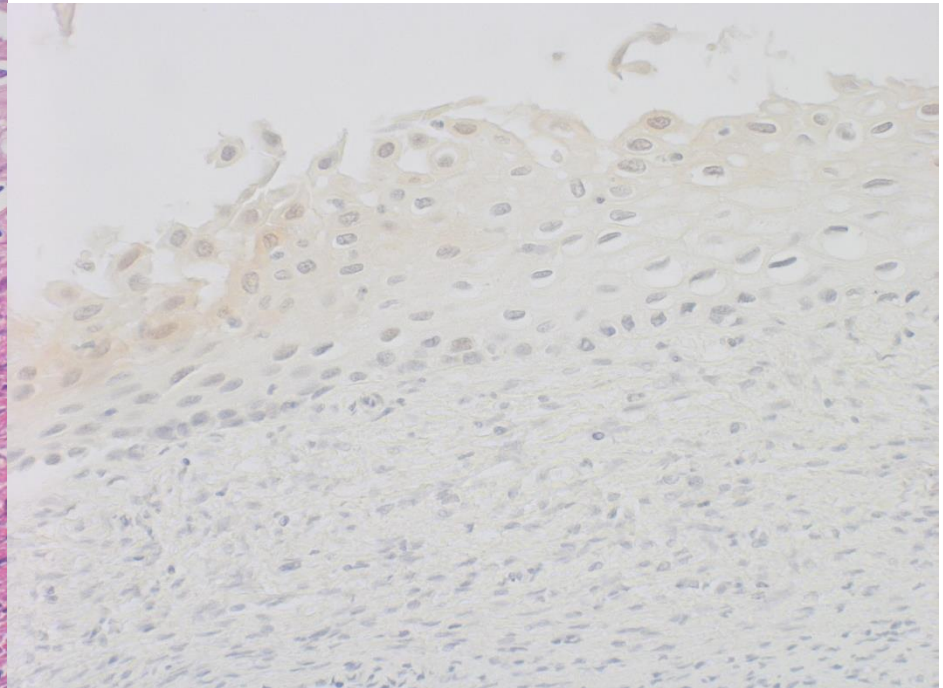
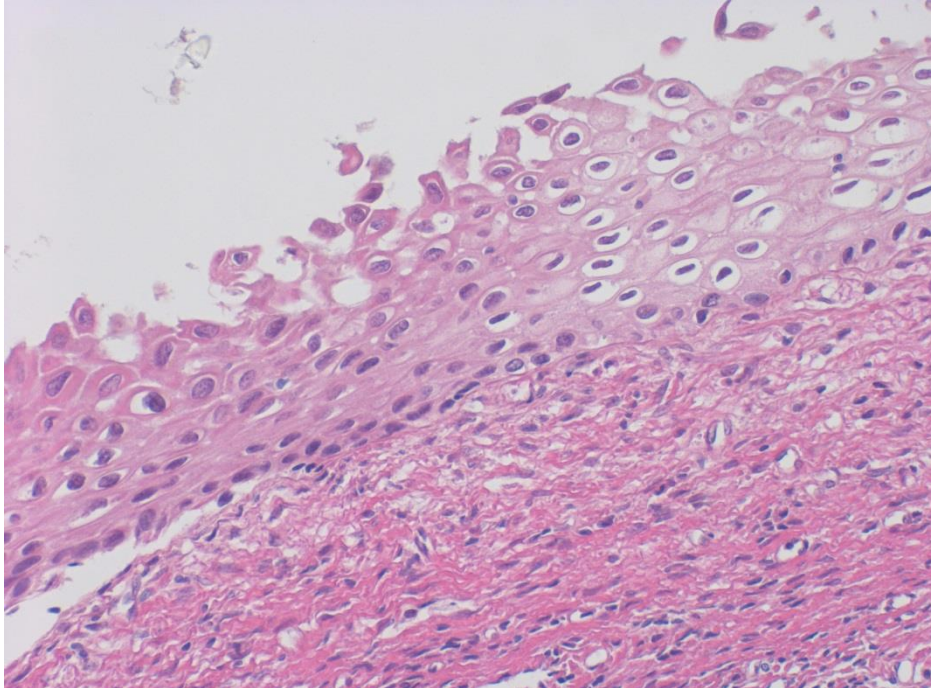
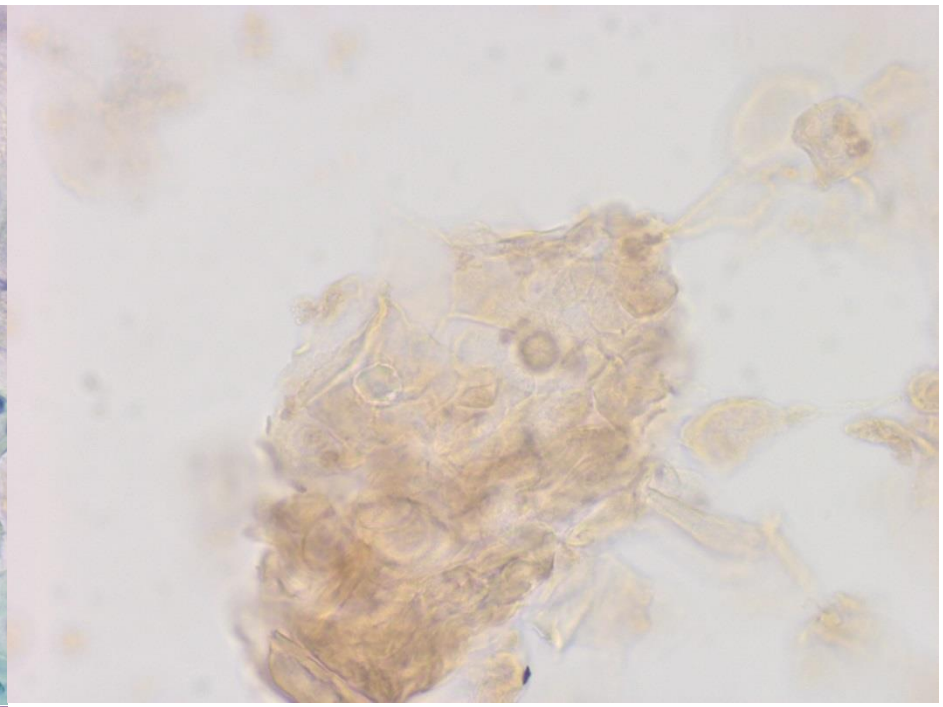
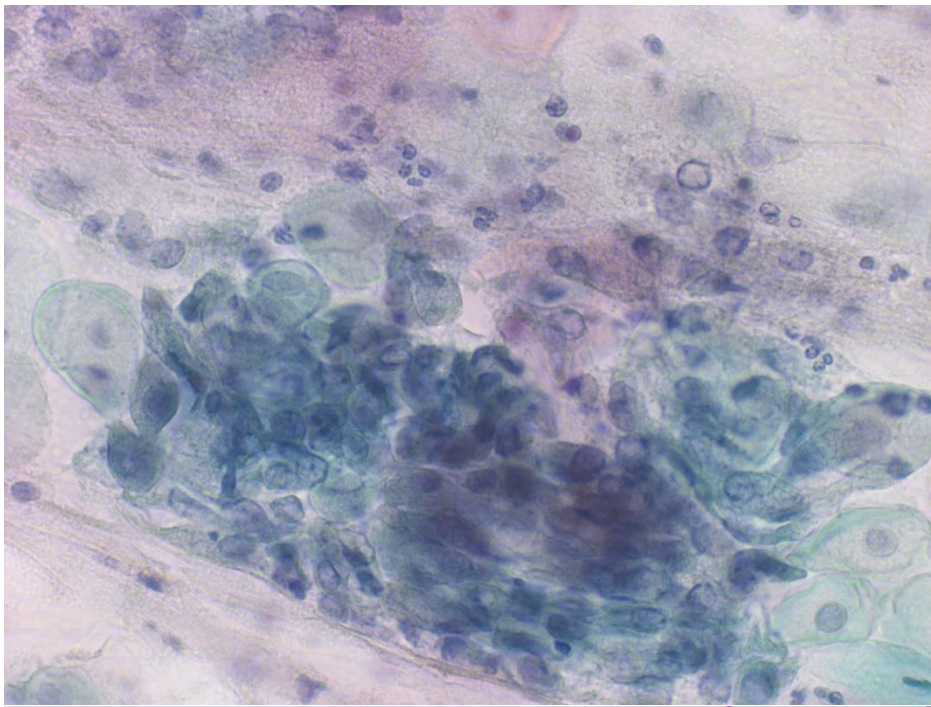


Two types of p16-negative senile colpititis: 1) Clusters of parabasal cells, and 2) Atypia in superficial cells in the background of inflammation.

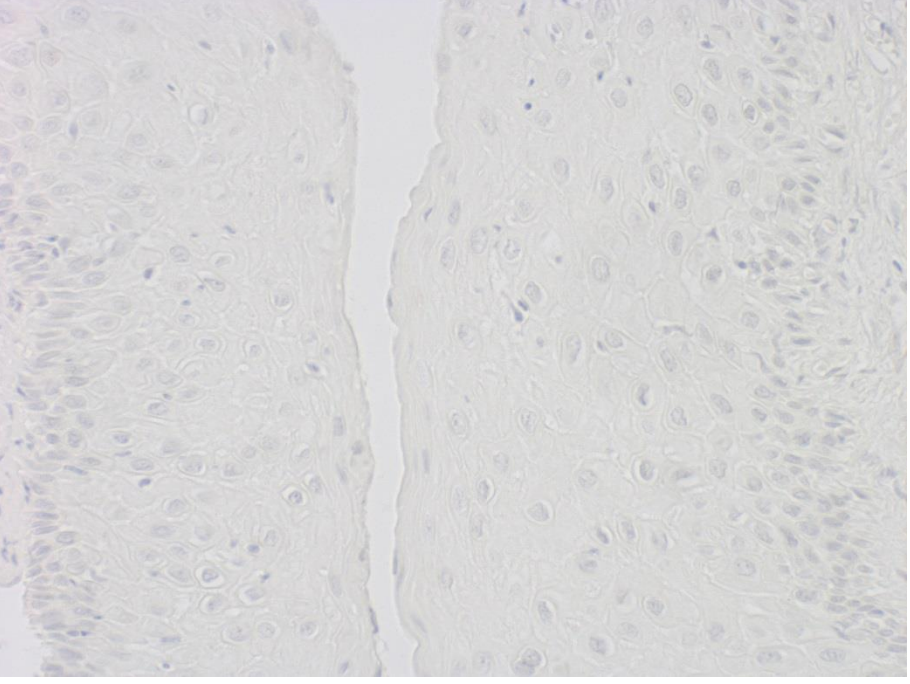
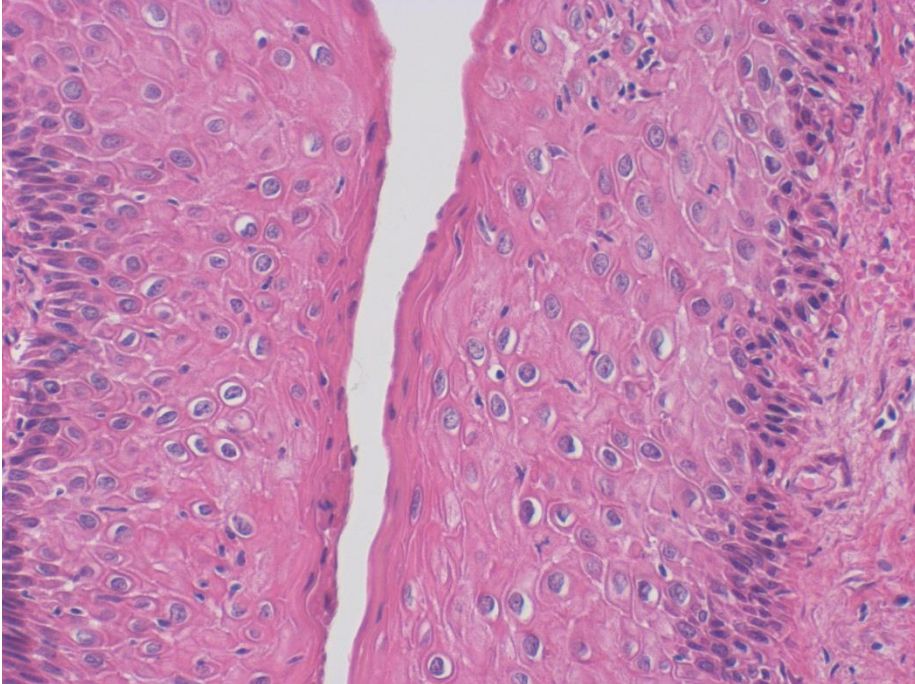
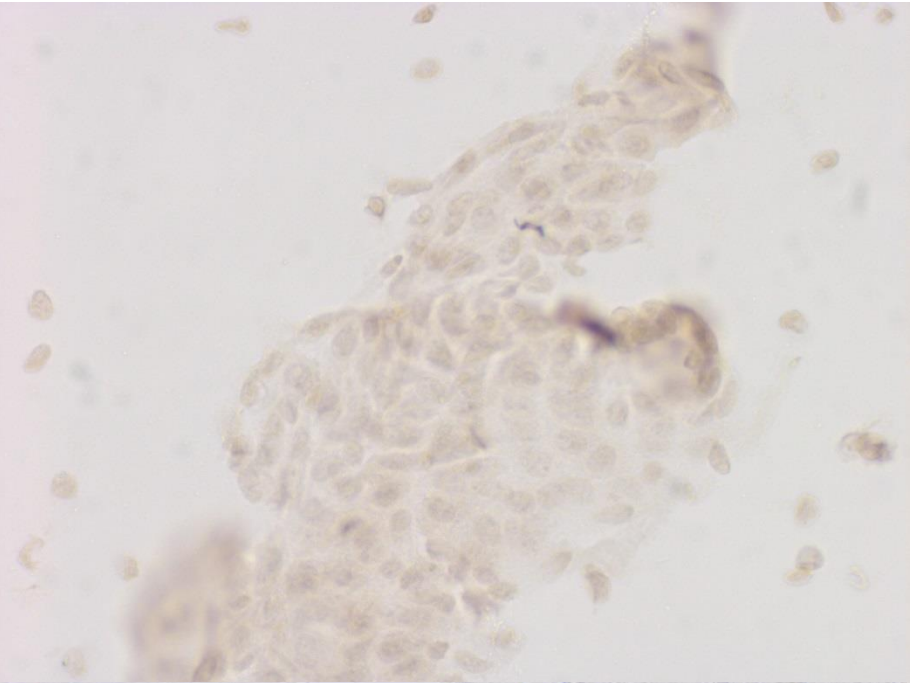
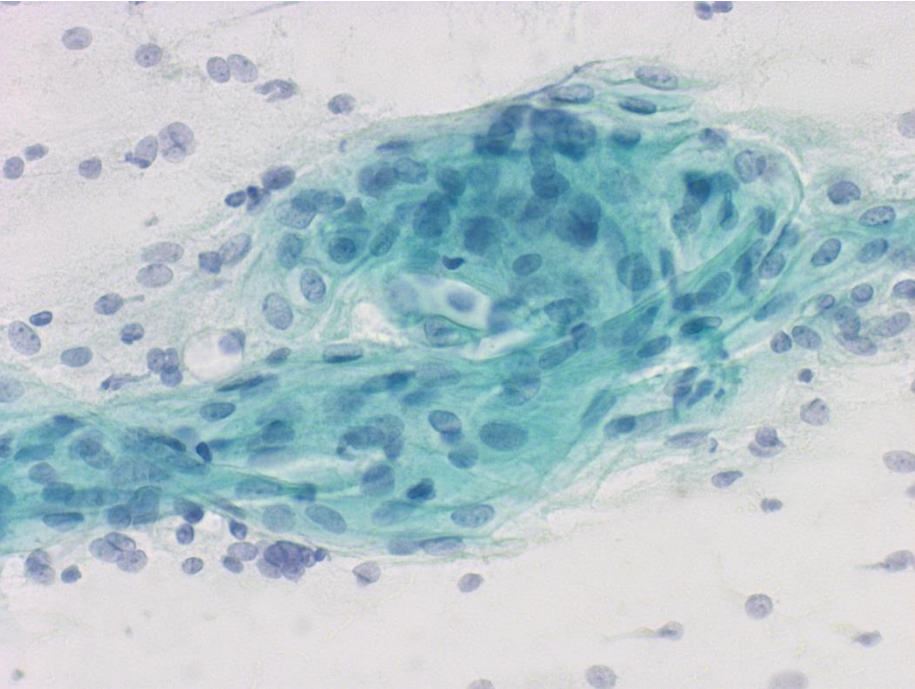


82 y-o F: HSIL (moderate dysplasia), p16-positive in parabasal cells

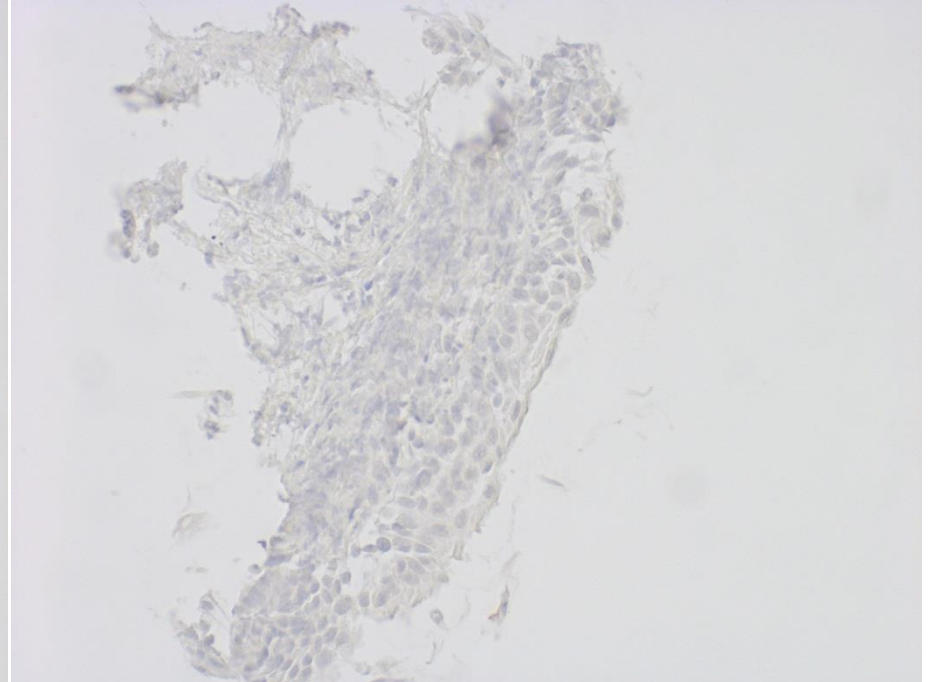
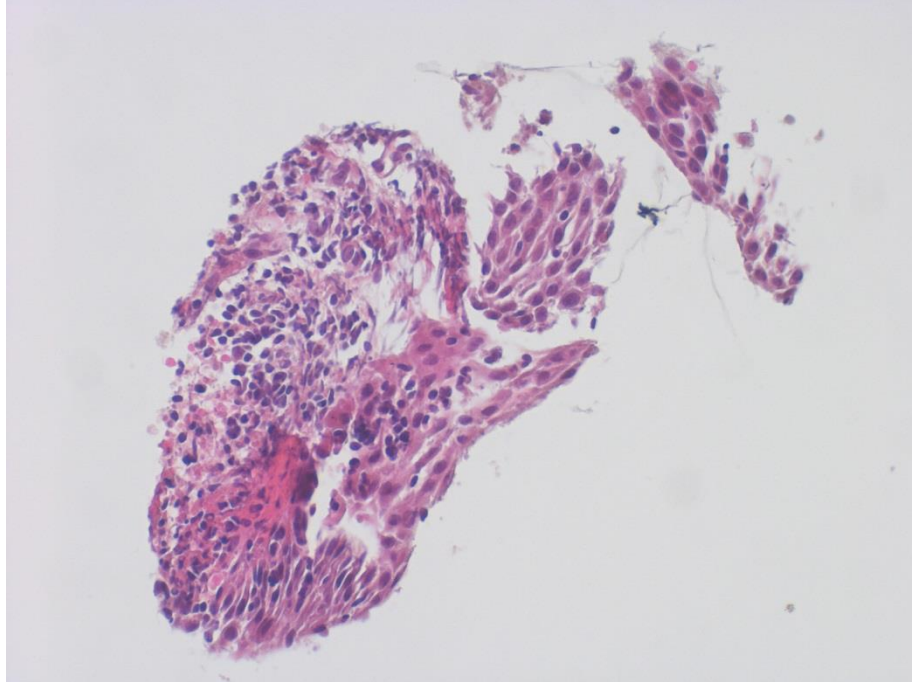
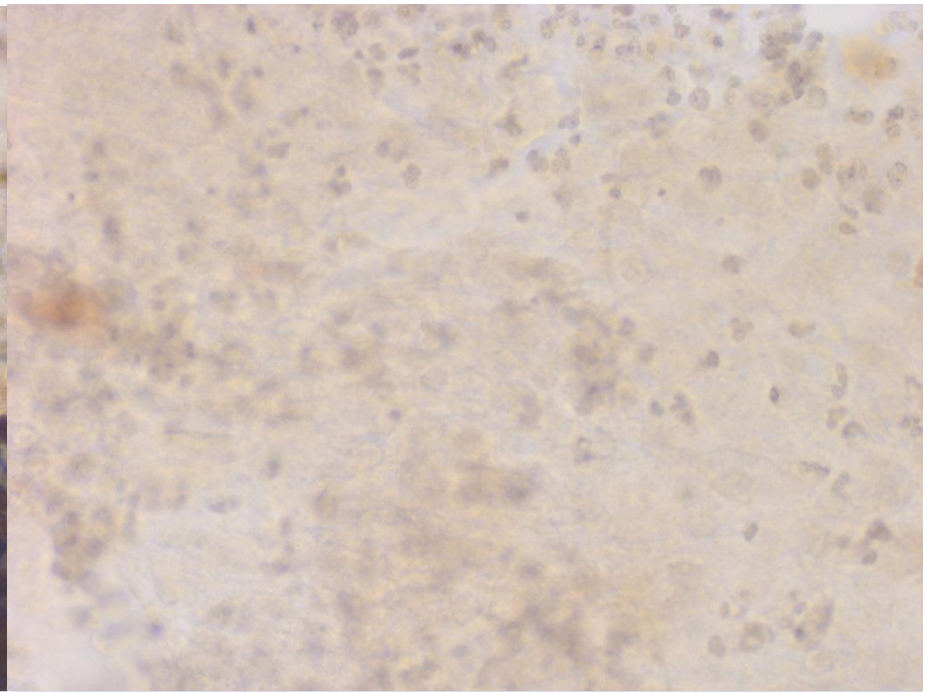
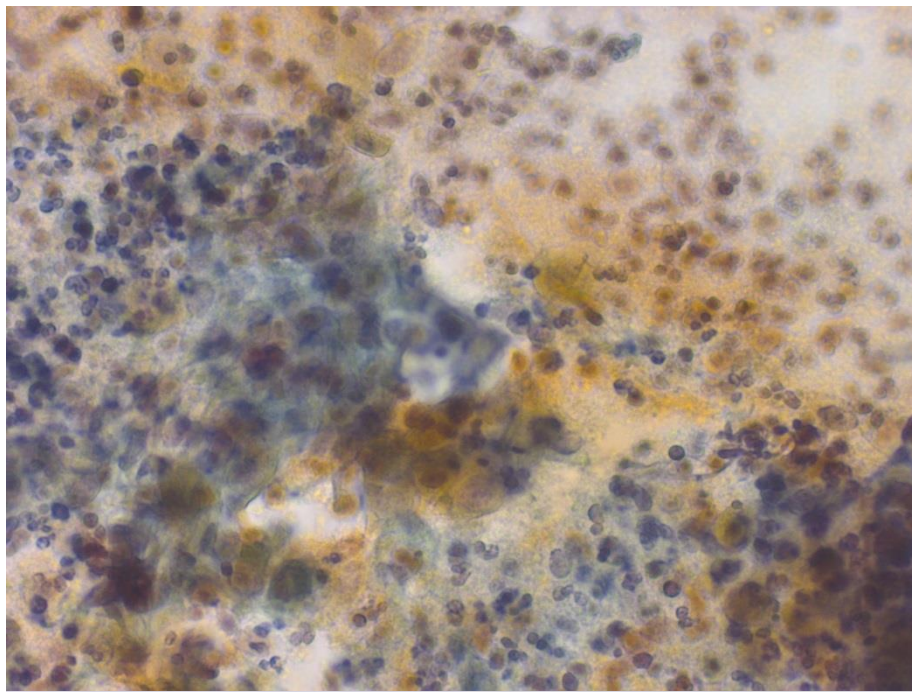
62 y-o F:
ASC-US
p16-
negative



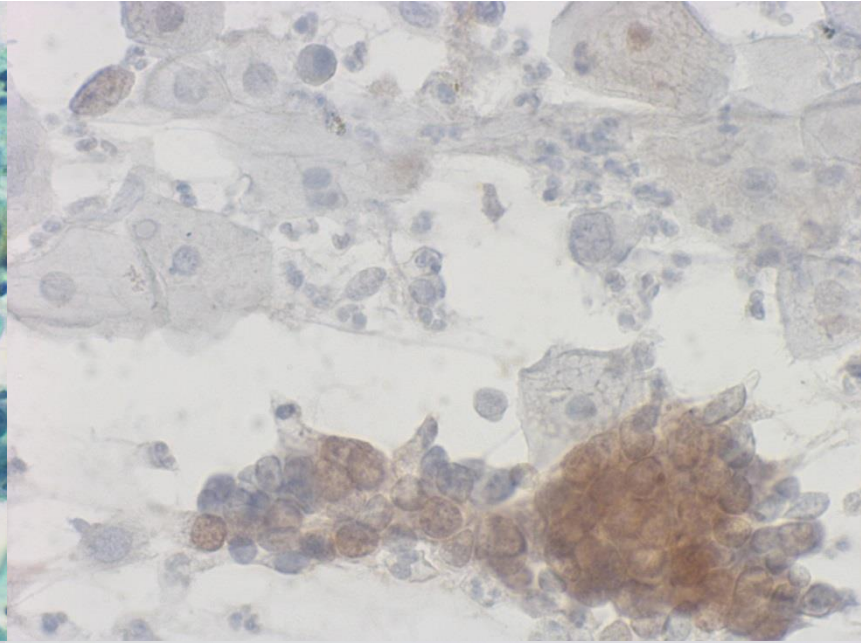
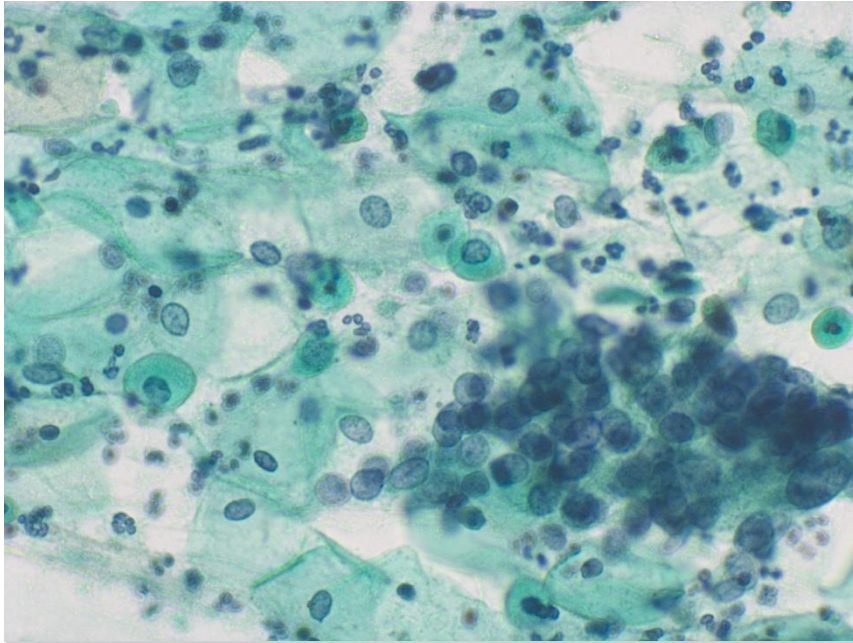
72 y-o F
ASC-US
p16-negative



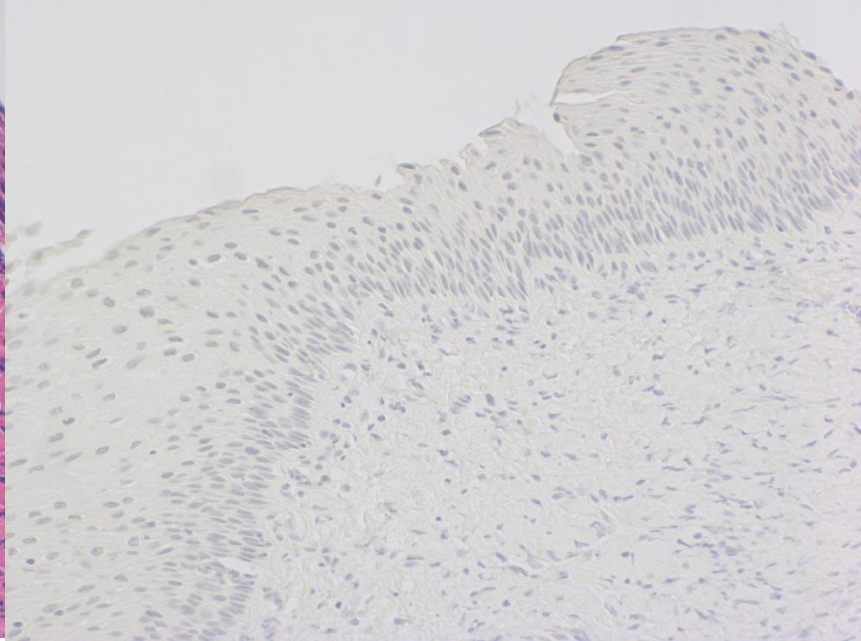
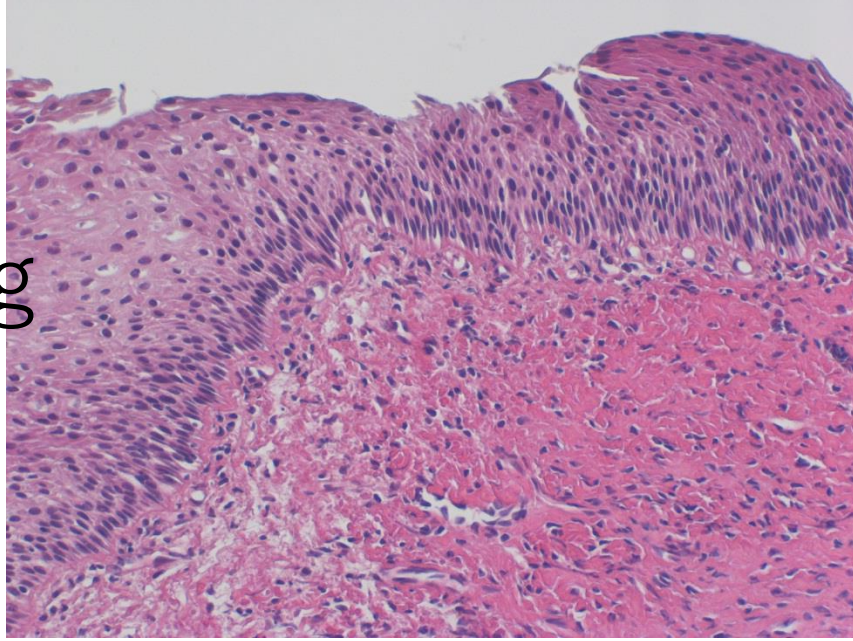
64 y-o F
ASC-US
p16-negative

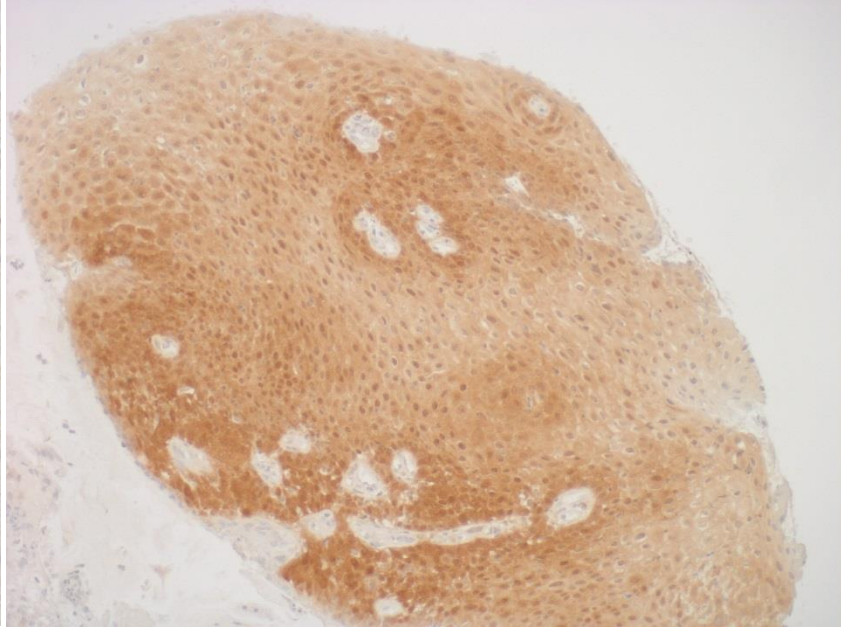
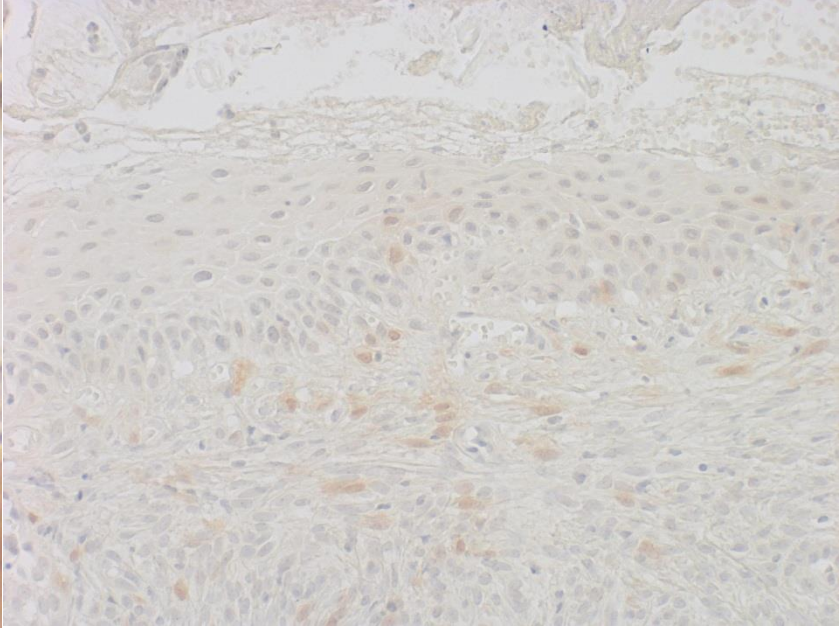
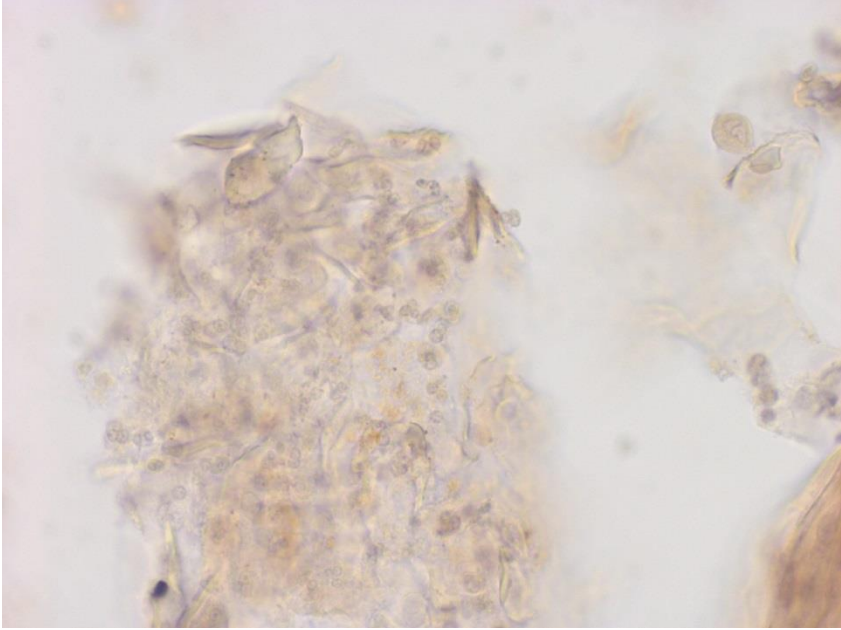
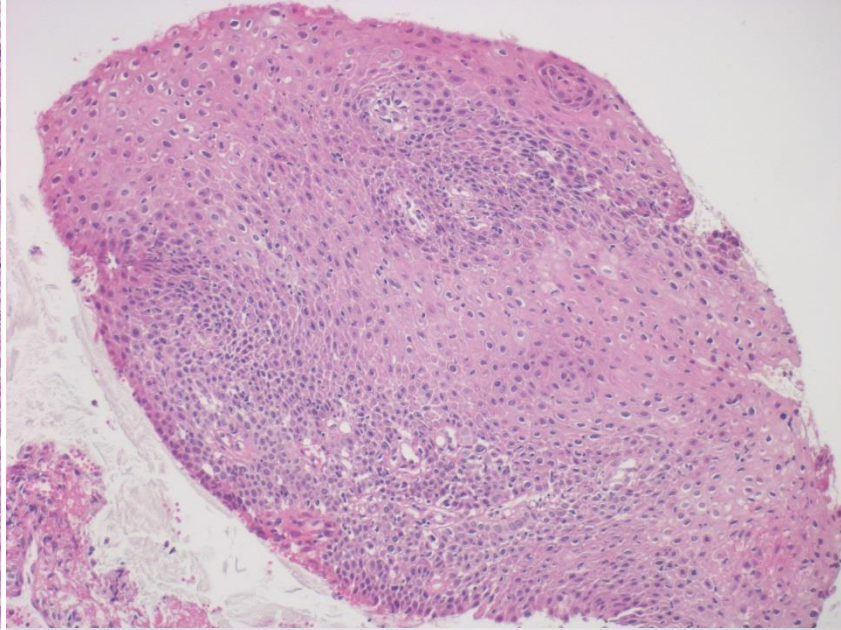
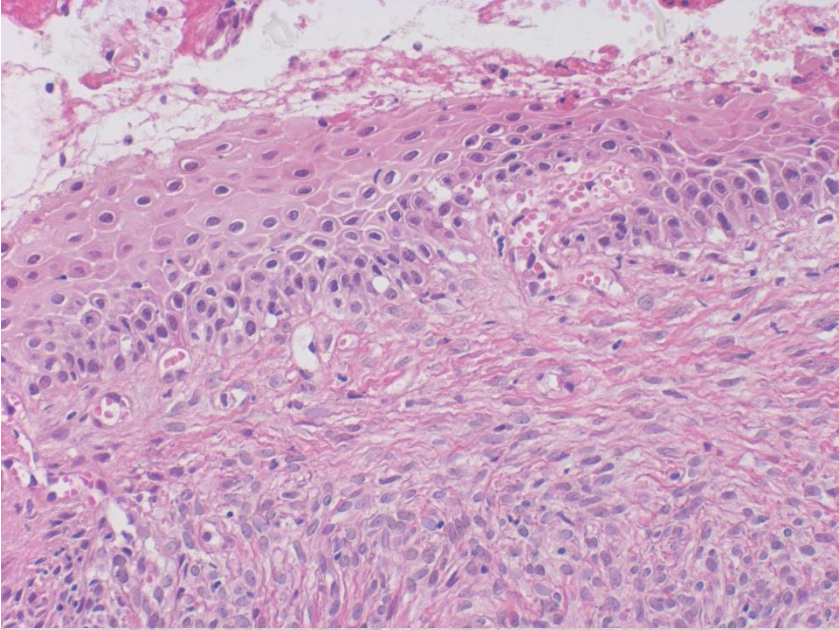
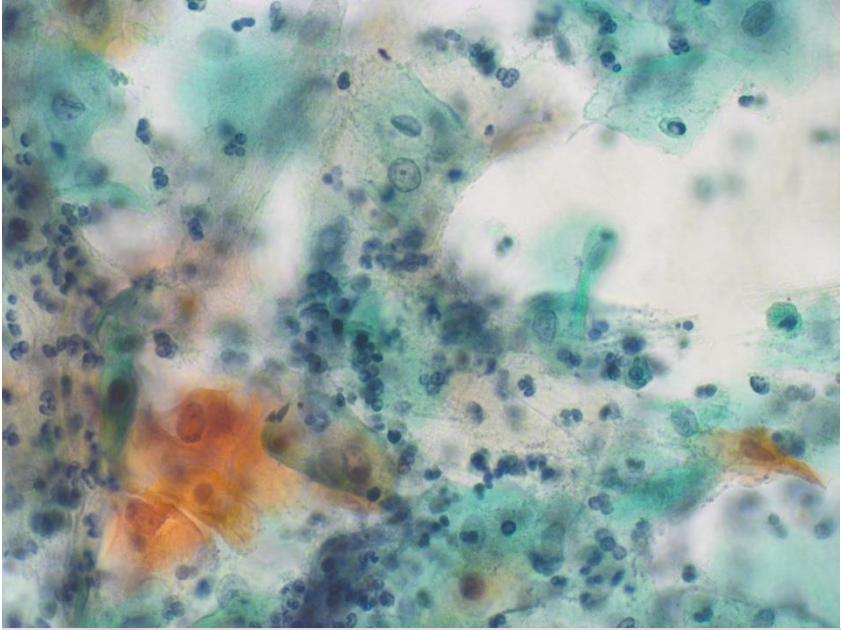


62歳
HSIL
p16陽性



生検は
偽陰性
(sampling
error)

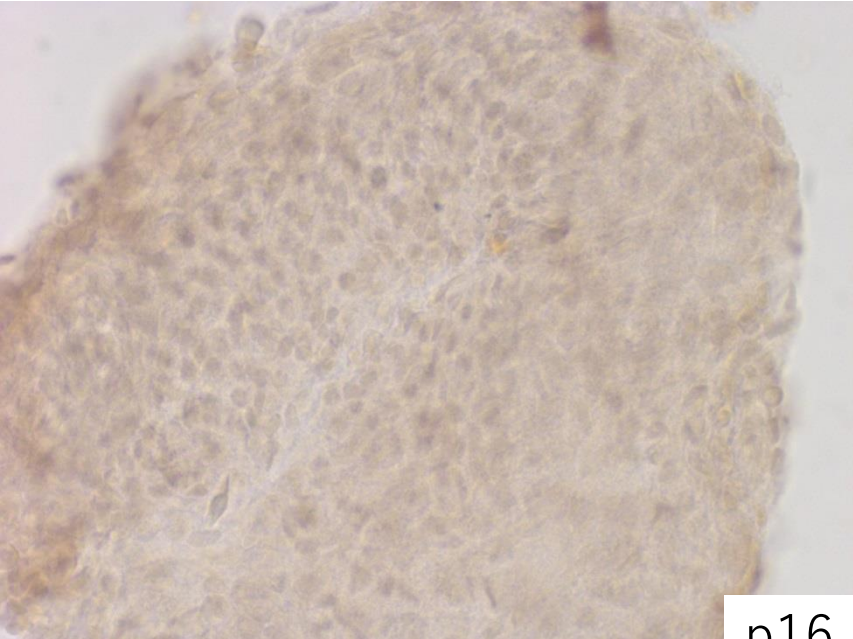
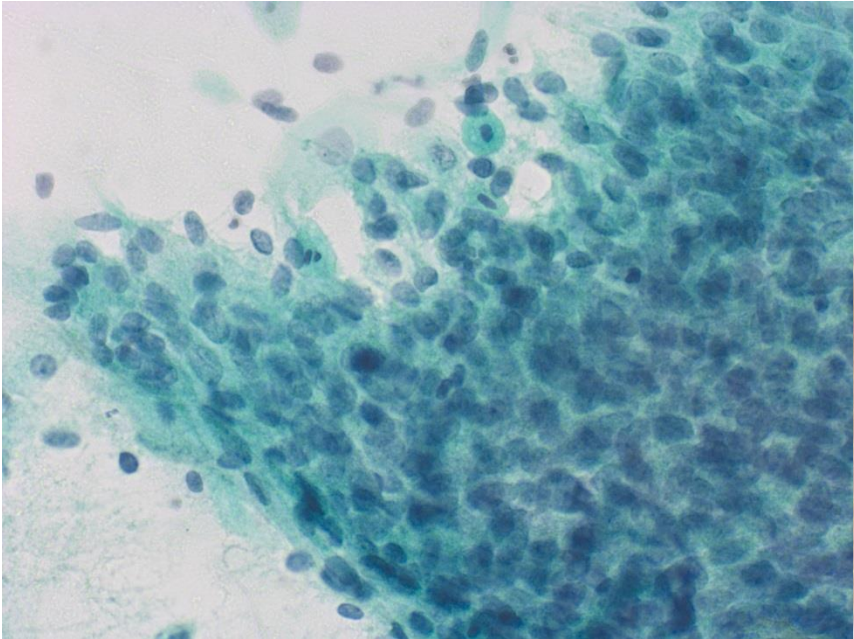




69 y-o F ASC-US, senile colpititis

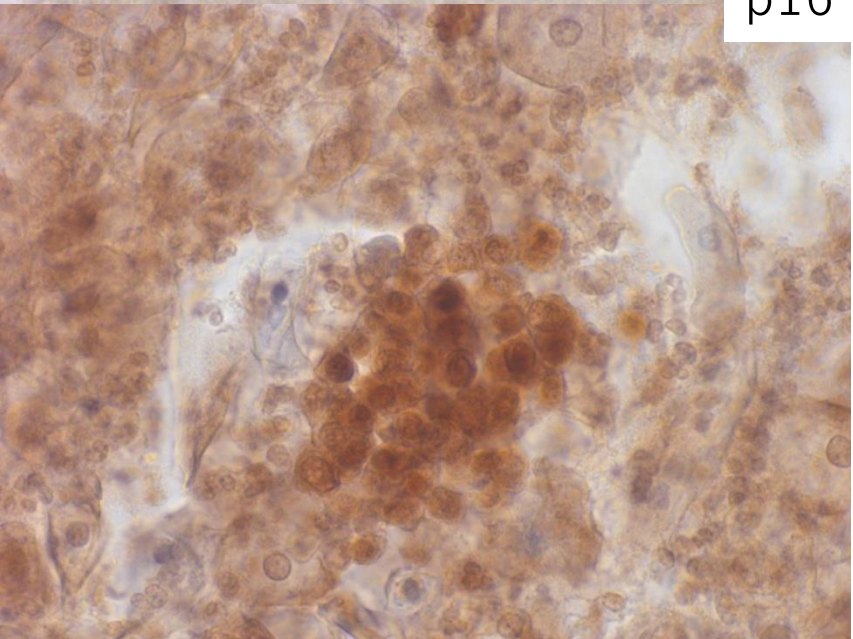
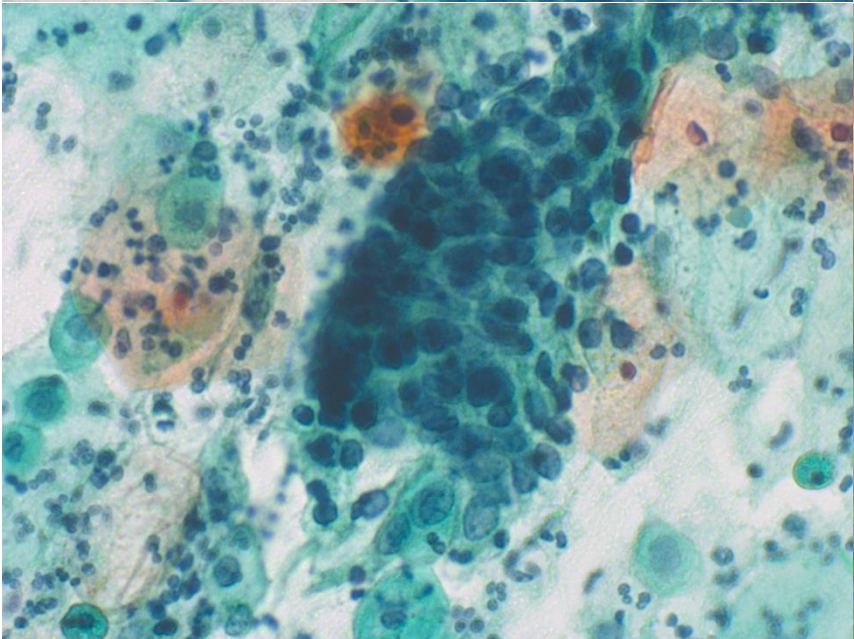
Biopsy: p16-positive (CIN1)

61F
ASC
-H



p16

82F
HSIL



**In Pap smears,
distinction between
senile colpitis and CIN
is not easy.**

**It is recommended to
make a diagnosis of
ASC-H instead of
ASC-US, and biopsy
should be requested.**