

Senile colpitis or dysplasia ? The usefulness of p16 immunostaining in post-menopausal cytology

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. A total of 29 cervical cytology specimens diagnosed as atypical squamous cells (ASC) or SIL were retrospectively sampled from 24 postmenopausal women (age range: 56–84 years, mean: 65.5, median: 65). Cytological diagnoses in the routine services included ASC-US 20, ASC-H 2, LSIL 4 and HSIL 3. All the specimens showed an atrophic background (senile colpitis). There were two microscopic patterns of senile colpitis with atypia: 1) parabasal cells were clustered (n = 23) and 2) dyskeratotic superficial cells were seen in a highly inflamed background (n = 6). Immunostaining for p16-INK4a (p16 in short) was performed, after the cells were transferred to trimethoxy[3-(phenylamino)propyl]silane-coated glass slides. Only three of the 29 cytology specimens (two of the 24 cases) judged as HSIL cytologically revealed p16 positivity in clustered atypical parabasal cells. Biopsy was performed in 11 cases, and chronic cervicitis without p16 expression was seen in nine. Two lesions showed p16-positive dysplasia (one mild dysplasia and another moderate dysplasia). In one lesion in an 84 year-old female, both cytology and histology specimens showed p16 positivity (HSIL). Discrepancy of p16 expression between the cytology and histology specimens was encountered in two lesions, representing sampling errors. It is of note that the overcytodiagnosis is avoidable with the aid of p16 immunostaining.

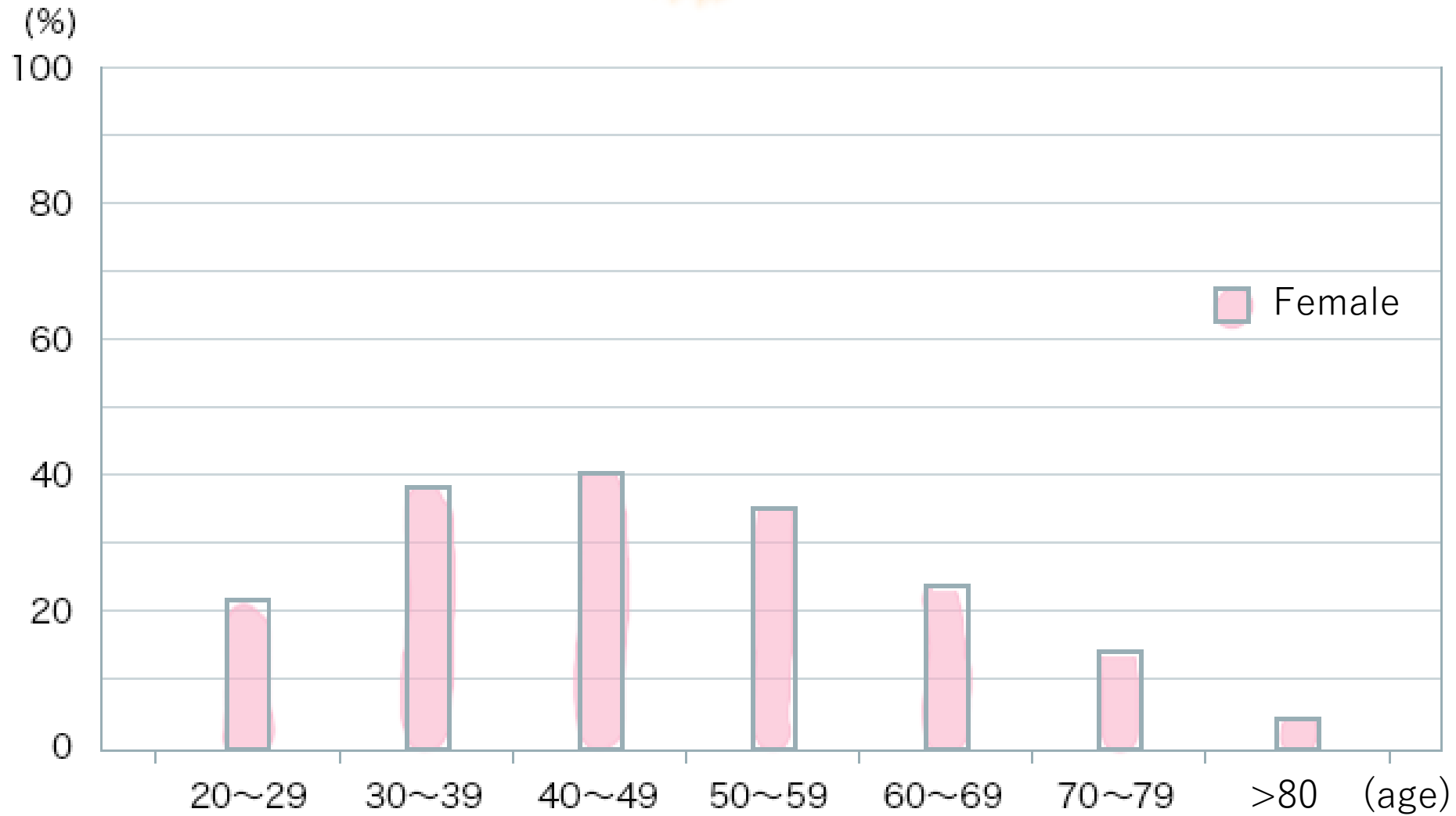
Ref.: Tsutsumi Y, et al. p16 immunostaining can avoid overdiagnosis in postmenopausal cervical cytology. *Int Res J Med Medic Sci* 2021; 9(1): 1-8. doi: 10.30918/IRJMMS.91.20.056

Cytology and HPV-DNA analysis

When the cytodiagnosis of ASC-US in Bethesda classification was made, HPV-DNA analysis should be strongly recommended in routine cytology practice. Under the diagnosis of ASC-H or more, cervical biopsy is prompted.

Clinicians will regard that the HPV-DNA analysis is necessary, when cytopathologists make a diagnosis of ASC-US.

Cervical cancer screening rate in Japan (according to the age group)

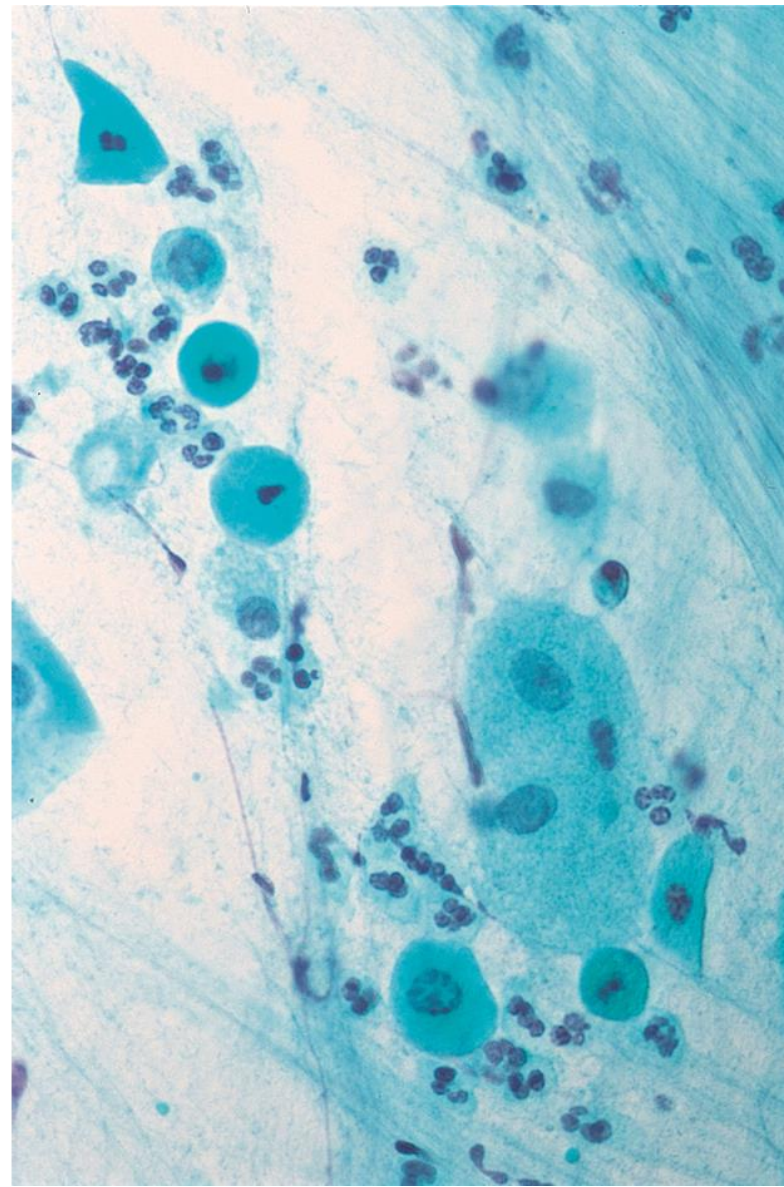
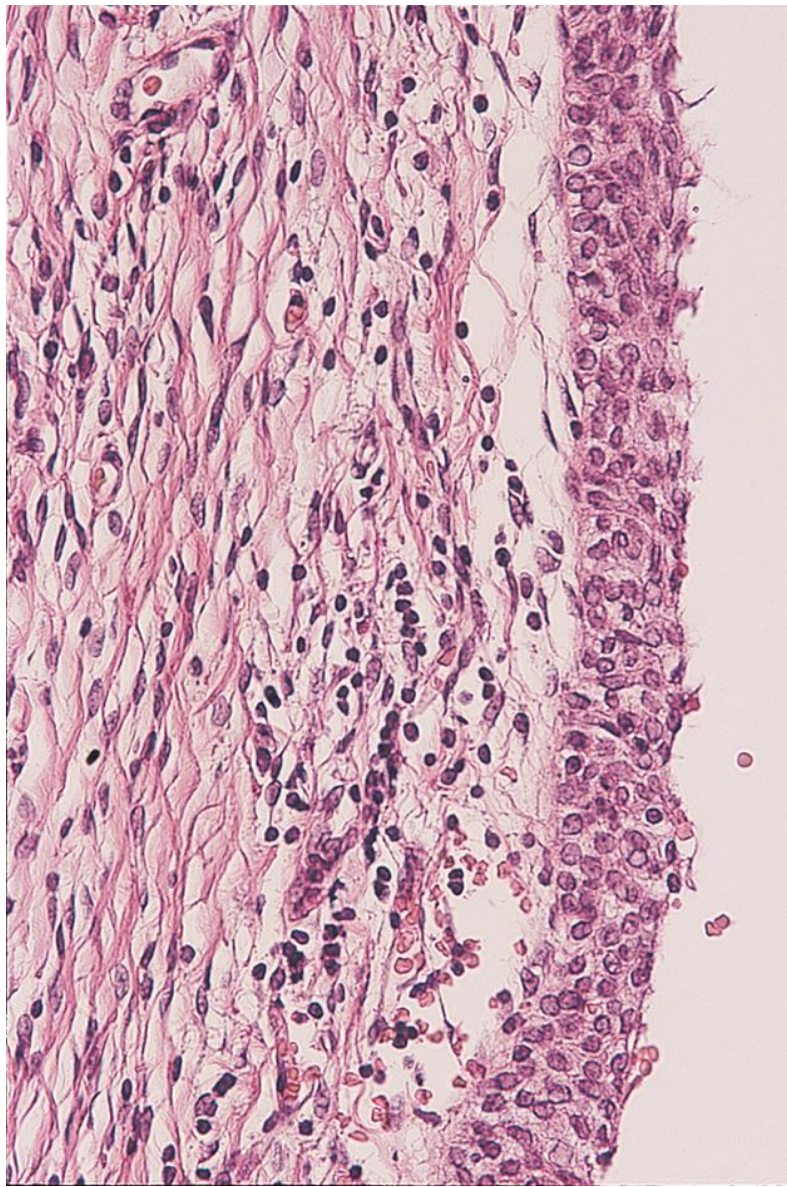


1/4 - 1/3 of the cervical cytology specimens were sampled from the postmenopausal women.
(cited from the HP of the Japan Medical Association)

Senile colpitis (atrophic colpitis)

Senile colpitis is featured by atrophy of the vaginal squamous mucosa due to decreased secretion of estrogen after menopause. Secondary bacterial infection may occur.

- 1) Superficial cells are lost.
- 2) Parabasal cells are predominant.
- 3) Nuclear pyknosis is seen in the remaining superficial cells.
- 4) Döderlein bacilli and *Gardnerella vaginalis* are lost.
- 5) Neutrophils appear due to secondary infection of *E. coli* or *Pseudomonas aeruginosa*.
- 6) The diagnosis of ASC-US should be avoided in the cervical cytology specimens from postmenopausal women as far as possible, because senile colpitis is unrelated to HPV infection.



Histology and cytology of senile colpitis (left: H&E, right: Papanicolaou). Parabasal cells predominate in the atrophic squamous mucosa.

Cytological diagnosis of senile colpitis

- 1) Parabasal keratinocytes seen in senile colpitis often show nuclear enlargement due to dryness of the vaginal mucosa and/or inflammatory change.
- 2) Parabasal keratinocytes may show clusters.
- 3) Senile colpitis is a benign and reactive condition, and therefore the diagnosis of NILM should be made. However, the presence of nuclear atypia may suggest the possibility of ASC. When the differential diagnosis from dysplastic lesions is difficult, the diagnosis of ASC-H may be made.
- 4) The diagnosis of ASC-US should be avoided as far as possible, since HPV-DNA analysis is recommended under the diagnosis of ASC-US.
- 5) However, squamous cell carcinoma may be seen in postmenopausal women.

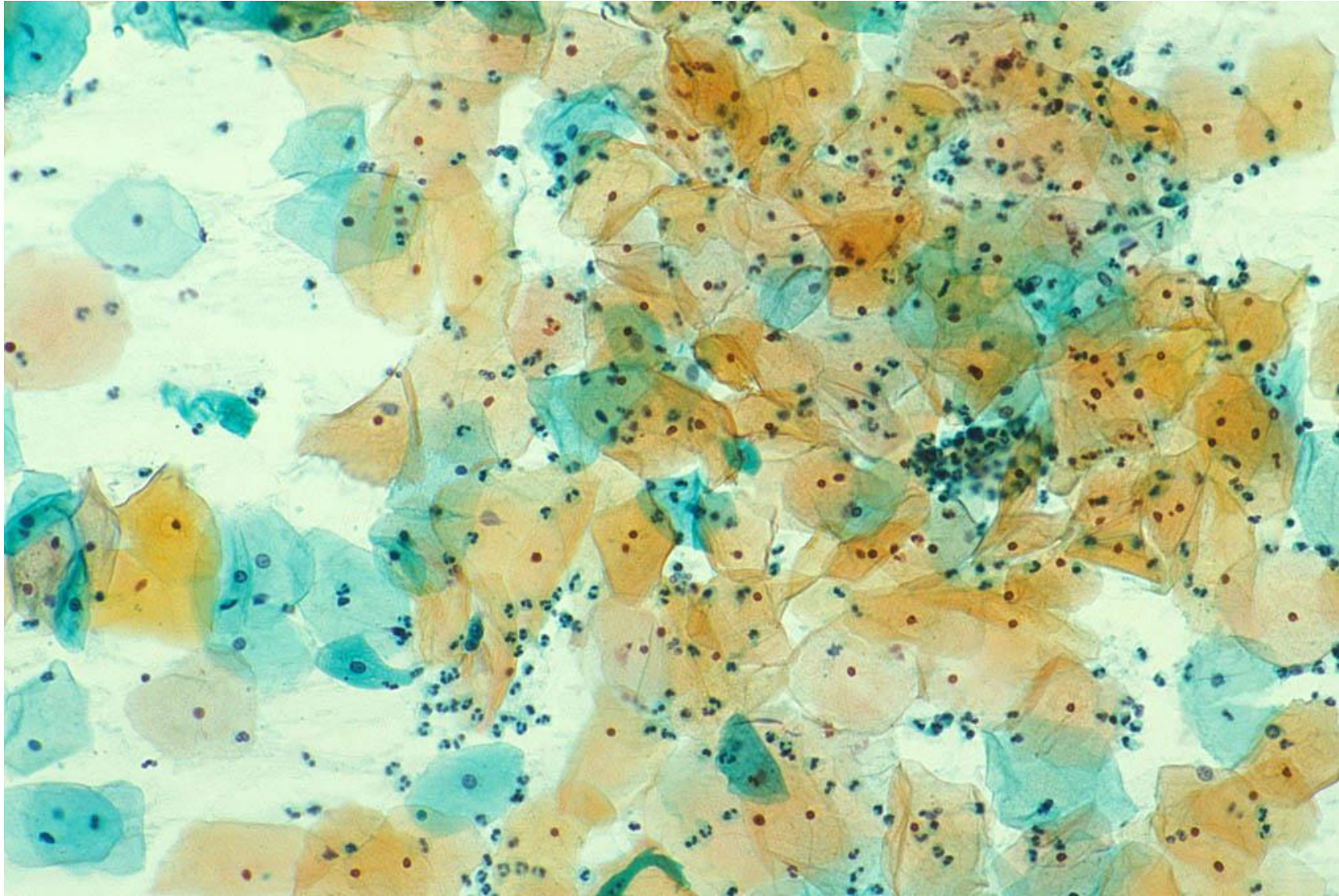
What happens when superficial keratinocytes predominate in cervical smear preparations sampled from postmenopausal women.

Parakeratotic superficial keratinocytes may be predominant in postmenopausal women, when ---

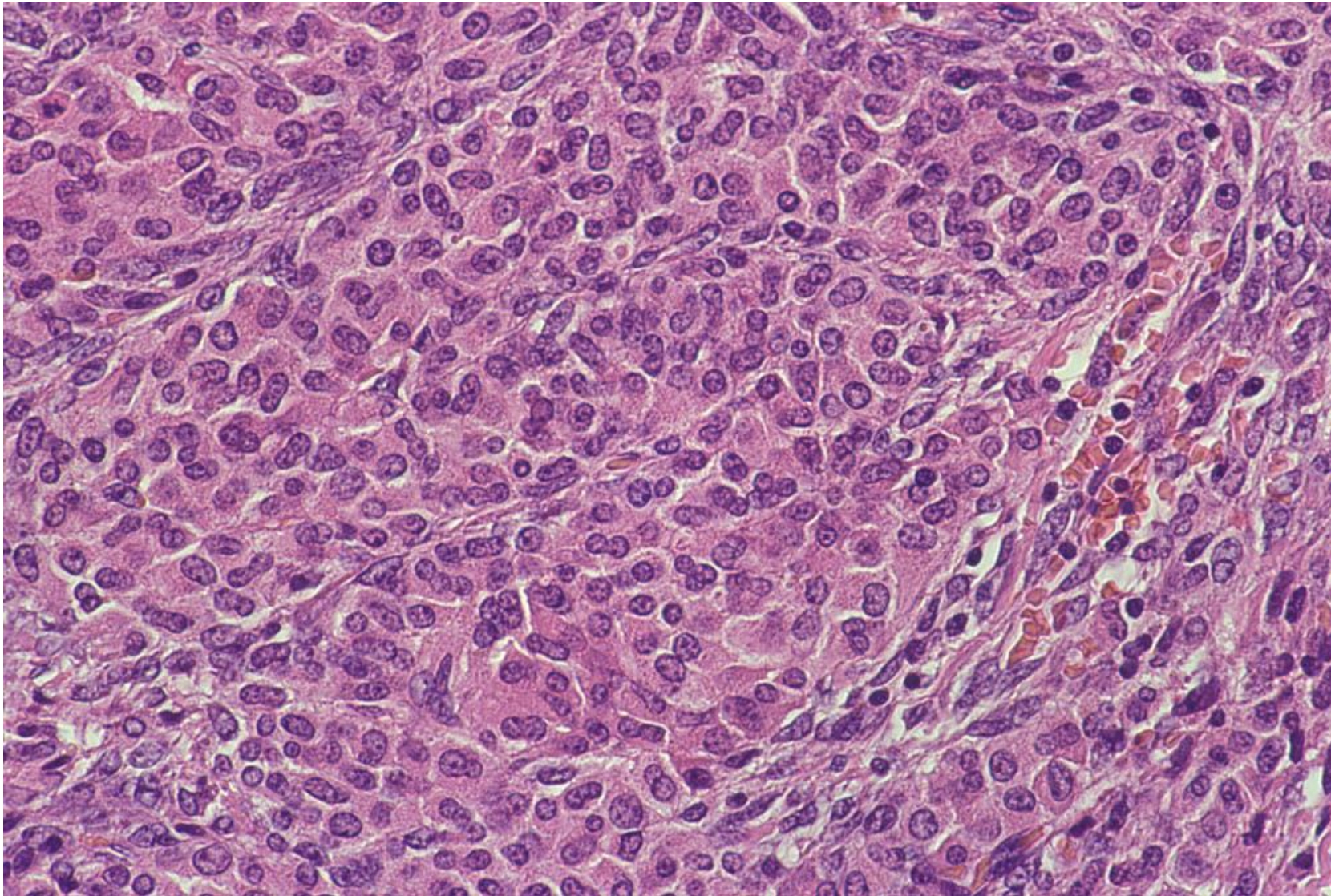
- 1) The patient takes hormonal pills or herbal medicines containing such phytoestrogens as isoflavonoids, flavonoids, stilbenes, and lignans.
- 2) Infrequently, the patient has an estrogen-secreting ovarian tumor such as thecoma.

A 70-year-old lady complained of irregular vaginal bleeding. She has been proud of her young and resilient skin.

- 1) Cervical cytology revealed numbers of superficial keratinocytes.
- 2) Endometrial biopsy reveals proliferative endometrial tissue.
- 3) Echo examination identified a small 1 cm-sized solid tumor.
- 4) The surgical removal of the uterus and bilateral ovaries was performed.
- 5) The histopathological diagnosis of the ovary was thecoma.
- 6) As a result, her young vivid skin soon turned to be “aged”, and she lost the cherished motivation of life.



The cervical smear preparation from a 70-year-old lady reveals young cells of the superficial keratinocytes. Papanicolaou stain



The thecoma brings to her:

- 1) The young skin
- 2) Vaginal bleeding
- 3) No threat to her life because of its benignity
- 4) Final histopathological diagnosis can be made after surgical removal.
- 5) An increased risk of endometrial and breast cancer may be associated.

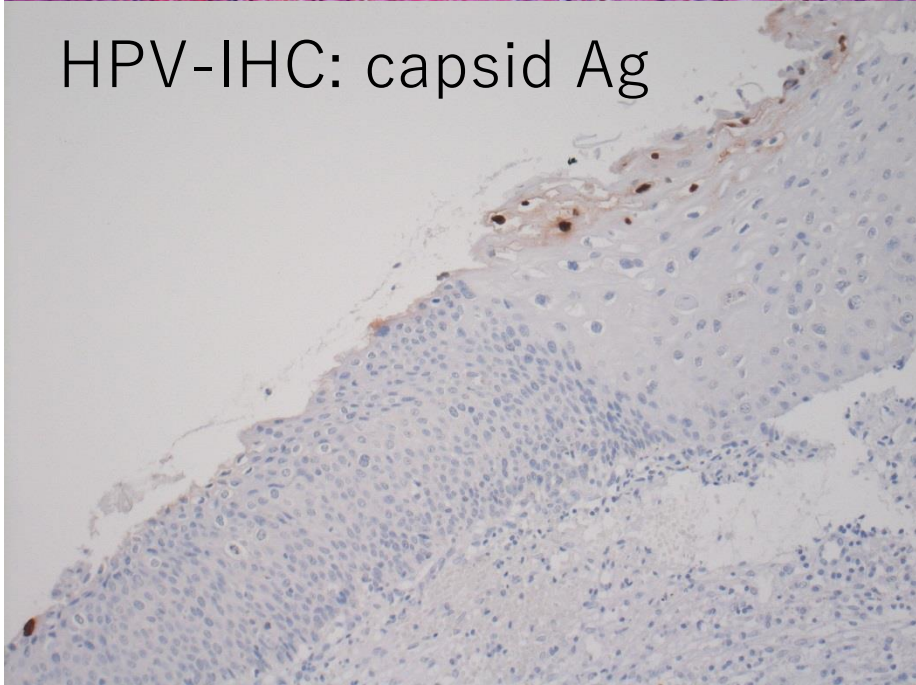
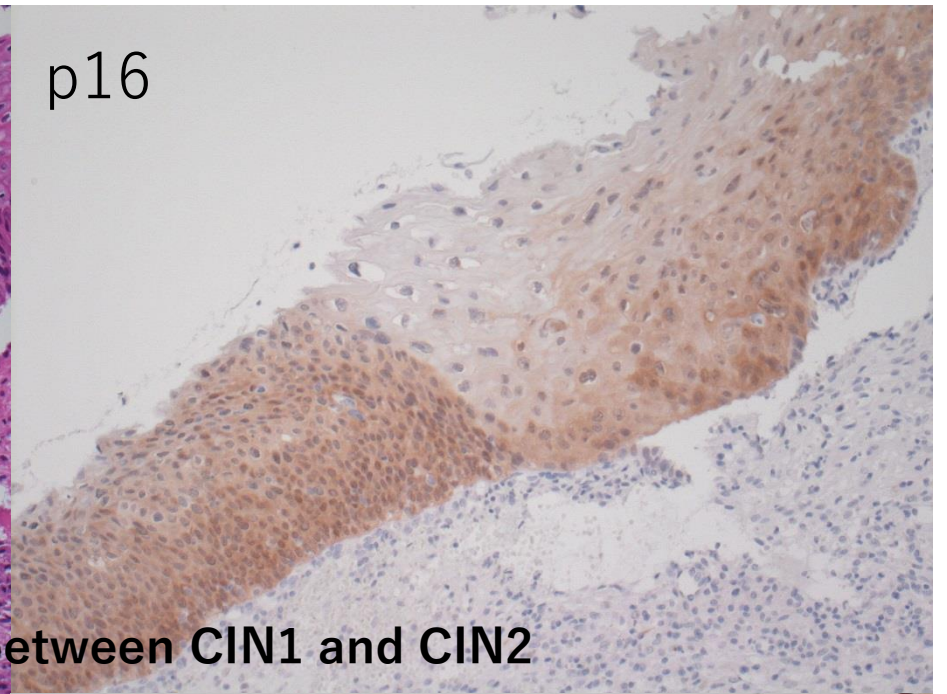
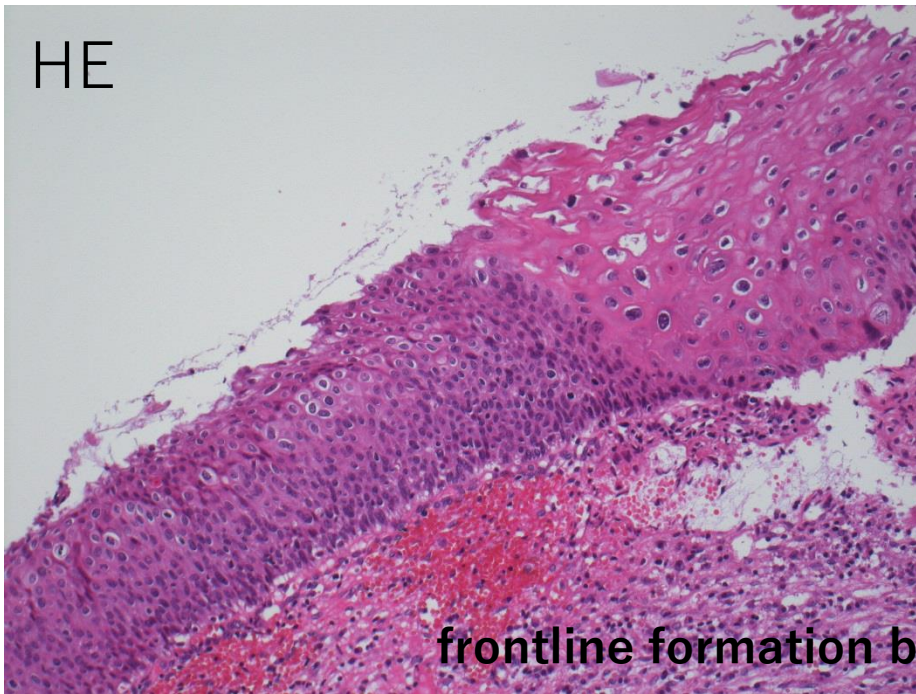
The ovary contained estrogen-secreting benign thecoma, a secret of youthfulness. The tumor had maintained patient's skin young.

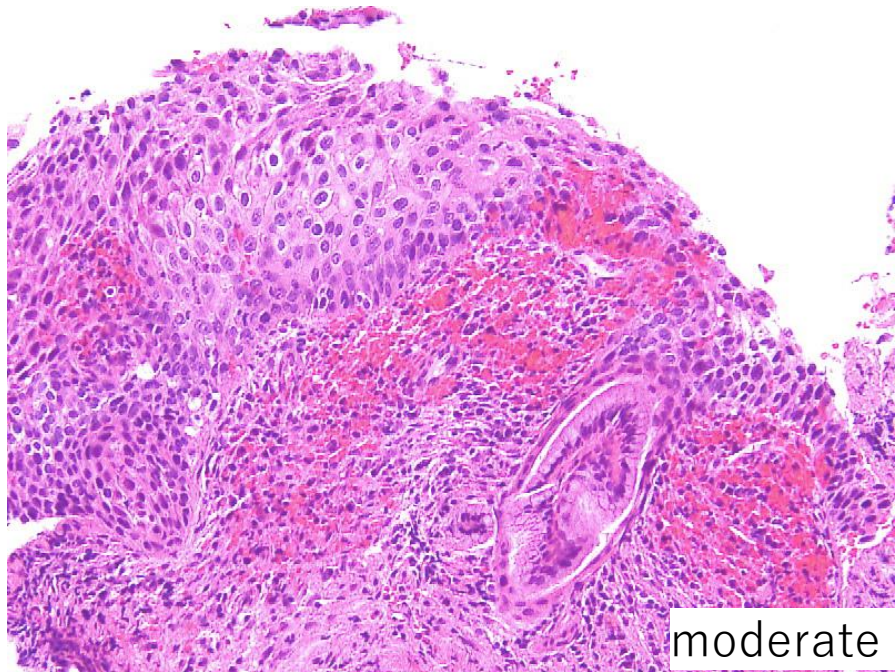
Application of p16 immunostaining for the differential diagnosis between senile colpitis and dysplasia

- 1) p16 (p16-INK4A) is expressed in the lesion of carcinogenic HPV infection.
- 2) The lesion of senile colpitis is negative for p16.
- 3) p16 immunostaining in the cytology preparation is useful for distinguishing dysplasia from senile colpitis.
- 4) For routine cytology smear preparations, cell transfer technique onto the Silene-coated glass slides is requested.
- 5) Liquid-based cytology (LBC) is especially useful for immunocytochemical staining. LBC is not popular in Japan.

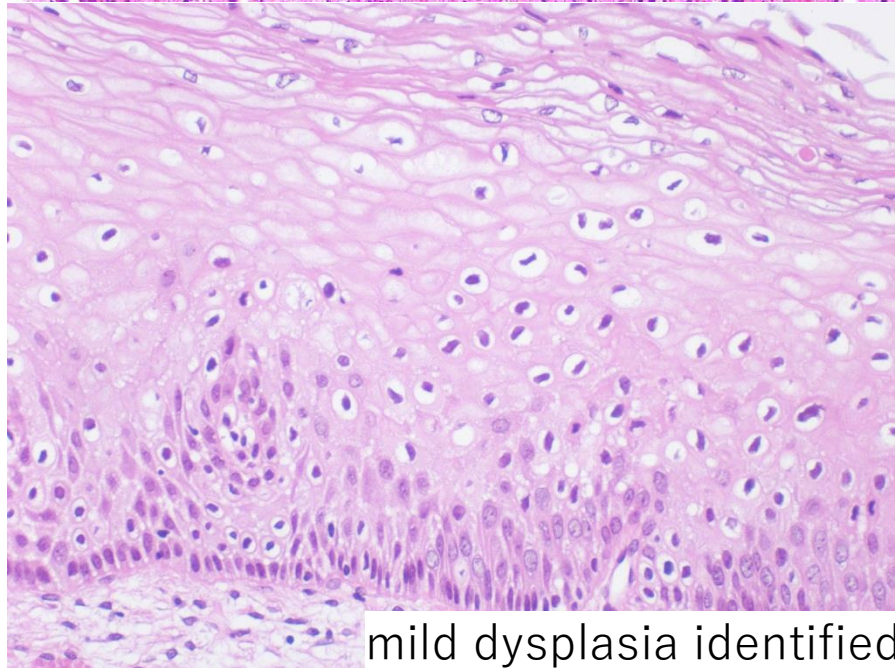
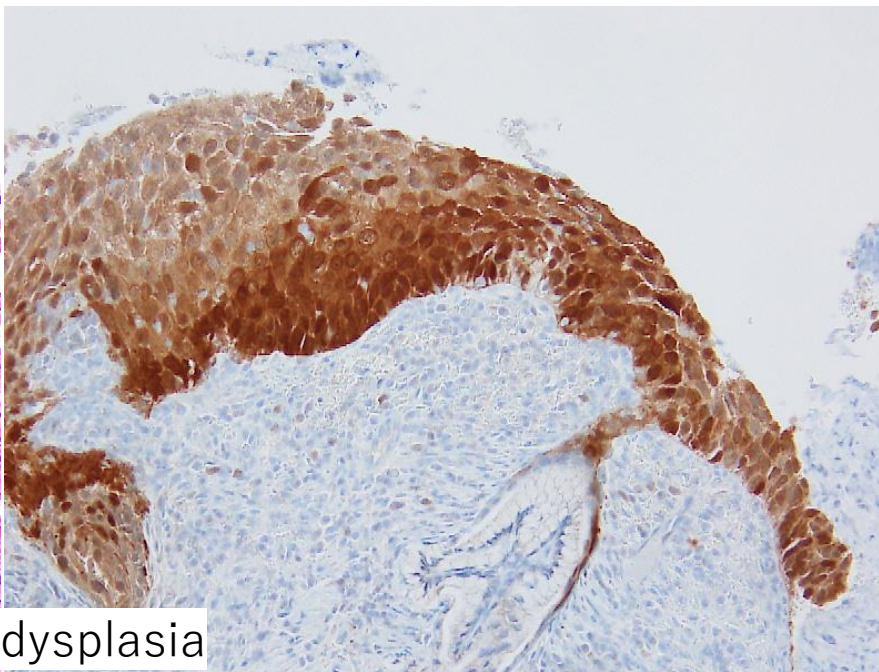
42 y-o F
CIN1+CIN2

p16 immuno-
staining is
much more
sensitive than
HPV histo-
chemistry.

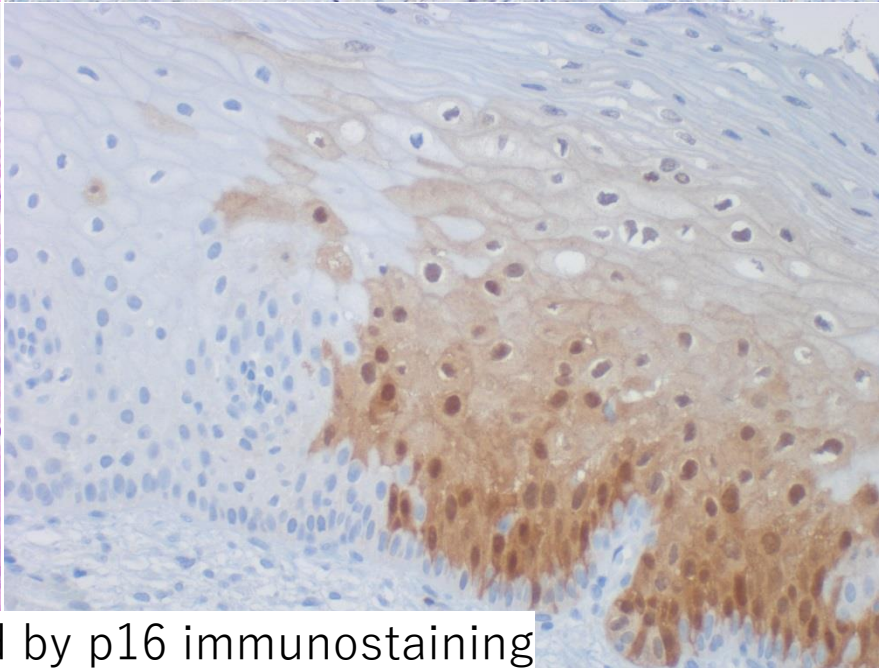




moderate dysplasia



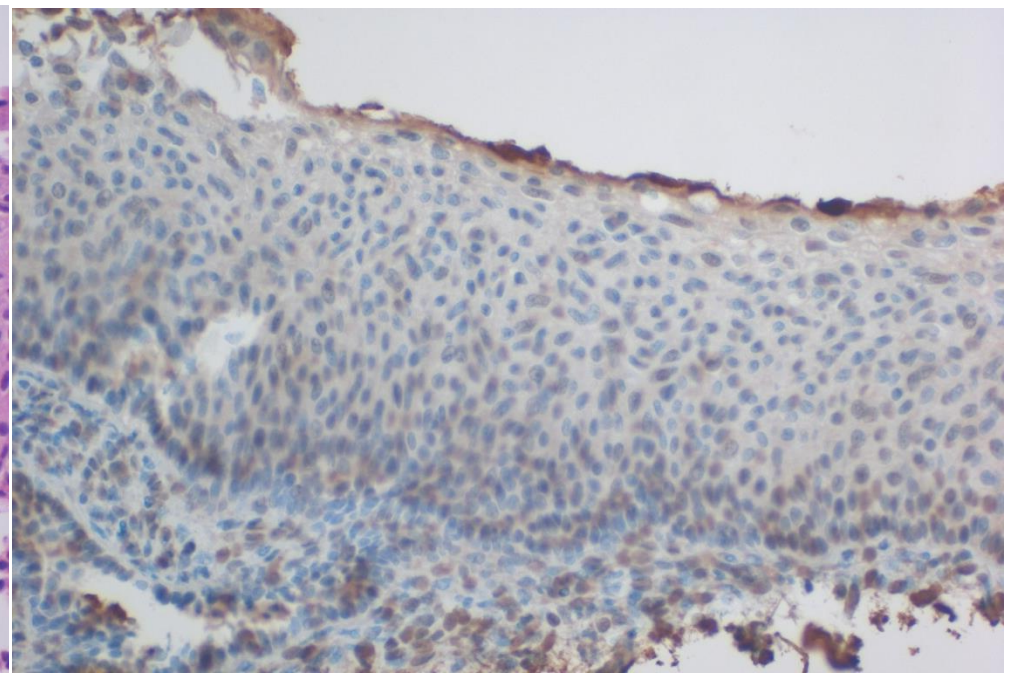
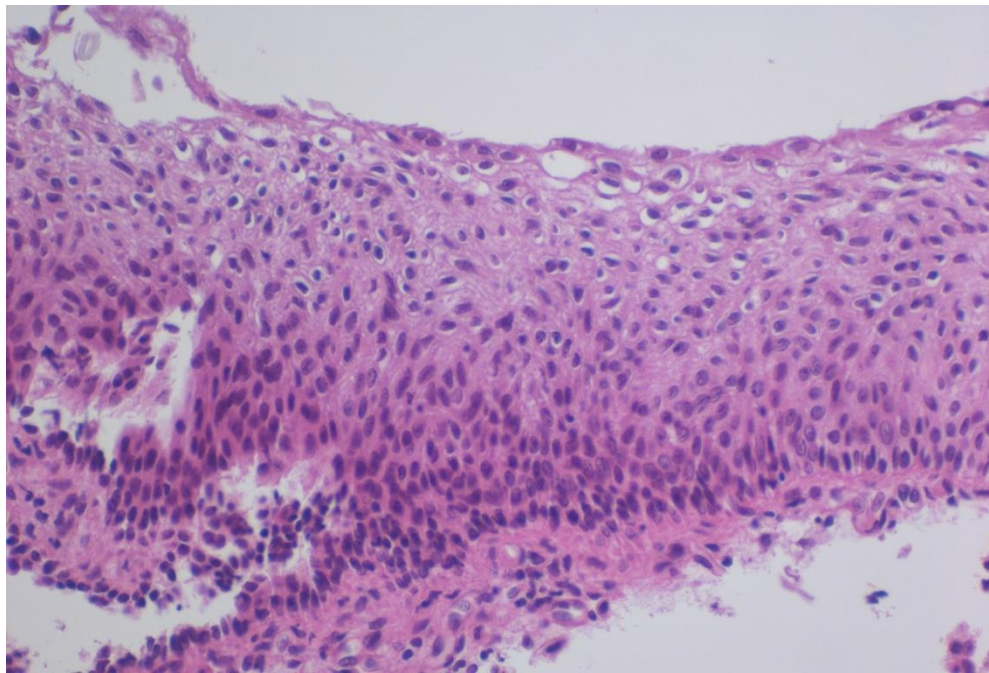
mild dysplasia identified by p16 immunostaining



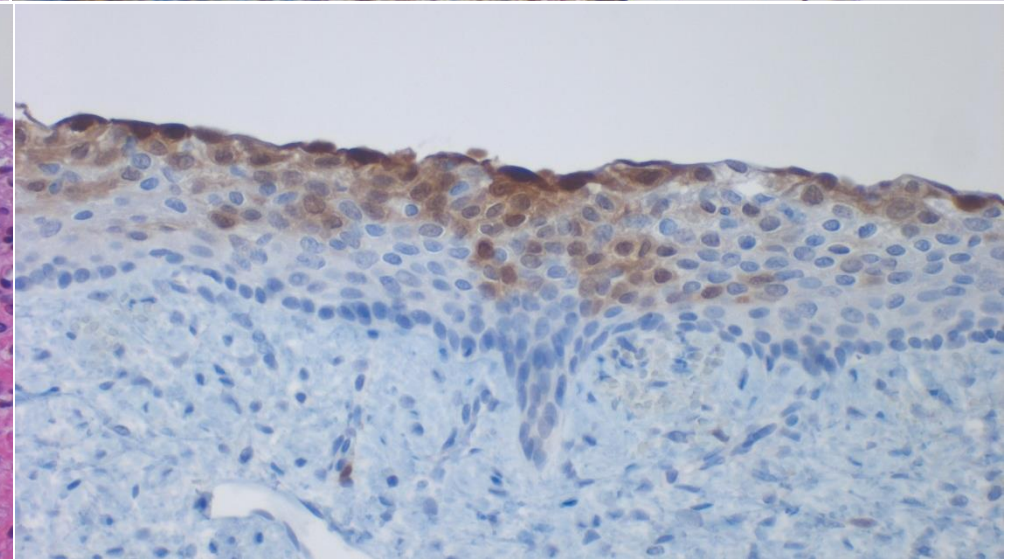
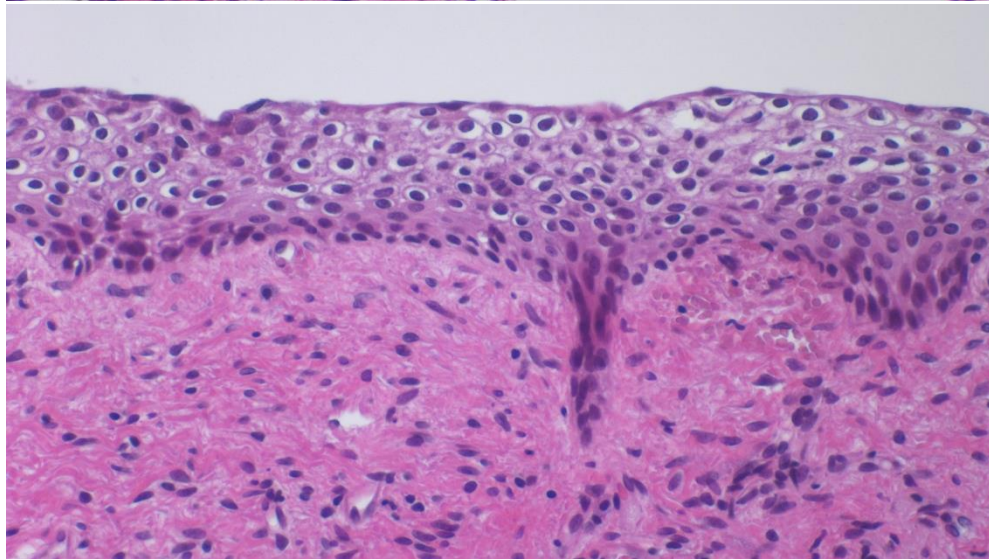
p16 immunostaining

(formalin-fixed, paraffin-embedded sections of the uterine cervix)

Case 1

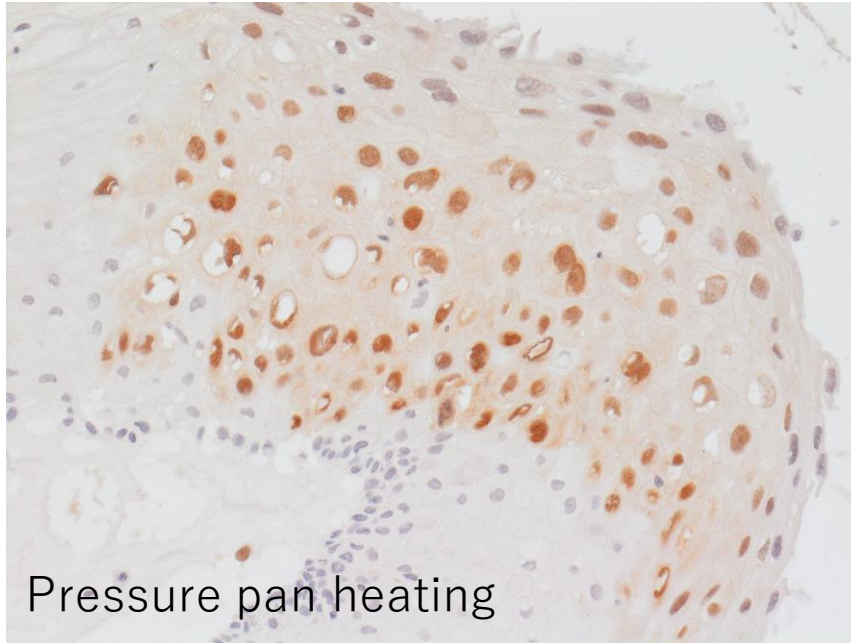


Case 2

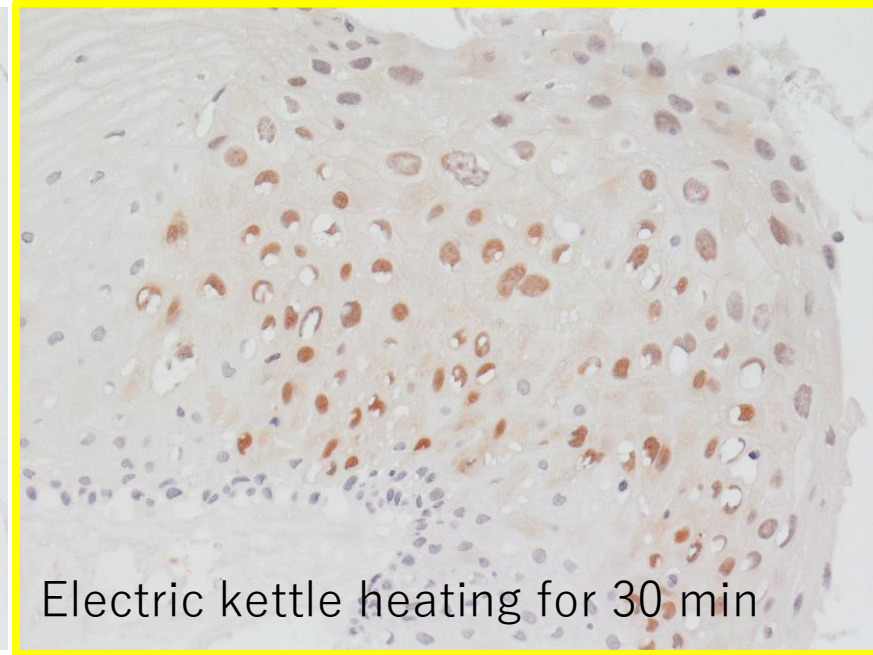


p16 immunostaining in senile colpitis (negative in the parabasal layer)

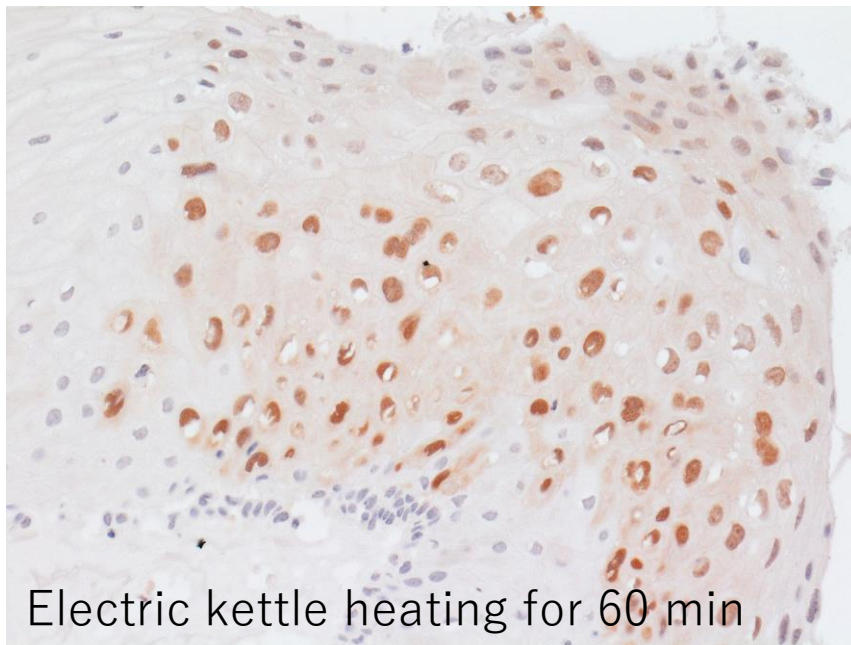
Suitable conditions for p16 immunostaining in cytology specimens



Pressure pan heating



Electric kettle heating for 30 min



Electric kettle heating for 60 min

Object :

Paraffin sections of moderate dysplasia

Staining conditions :

1st Ab: p16 MsMo Ab (E6H4), 1:10, overnight

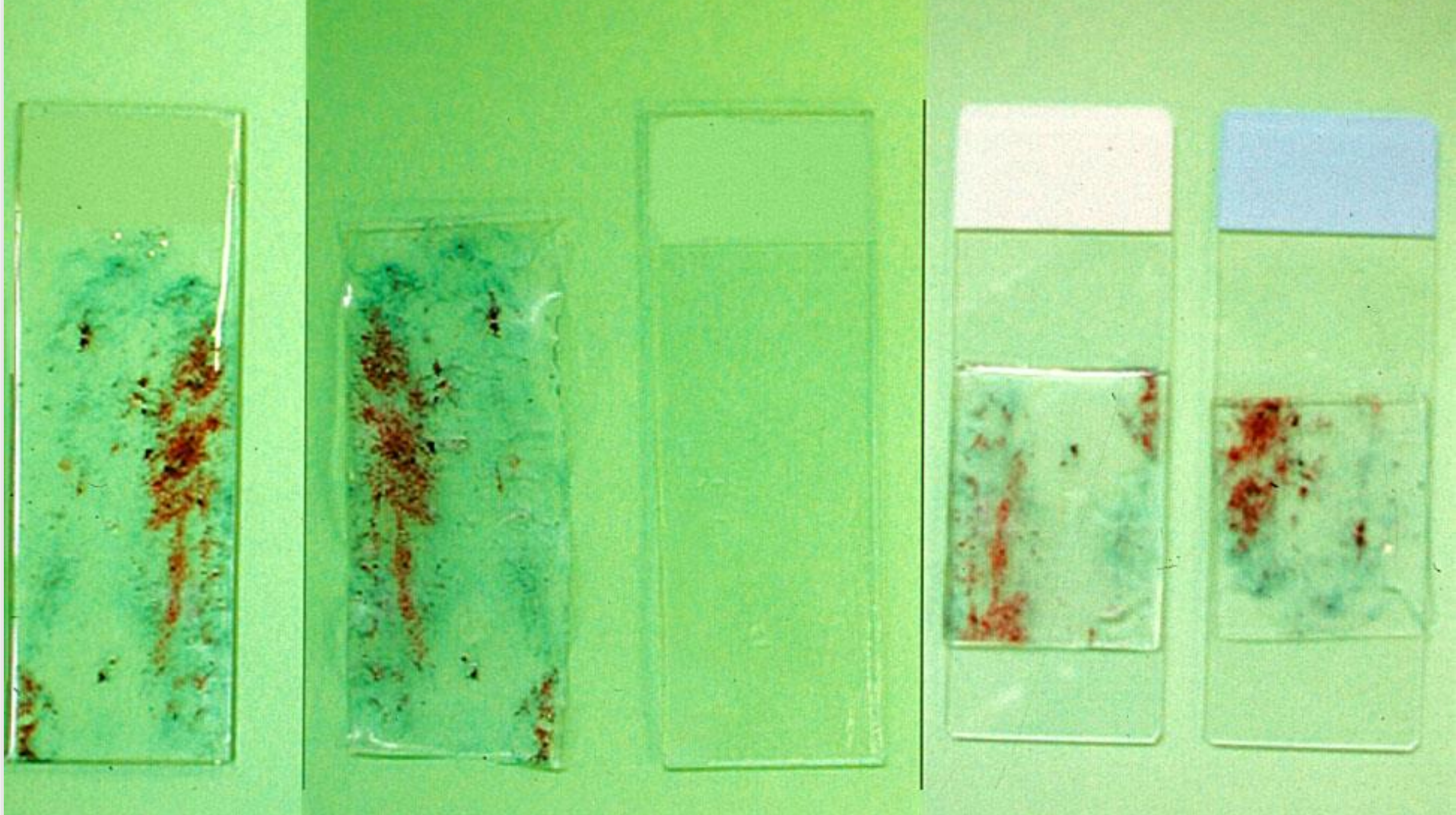
CINtec Histology kit (Ventana)

Retrieval solution: 1 mM EDTA, pH8.0

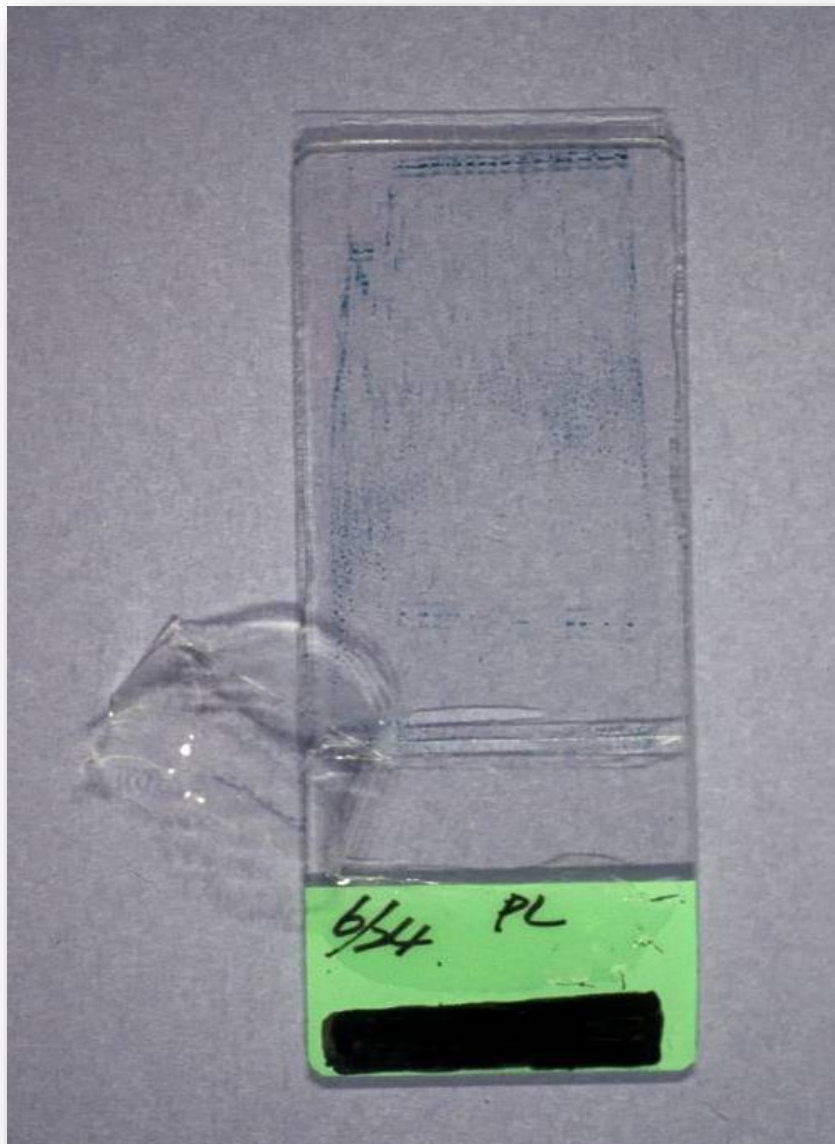
2nd Ab: Simple Stain MAX-PO (MULTI), 30min

Heating in an electric kettle for 30 min was chosen.
→p16 signals can be detected without detaching cells during the heating procedure.

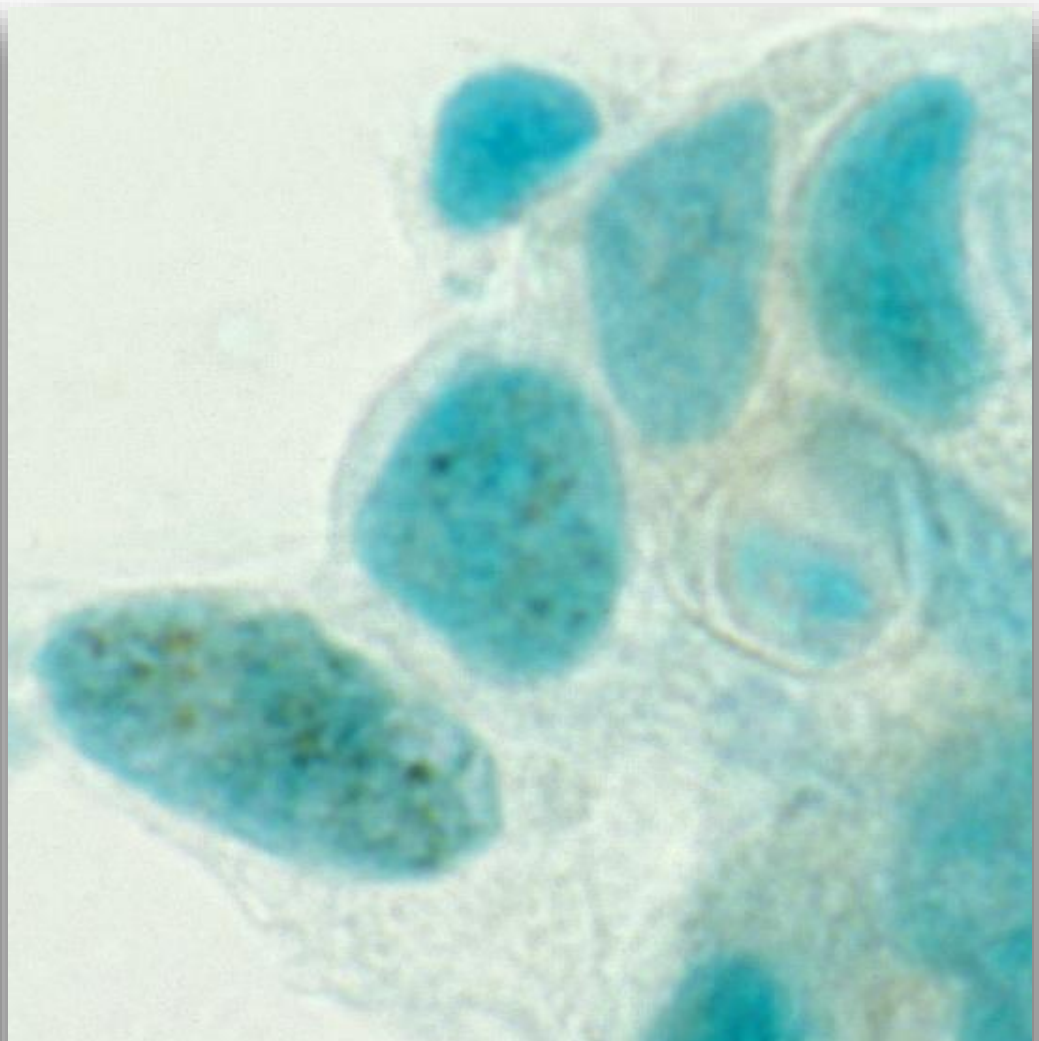
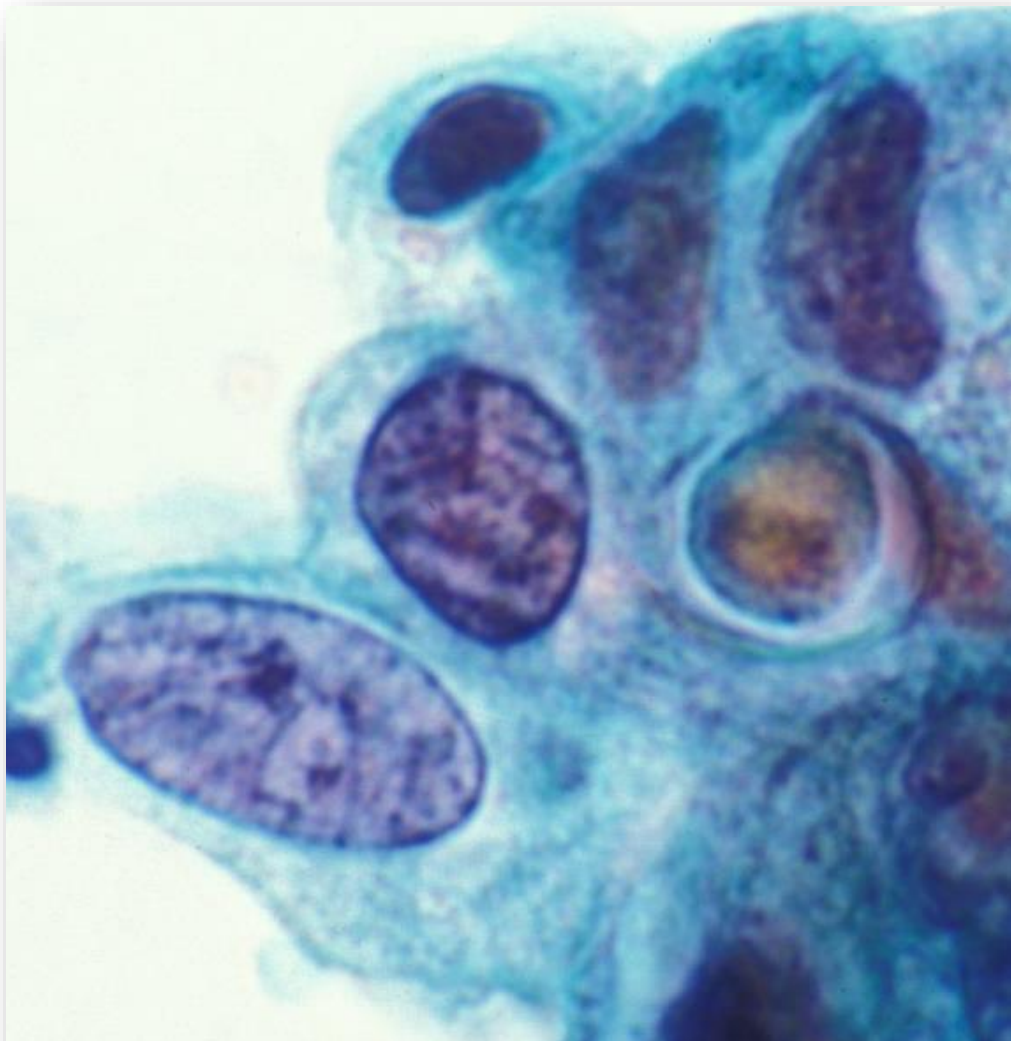
Cell transfer technique for cervical cytology preparations



The solidified Malinol membrane was cut into two pieces, and mounted onto the Silane-coated glass slides.



In cervical cytology preparations, the portion not covered by cover slip can be transferred onto the Silane-coated glass slide. In this case, time-consuming detachment of the cover slip is unnecessary.



In situ hybridization for HPV-16 genome in HSiL
(left : pap, right: ISH technique showing dotted signals)

ISH for HPV 16 employing heating treatment was performed after transfer to the Silane-coated glass slide.

Cervical smear preparations from postmenopausal women: analysis of 13 cases

Cytological diagnosis: done by 4 pathologists

Age: 56-82 years (mean: 64.7, median 64)

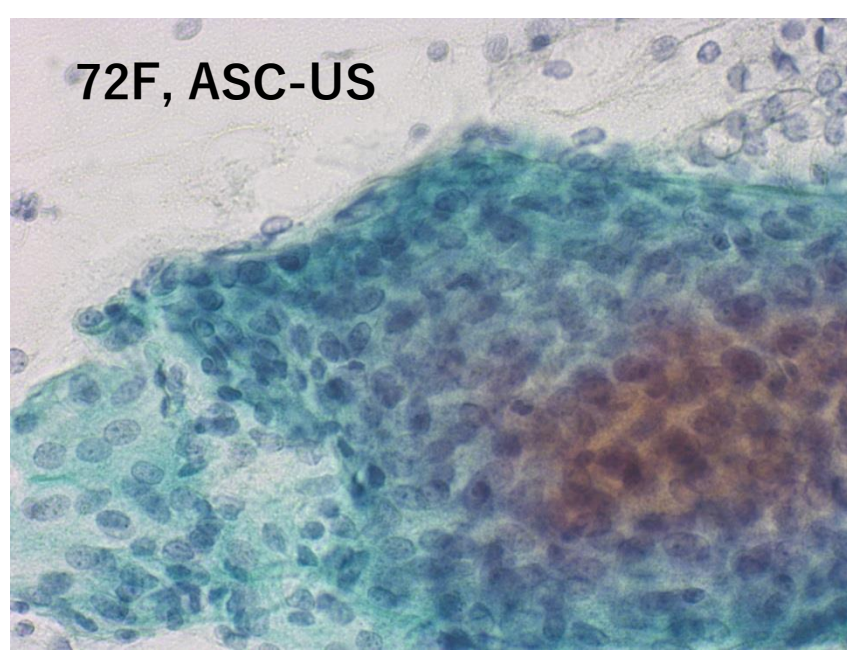
Number of specimens: 29 (1-6 specimens /case)

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

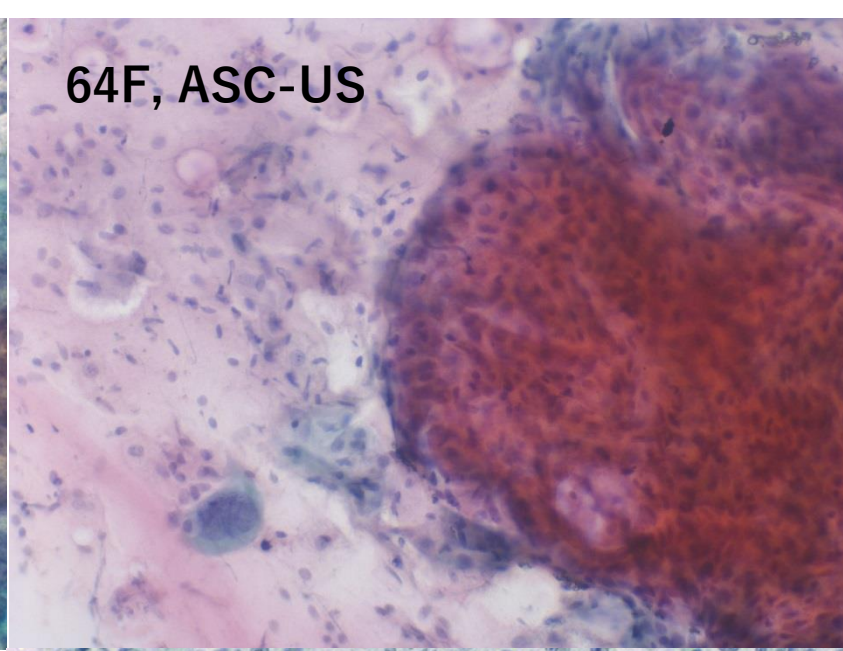
p16 positivity: 3 specimens from 2 cases of HSIL

Biopsy from 13 cases: CIN1 1 (ASC-US), CIN2 1 (HSIL)

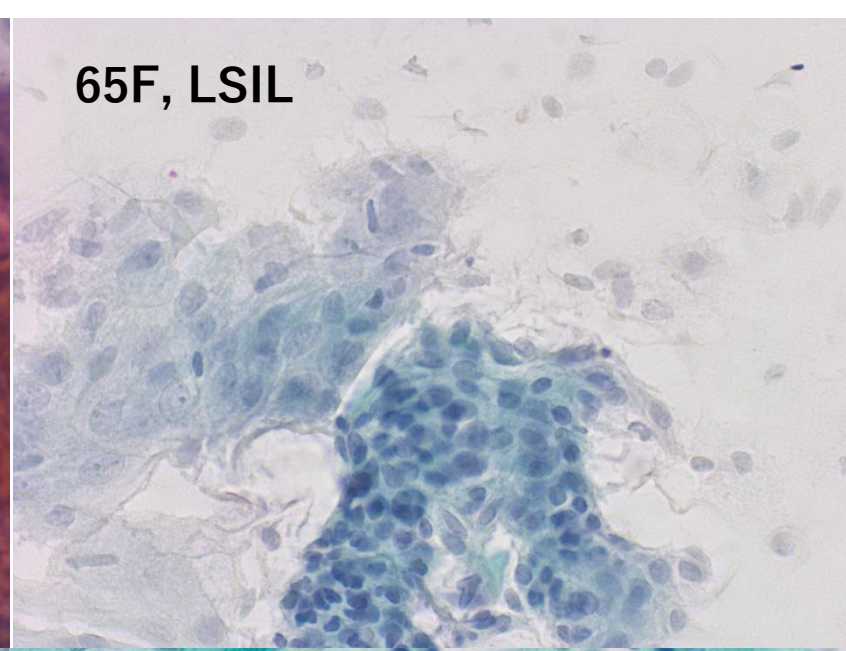
72F, ASC-US



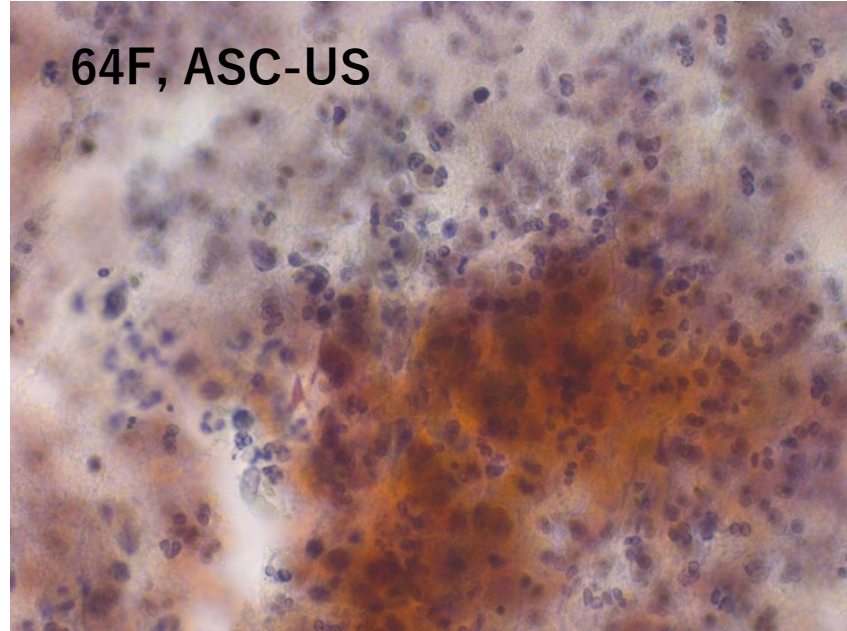
64F, ASC-US



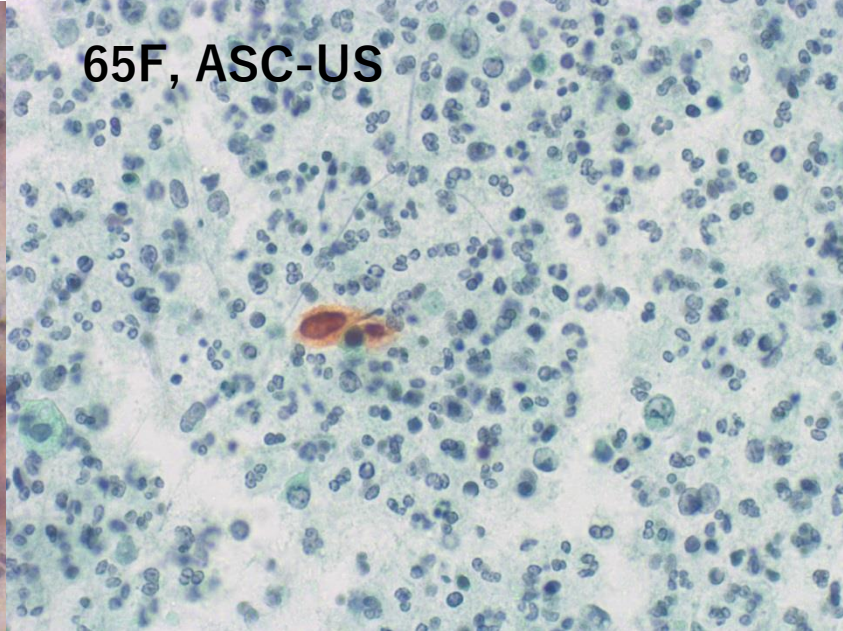
65F, LSIL



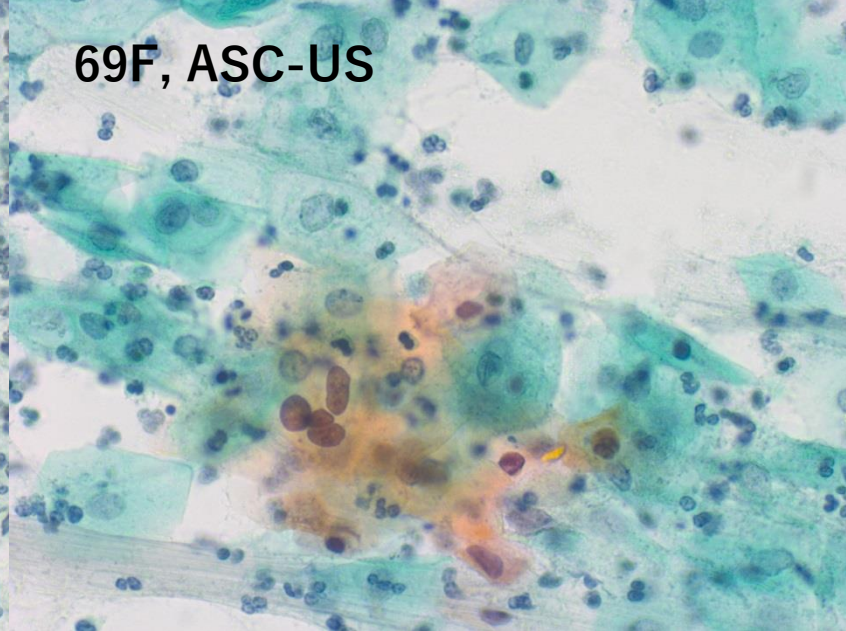
64F, ASC-US



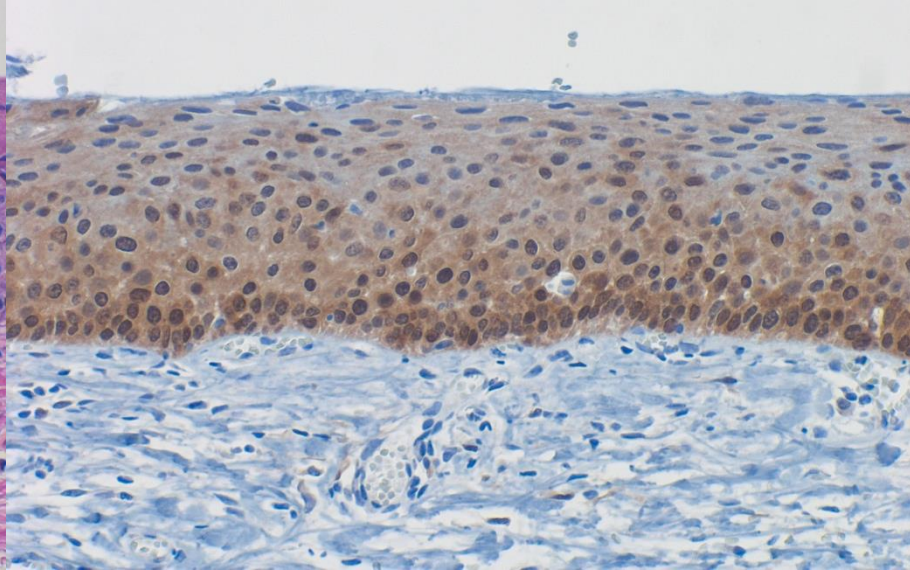
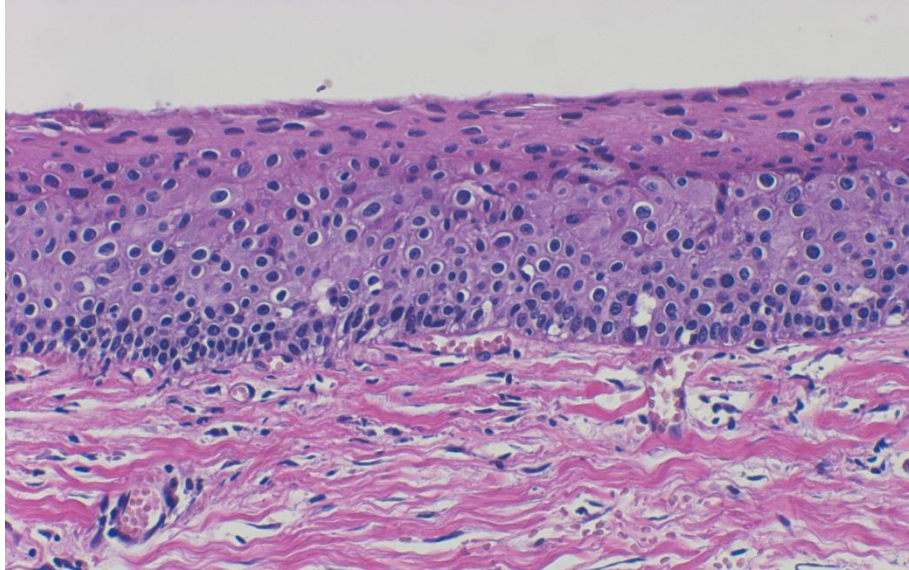
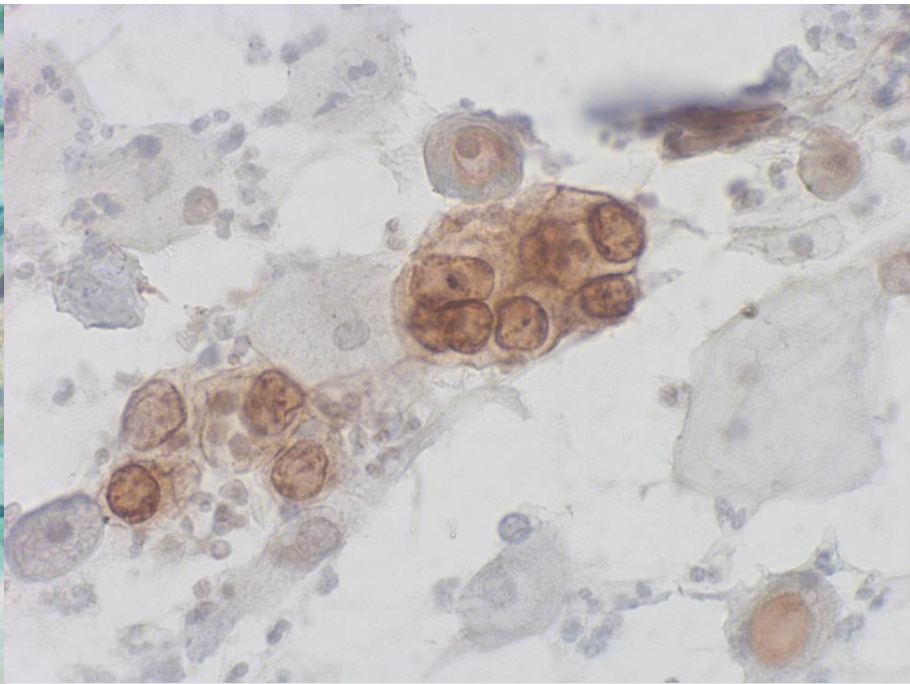
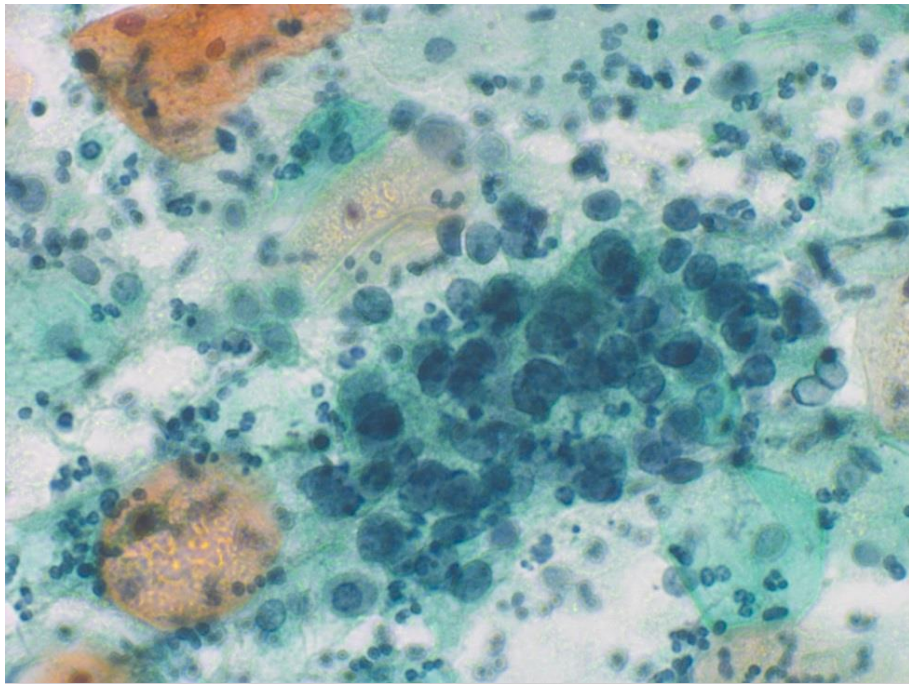
65F, ASC-US



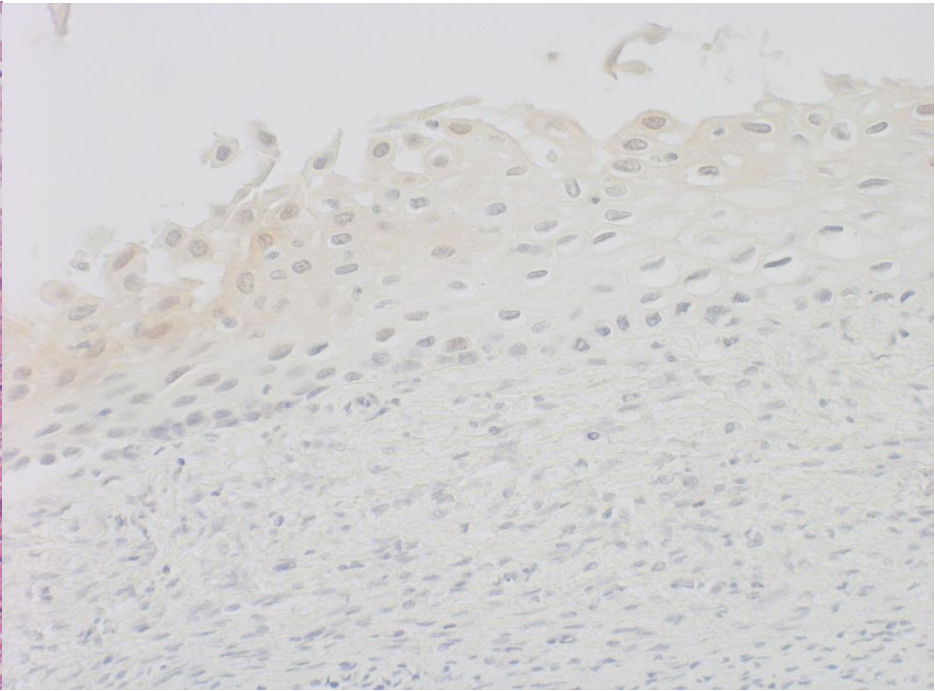
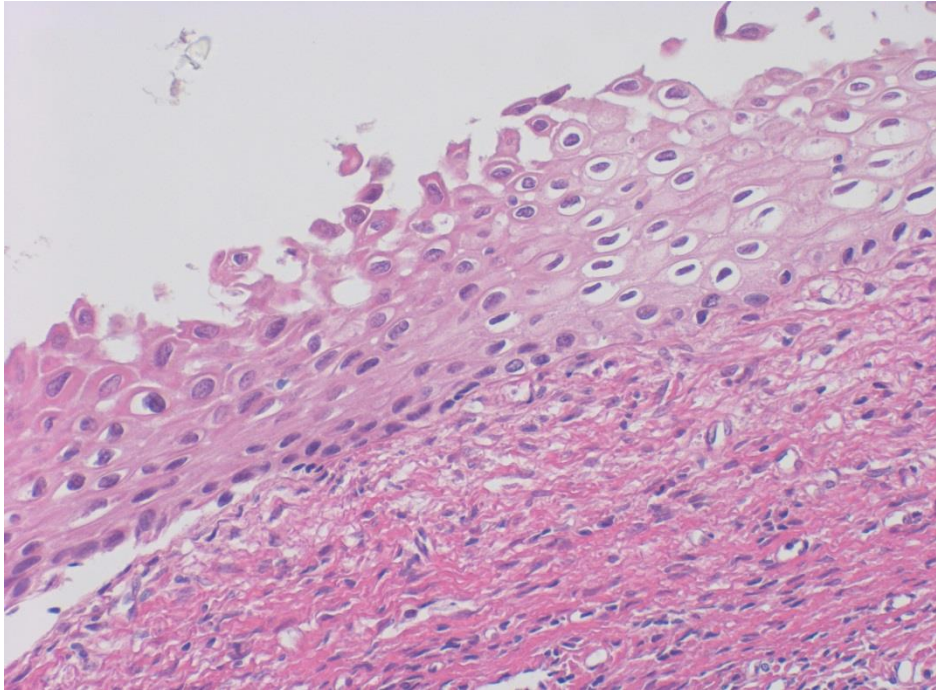
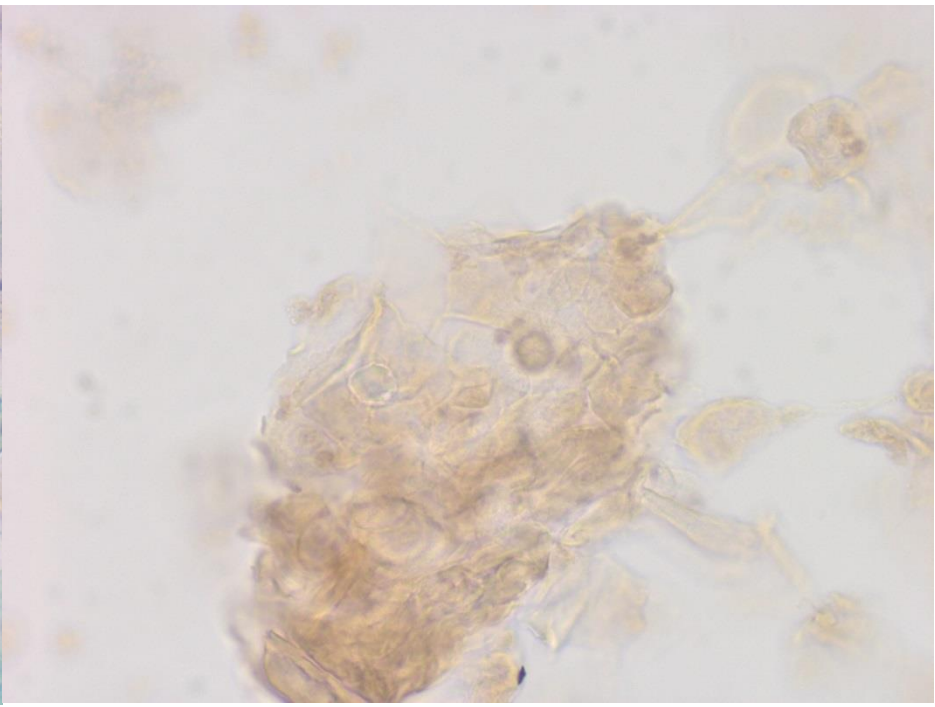
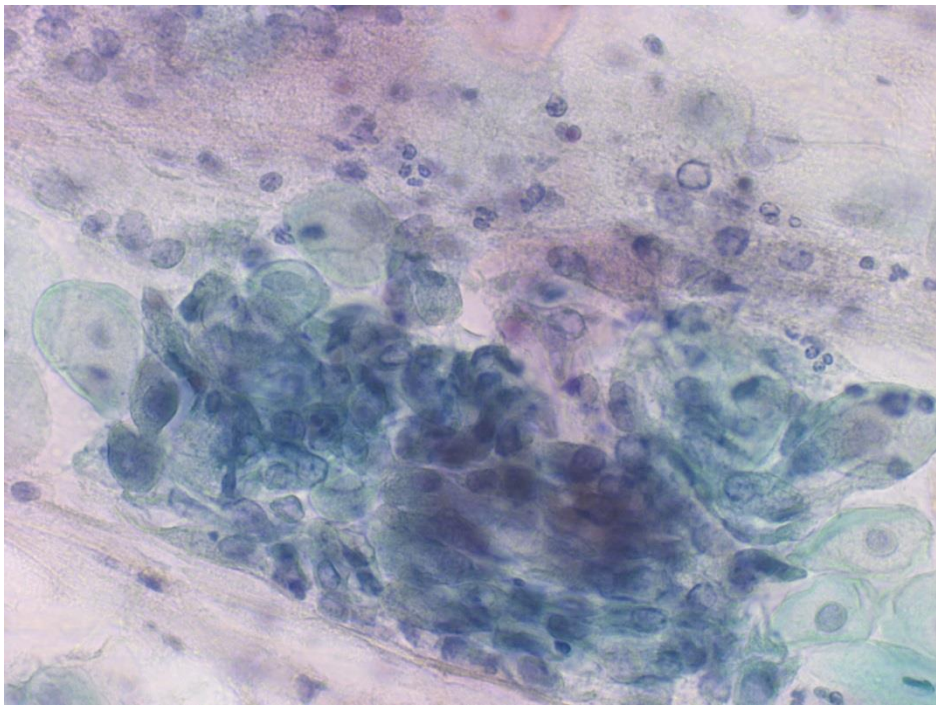
69F, ASC-US



Two faces of p16-negative senile colpitis: clustered parabasal cells (upper panels) vs. atypia in the superficial keratinocytes in inflammatory background (lower panels)

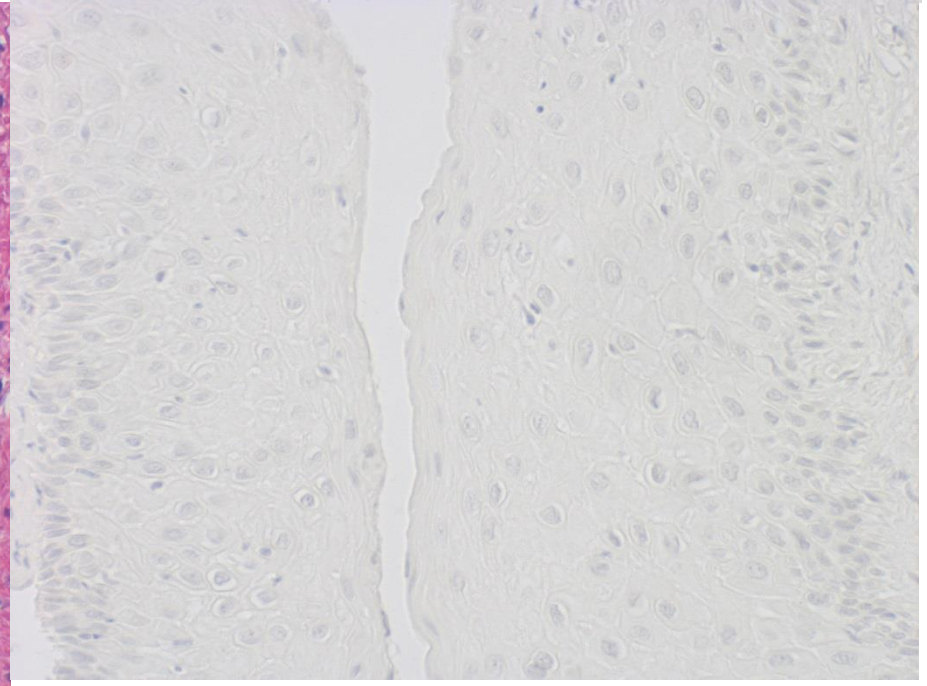
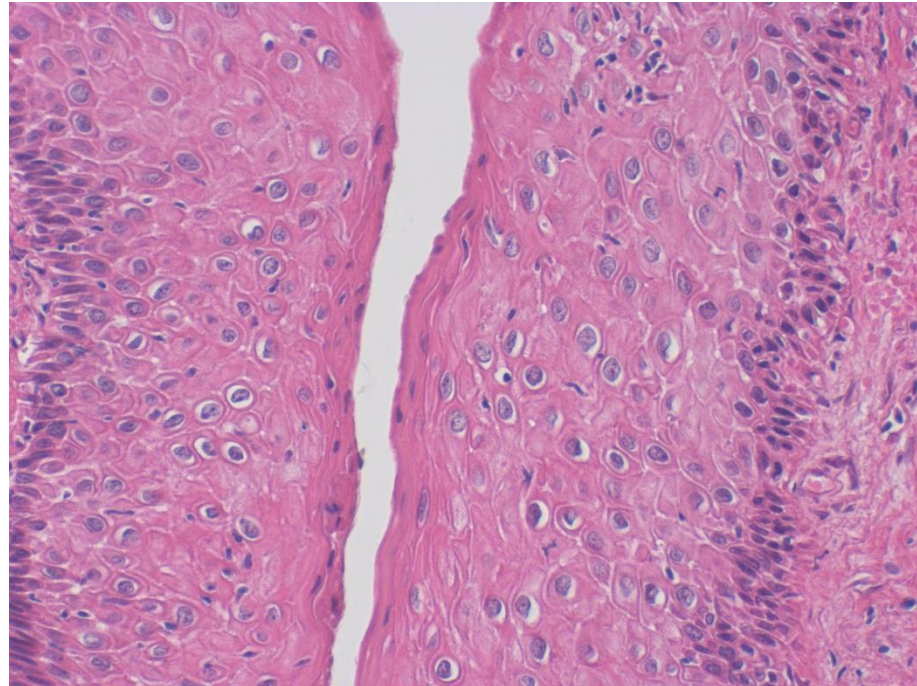
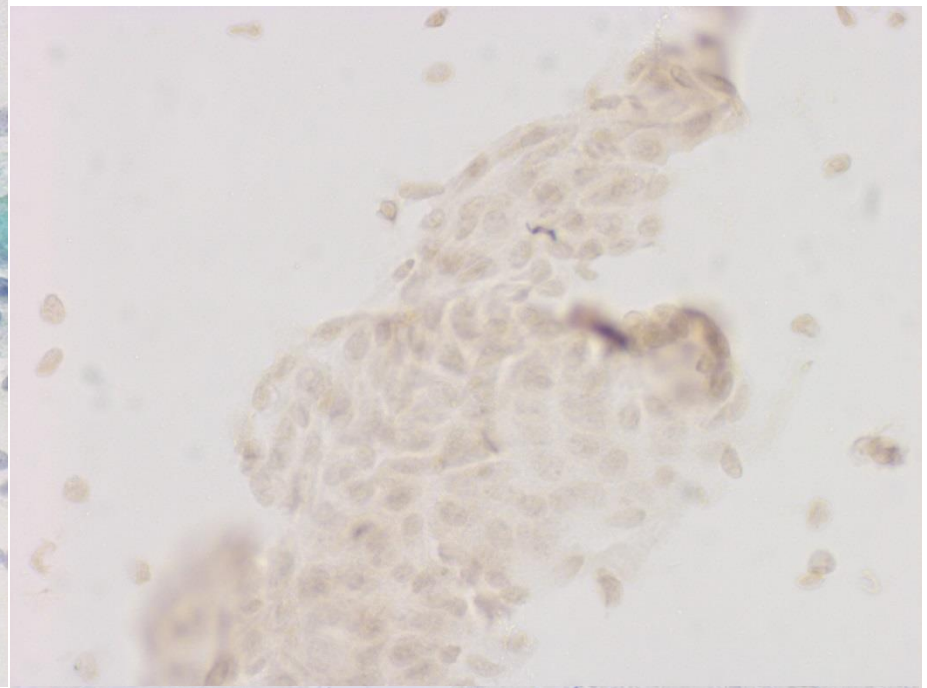
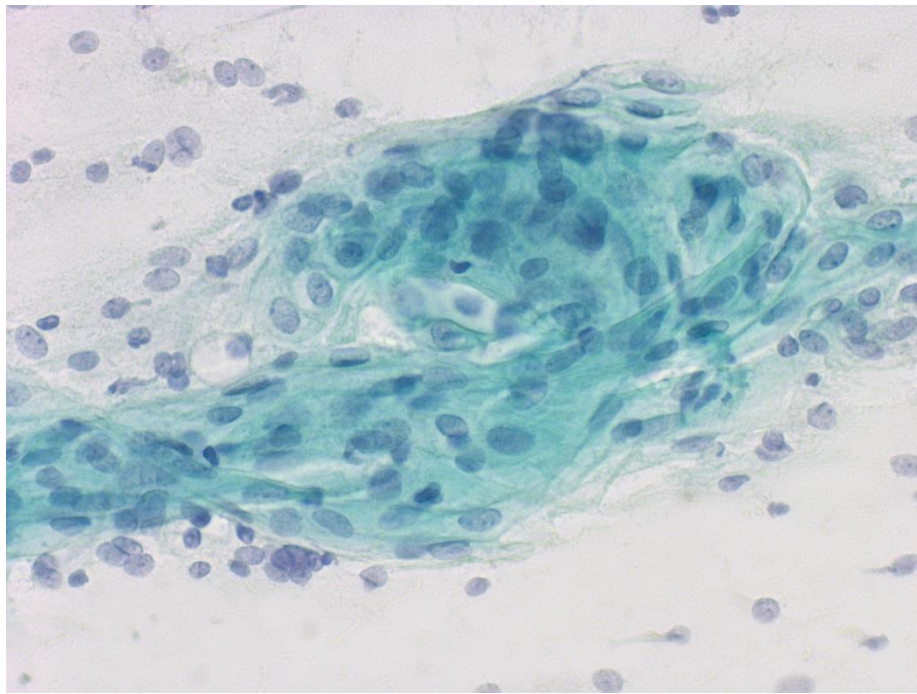


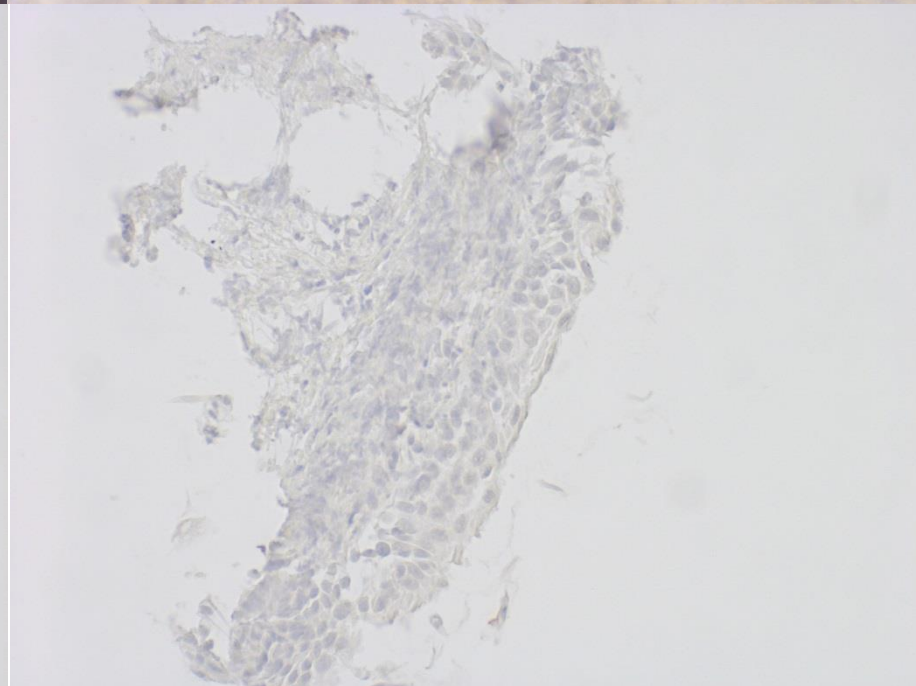
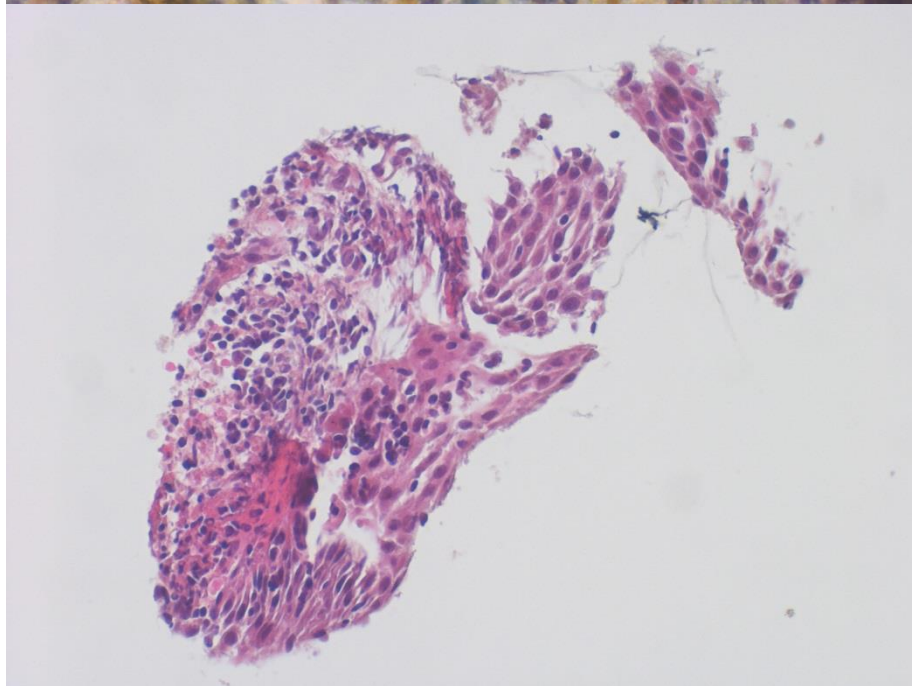
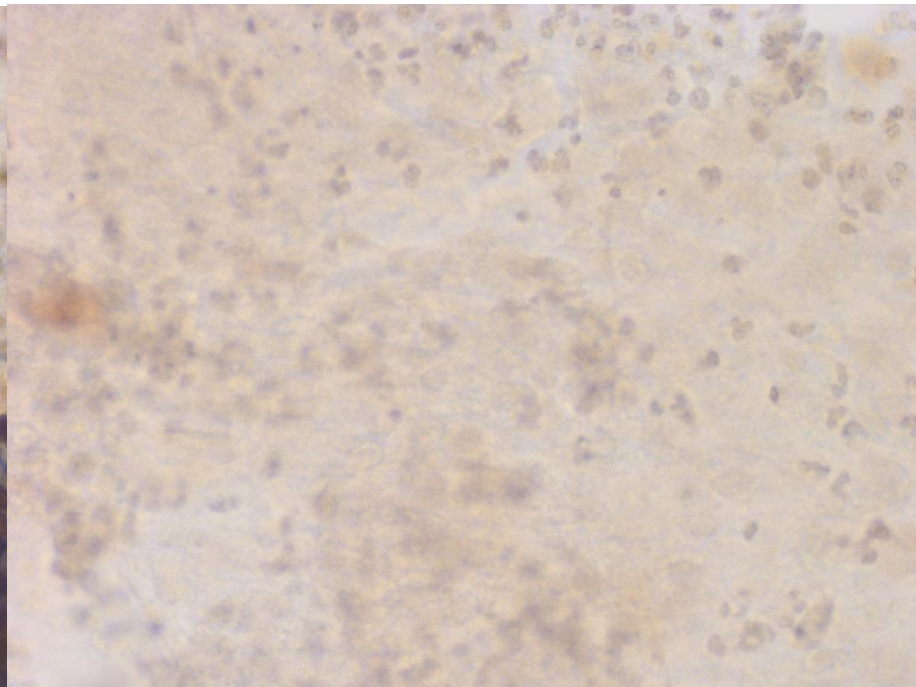
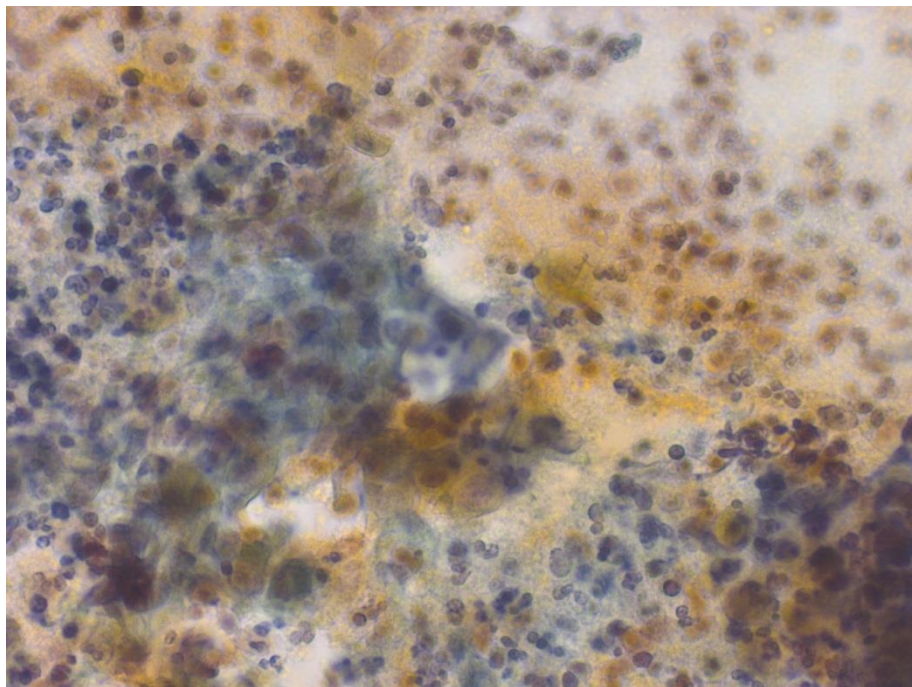
An 82 y-o lady: moderate dysplasia (HSIL) • p16 is expressed in parabasal keratinocytes.



62 y-o
lady:
ASC-US
P16 (-)

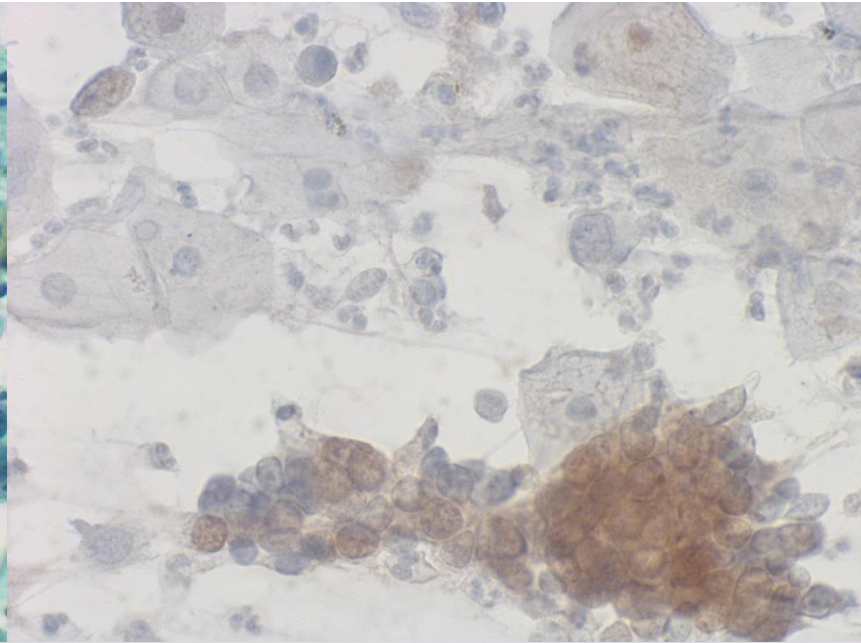
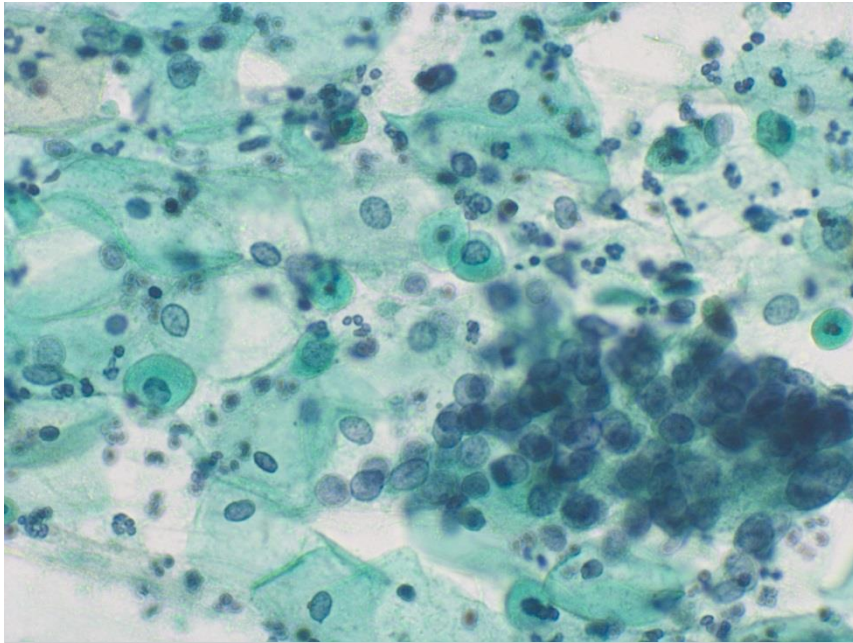
72 y-o
lady
ASC-US
P16 (-)



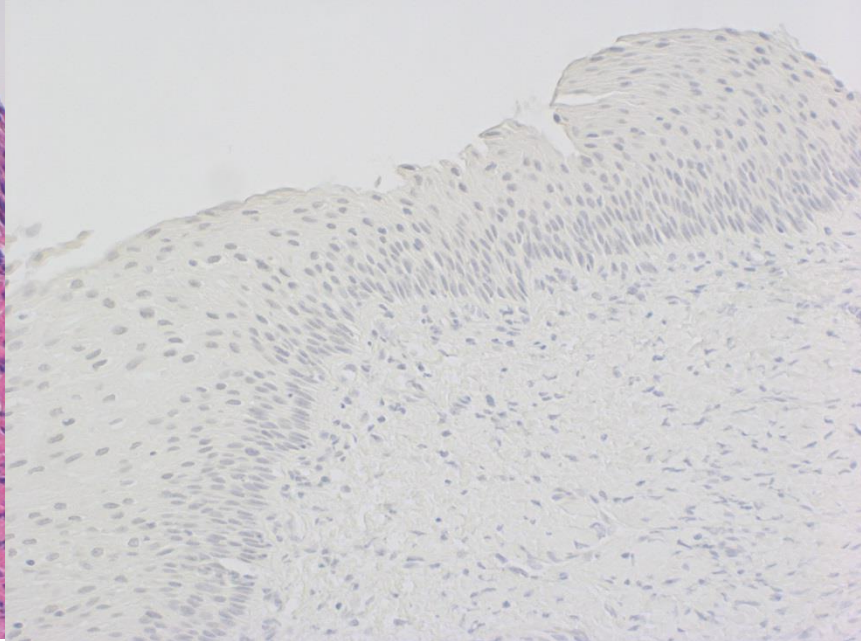
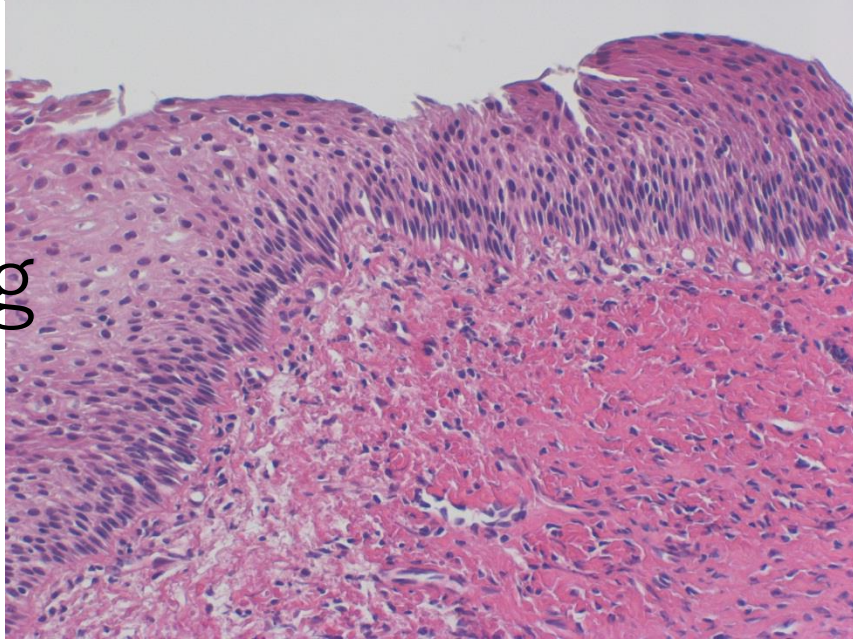


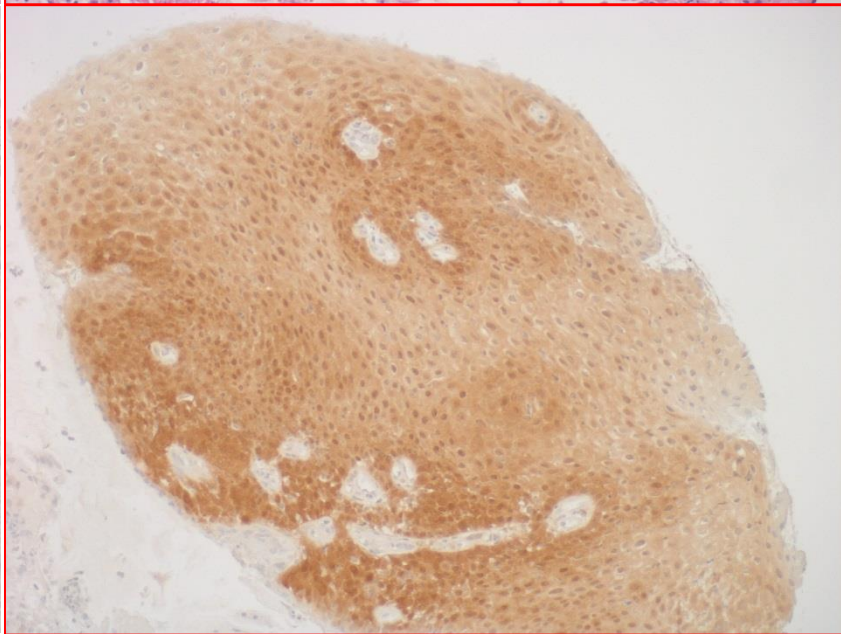
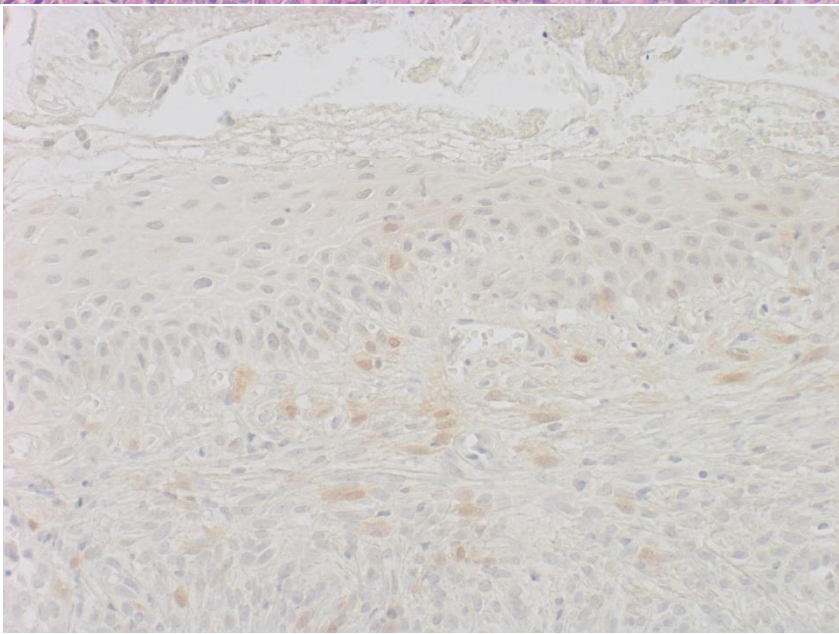
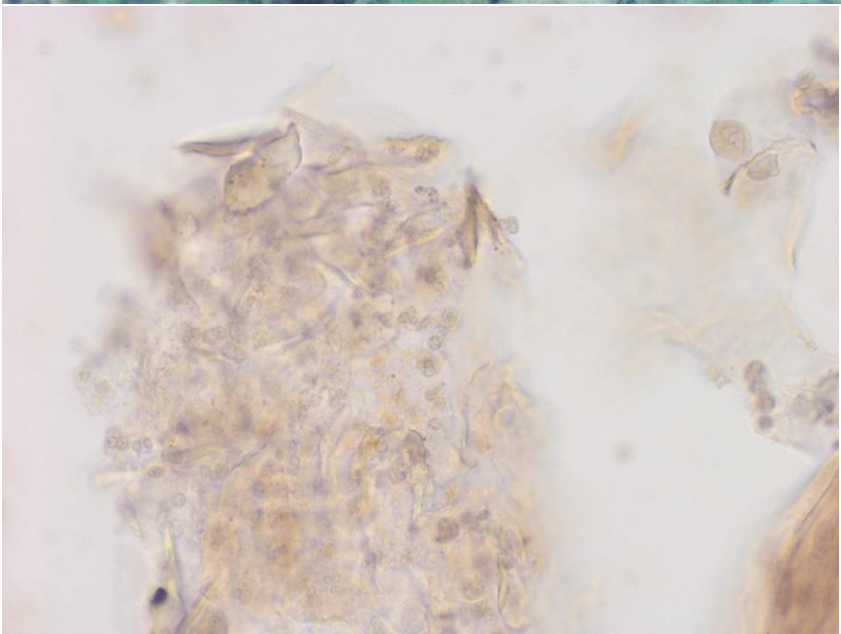
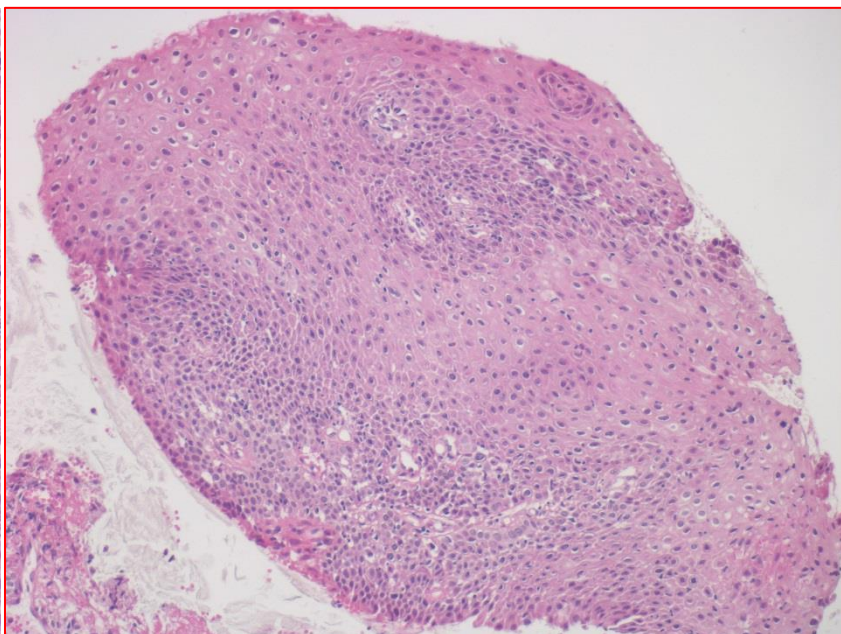
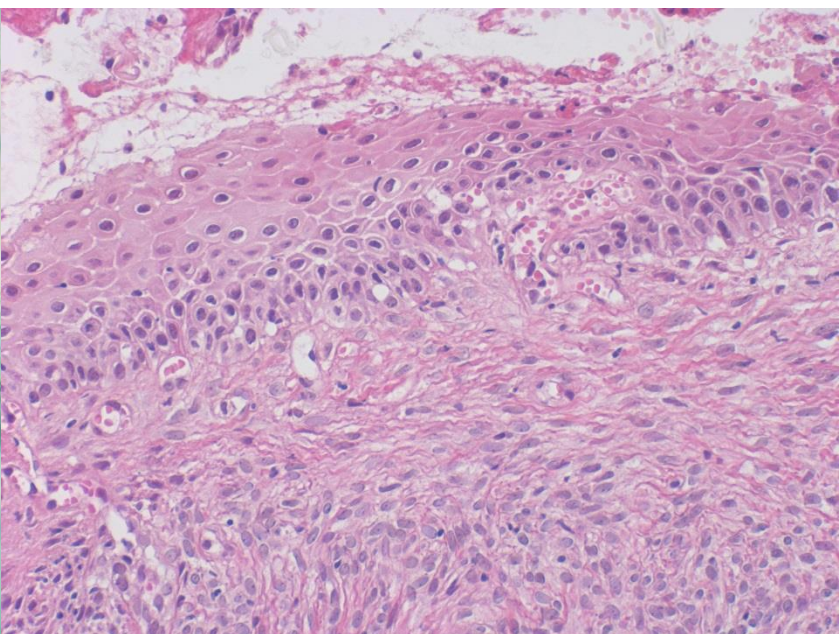
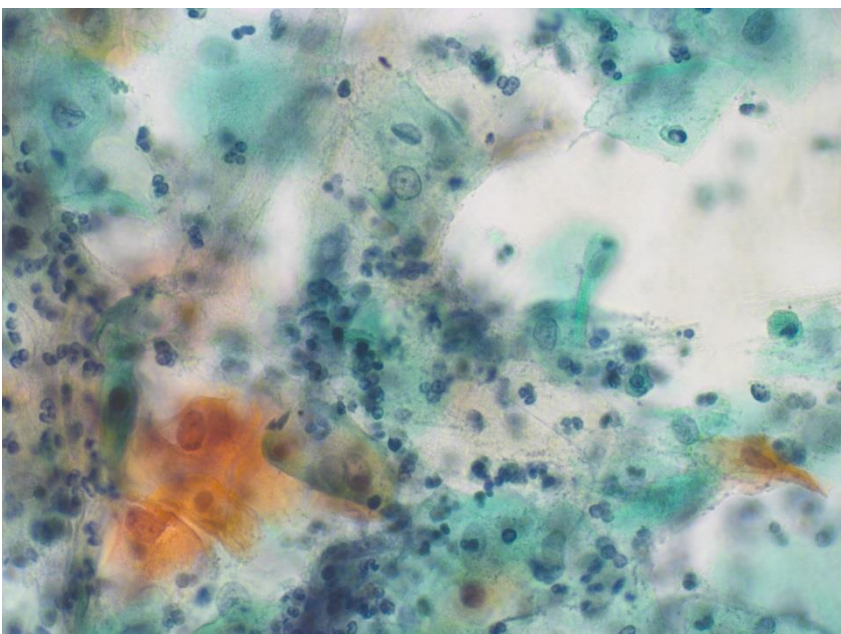
64 y-o
lady
ASC-US
P16 (-)

62歳
HSIL
p16陽性



生検は
偽陰性
(sampling
error)

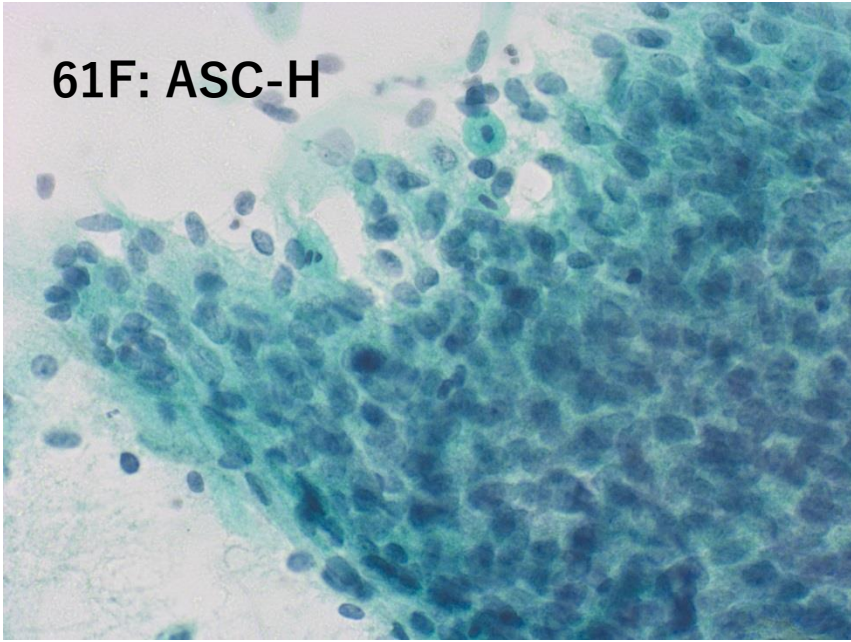




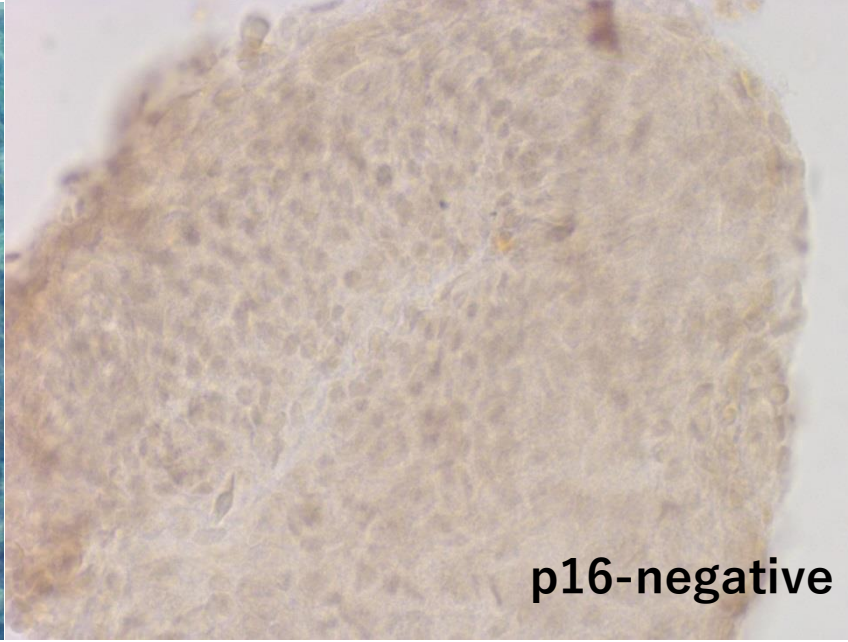
69F ASC-US (p16-negative senile colpititis)

**another Bx specimen:
p16-positive (CIN1)**

61F: ASC-H

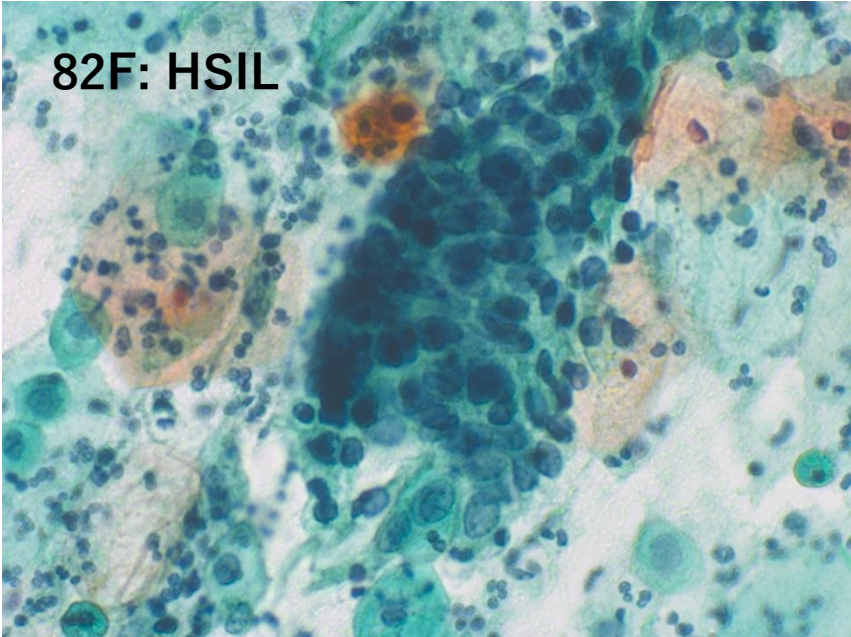


p16-negative

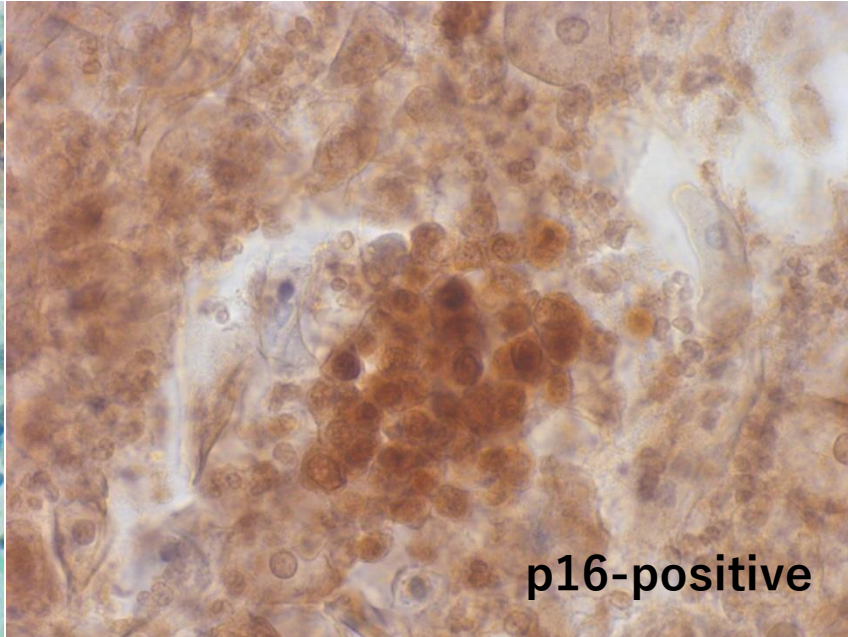


Distinction between senile colpitic and dysplasia in pap smear is difficult.

82F: HSIL



p16-positive



Avoid the cytodiagnosis of ASC-US. Instead, the diagnosis of ASC-H should be made to recommend biopsy.