Immunostaining in cytological specimens

For performing immunostaining in cytological specimens, extra-slides must be prepared in advance or liquid-based cytology should be applied. For detecting antigenicities, heating pretreatment is needed for a number of antigens. Therefore, the cells should be smeared on silane-coated glass slides to prevent detachment during immunostaining. When only one glass slide is available, meticulous techniques are requested for immunocytochemical evaluation. See Inf-0-2-general. “How to identify pathogens in cytology specimens. Often times, only one specimen is available”.

Here presented are representative cases, in which immunostaining application was useful for making a final cytodiagnosis.

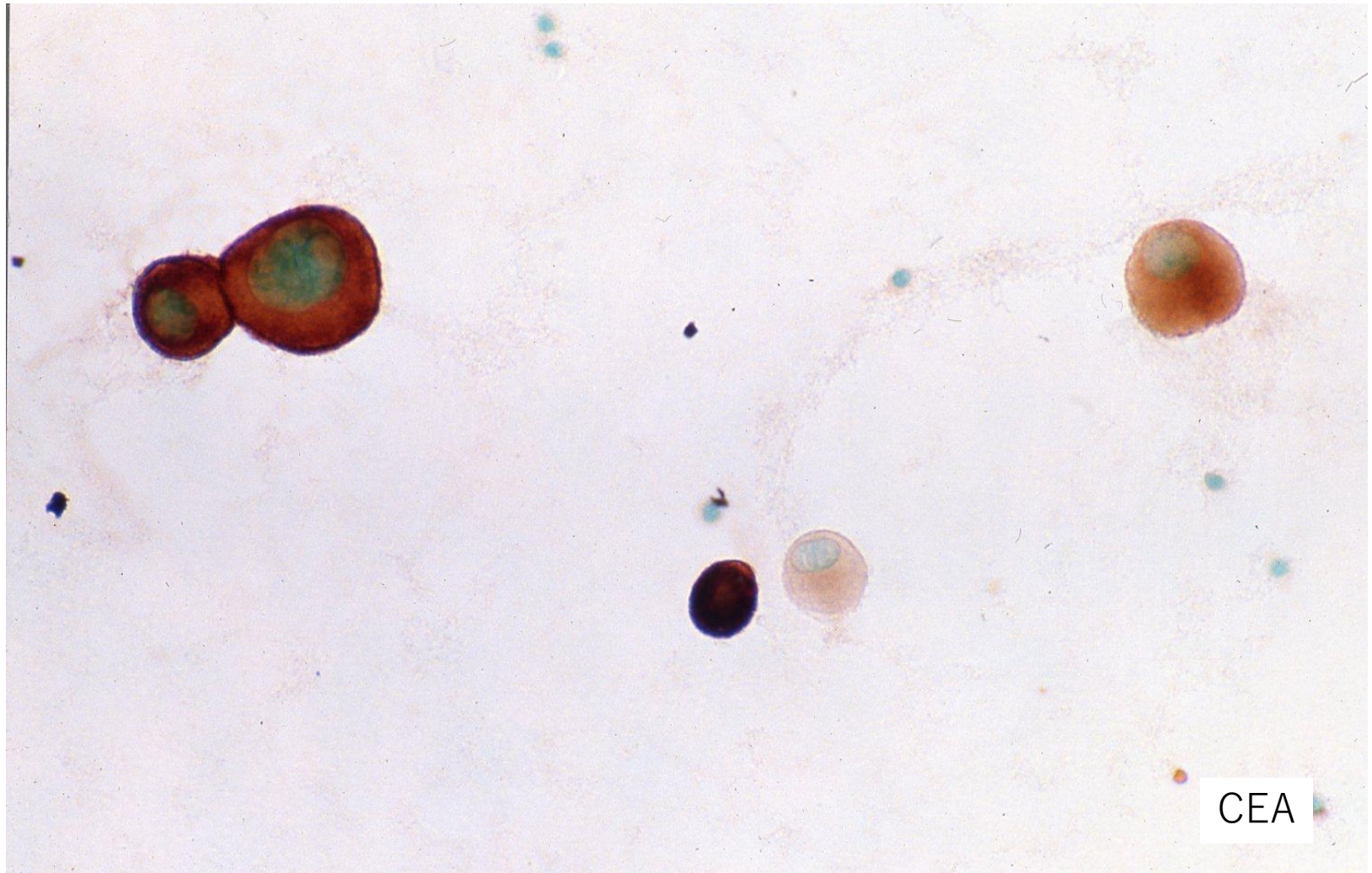
Ref.: Zhang X, Elhosseiny A. Role of immunostaining in cytopathology. Diagnost Histopathol 2012; 18(8): 335-340. doi: 10.1016/j.mpdhp.2012.06.002

Inf-0-2-general

How to identify pathogens in cytology specimens: Often times, only one specimen is available

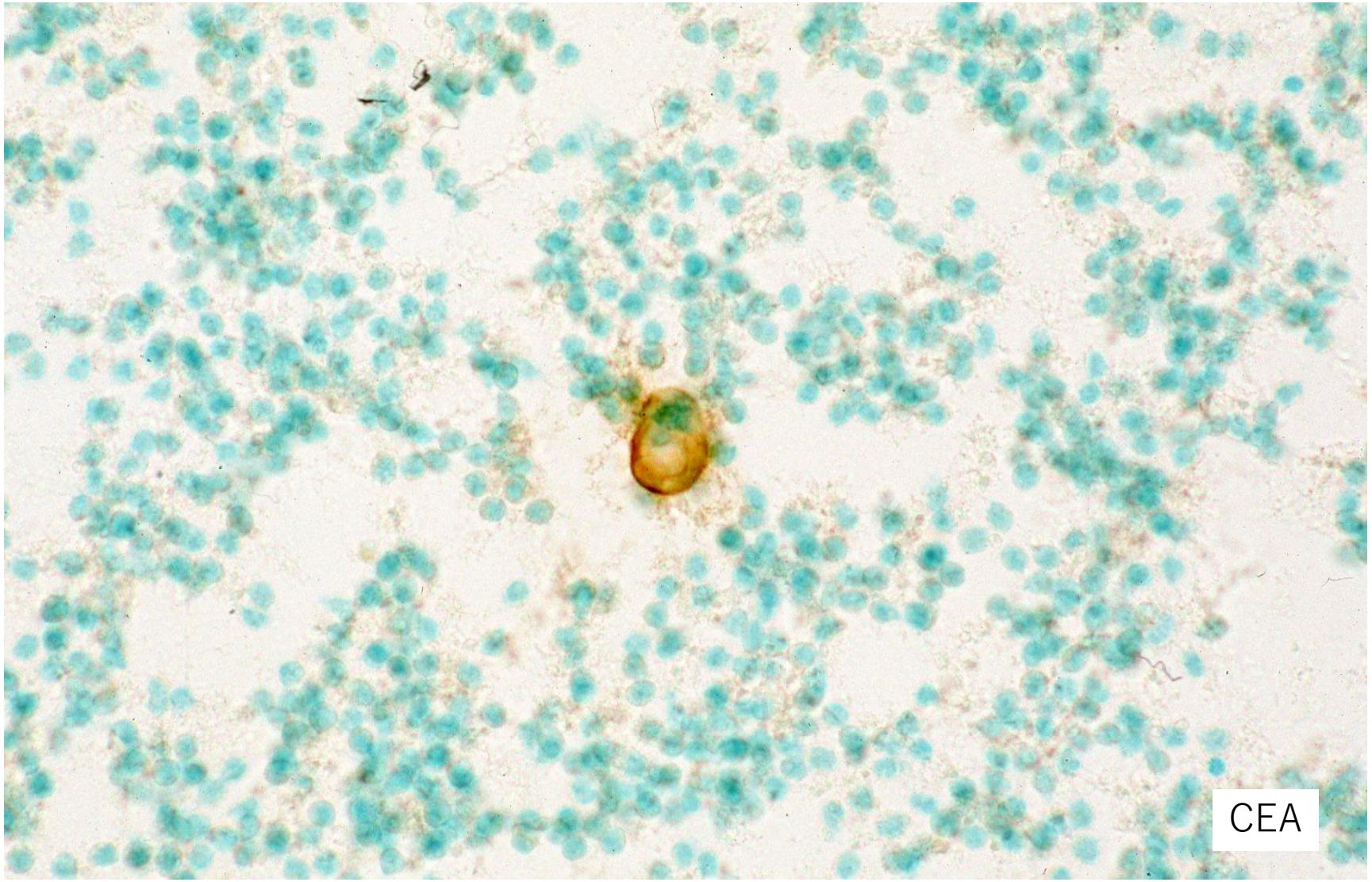
Several methods can be applied to demonstrate pathogens in cytology preparations. The following items are presented. 1) Cell block preparations, 2) restaining method, 3) cell transfer technique (transfer of smears to another glass slide), and 4) applications of the cell transfer technique (repair of broken slides and evaluation with PCR).

Ref.: Tsutsumi Y. Cytological diagnosis of infectious diseases: identification of pathogens and recognition of cellular reactions. IntechOpen 2020. In: Innate Immunity in Health and Disease (eds: Saxena SK, Prakash H). doi: 10.5772/intechopen.95578

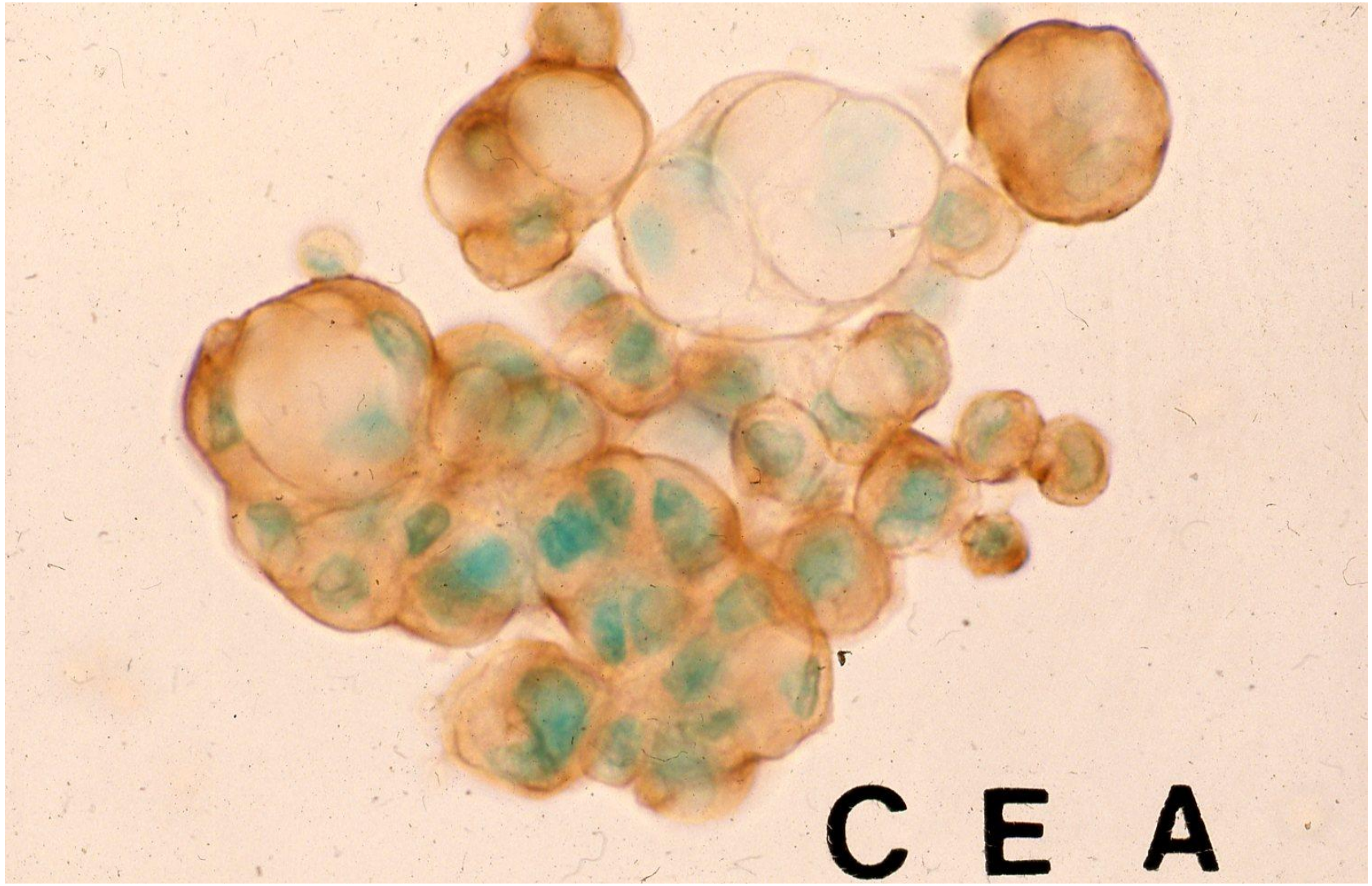


Lung adenocarcinoma cells in pleural effusion in a male patient aged 50’s. The isolated and partially cohesive cancer cells express CEA. A mouse monoclonal antibody was utilized for specific detection of CEA.

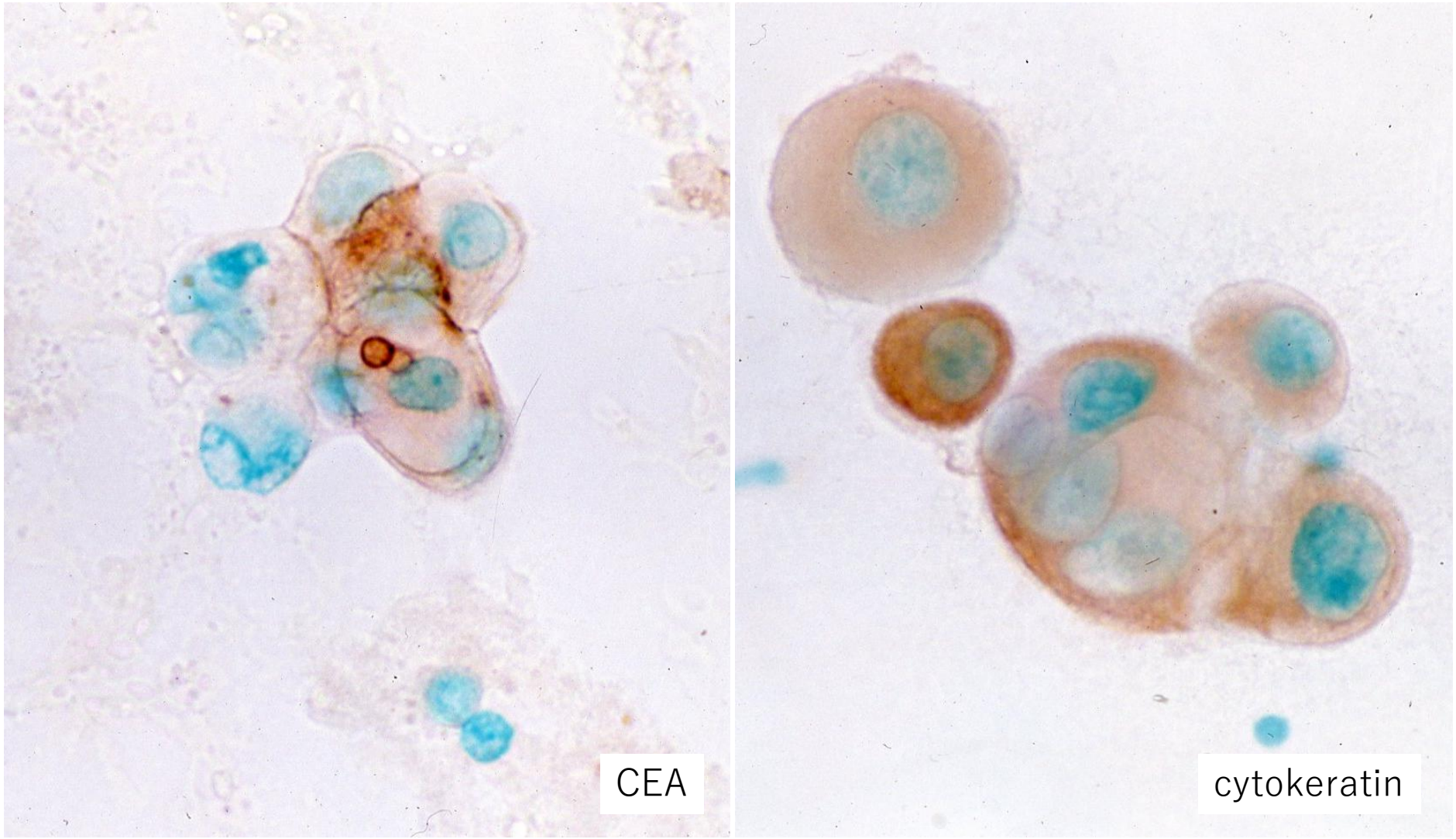
Lung adenocarcinoma cells in pleural effusion in a male patient aged 50’s. The isolated and partially cohesive cancer cells express CEA. Microvillous surface is observed on the cancer cells.



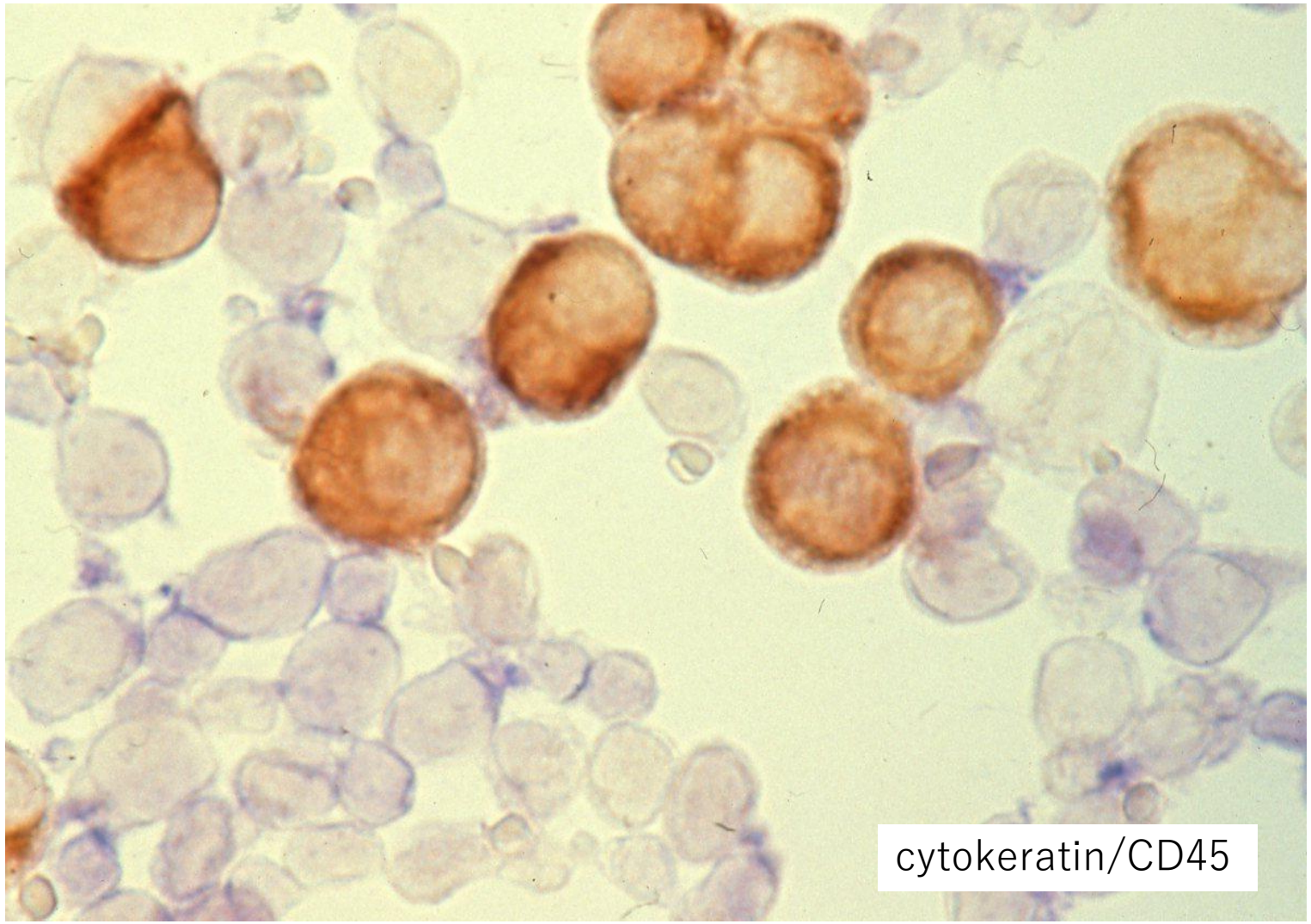
Lung adenocarcinoma cells in pleural effusion in a male patient aged 70’s. An isolated cancer cell can be detected with the aid of immunostaining for CEA. In the present case, CEA antiserum absorbed with the splenic abstract was used. The cross-reactivity to nonspecific cross-reacting antigen (NCA) expressed in neutrophils had been absorbed. The background neutrophils are thus negative.



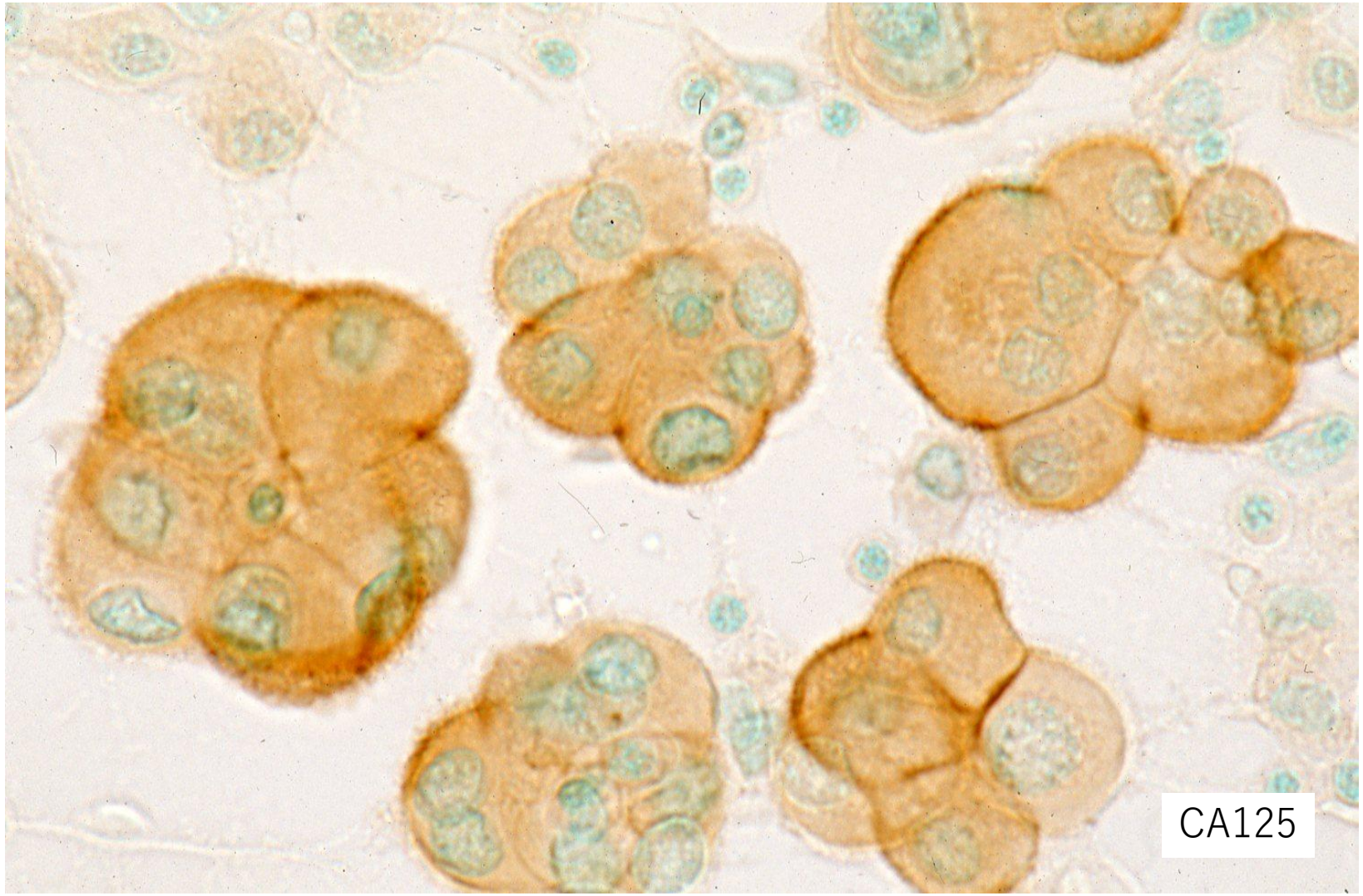
Gastric adenocarcinoma cells in pleural effusion in a female patient aged 60’s.
The clustered cohesive cancer cells express CEA on the plasma membranes.



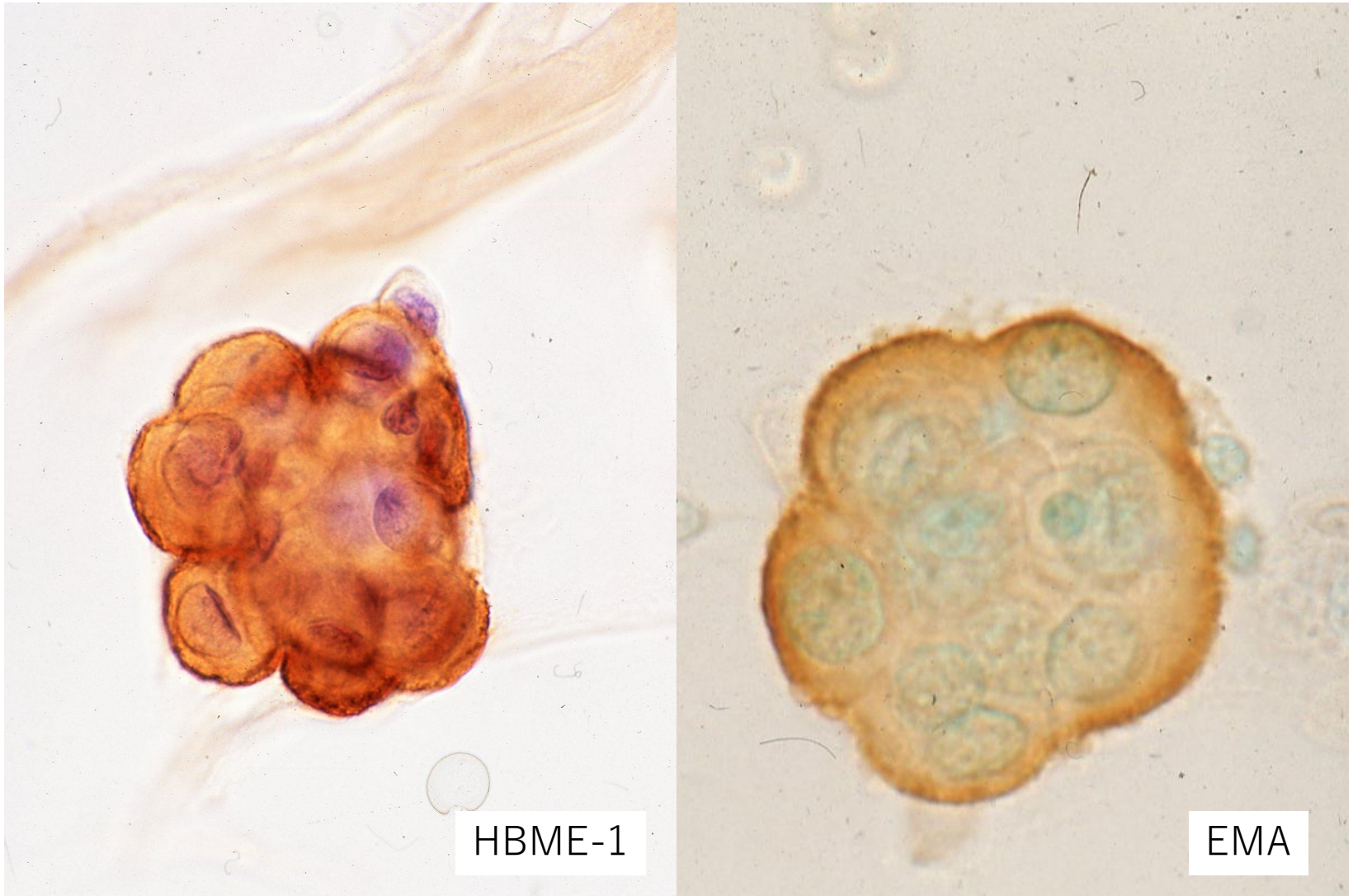
Mammary ductal carcinoma cells in pleural effusion in a female patient aged 40’s. The clustered cohesive cancer cells express CEA on the plasma membranes and in the Golgi area. Cytokeratin is expressed in both cancer cells and mesothelial cells. Small-sized mesothelial cells are negative for CEA but positive for cytokeratin.



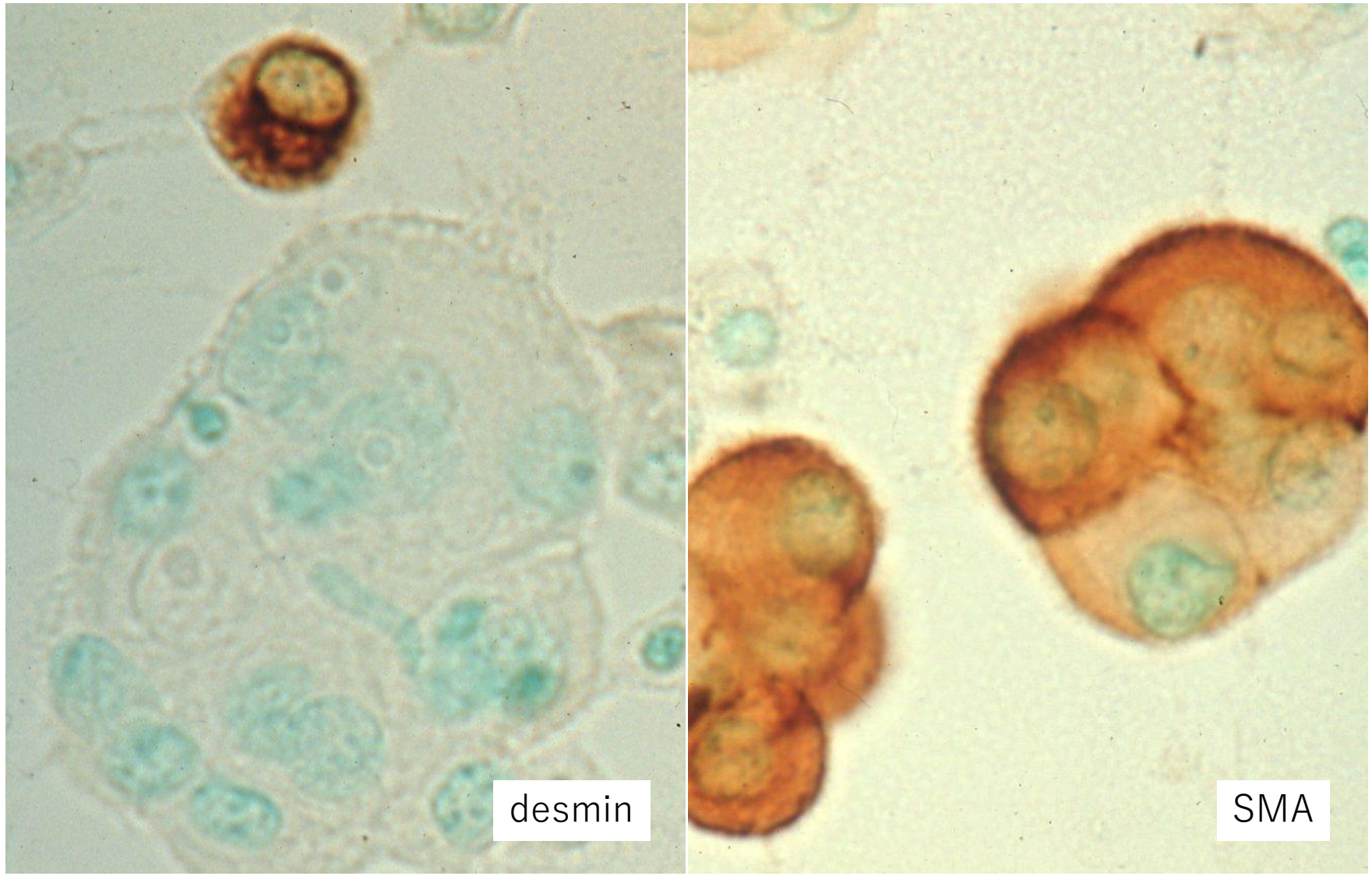
Double immunostaining for cytokeratin (brown) and CD45 (leukocyte-common antigen) (blue) in pleural effusion. The cytoplasm of reactive mesothelial cells is stained brown, while the plasma membrane of lymphocytes is stained blue.



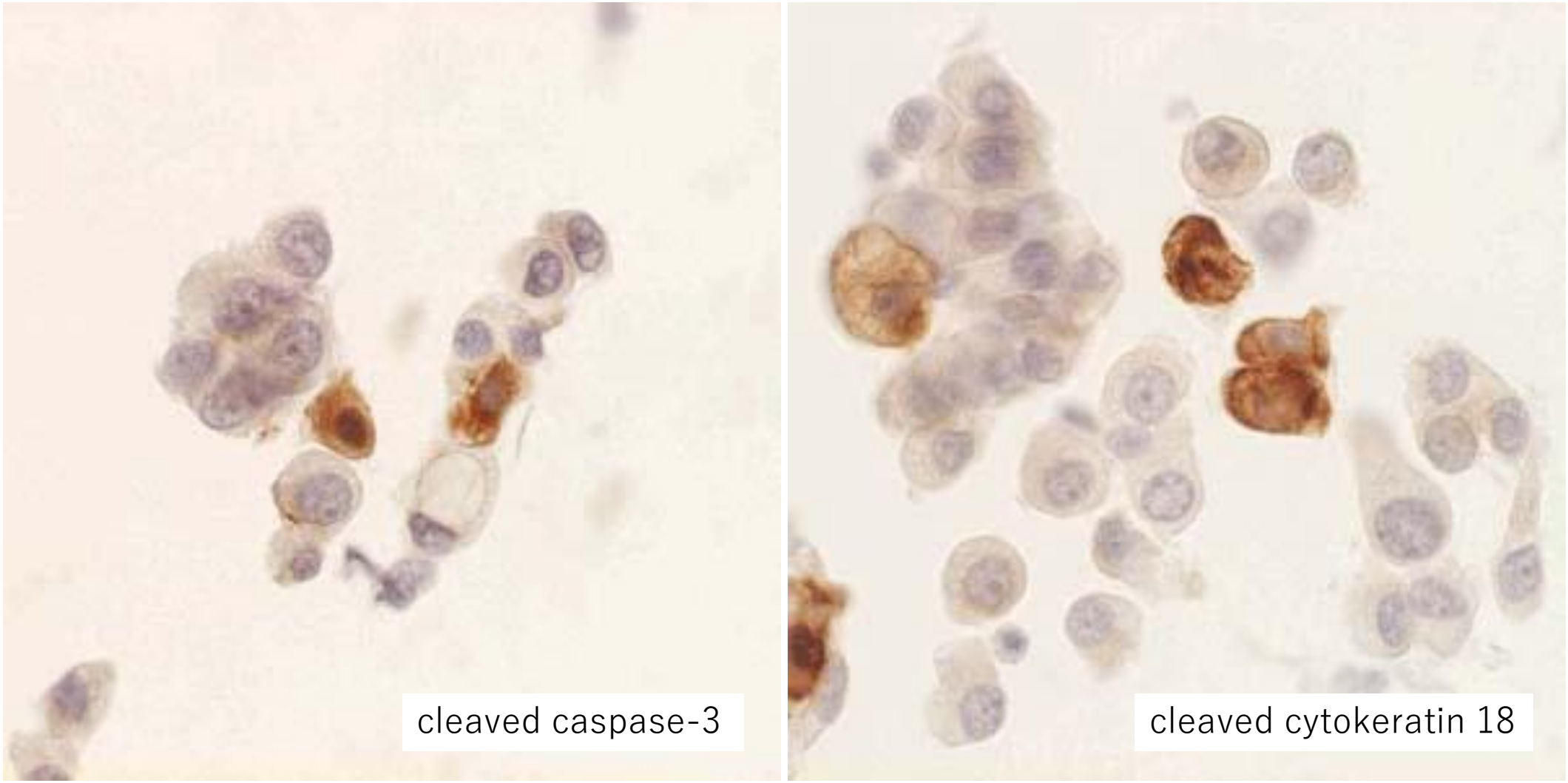
Malignant mesothelioma in pleural effusion seen in a male patient aged 60’s. CA125 is clearly expressed on the plasma membrane of clustered large atypical cells of mesothelial origin.



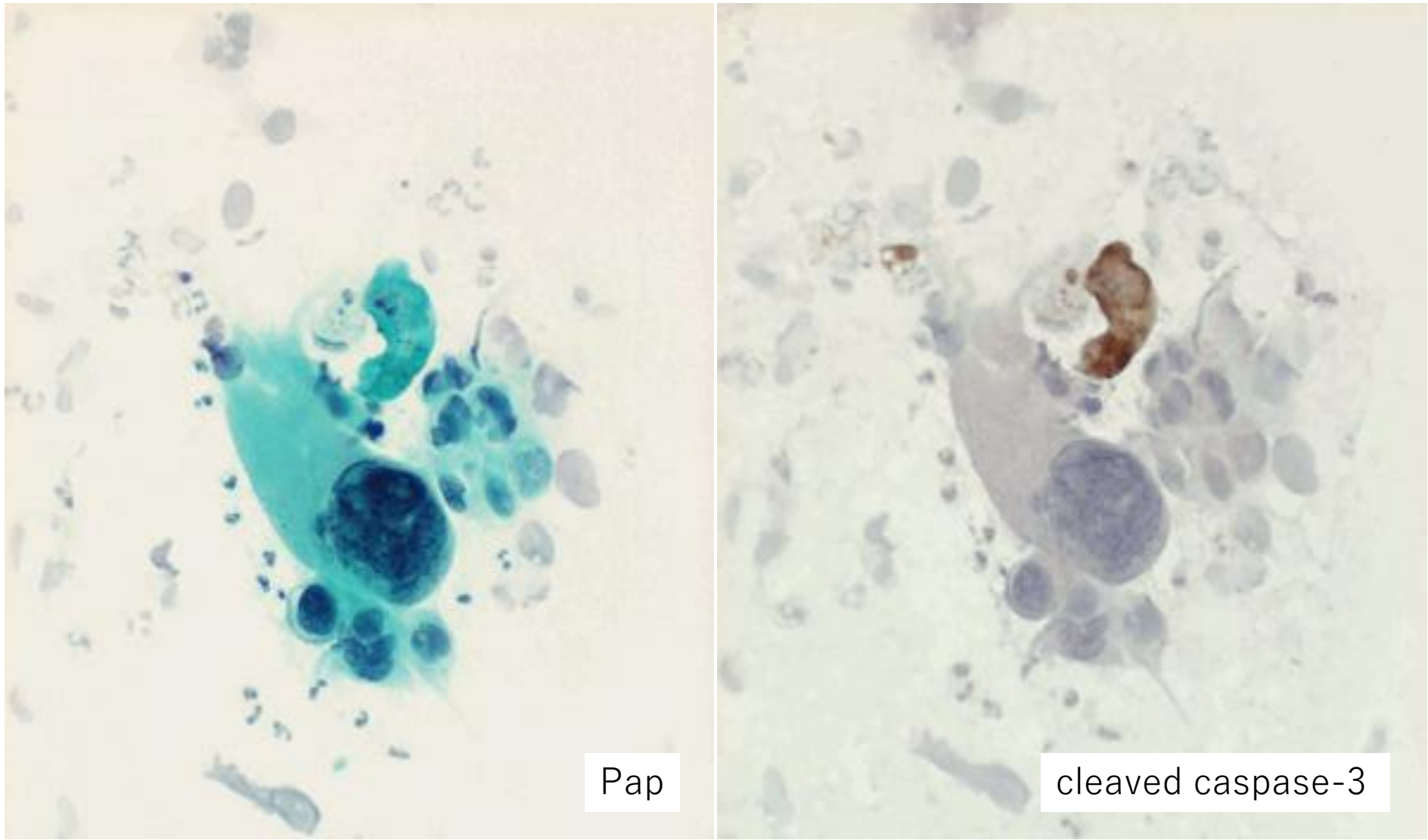
Malignant mesothelioma in pleural effusion seen in a male patient aged 60's. HBME-1 (left) and EMA (right) are expressed on the plasma membrane of clustered large atypical cells of mesothelial origin.



Malignant mesothelioma in pleural effusion seen in a male patient aged 60's. Desmin (left) is negative but SMA (right) is expressed in the cytoplasm large atypical cells of mesothelial origin. A non-neoplastic mesothelial cell is desmin-positive.

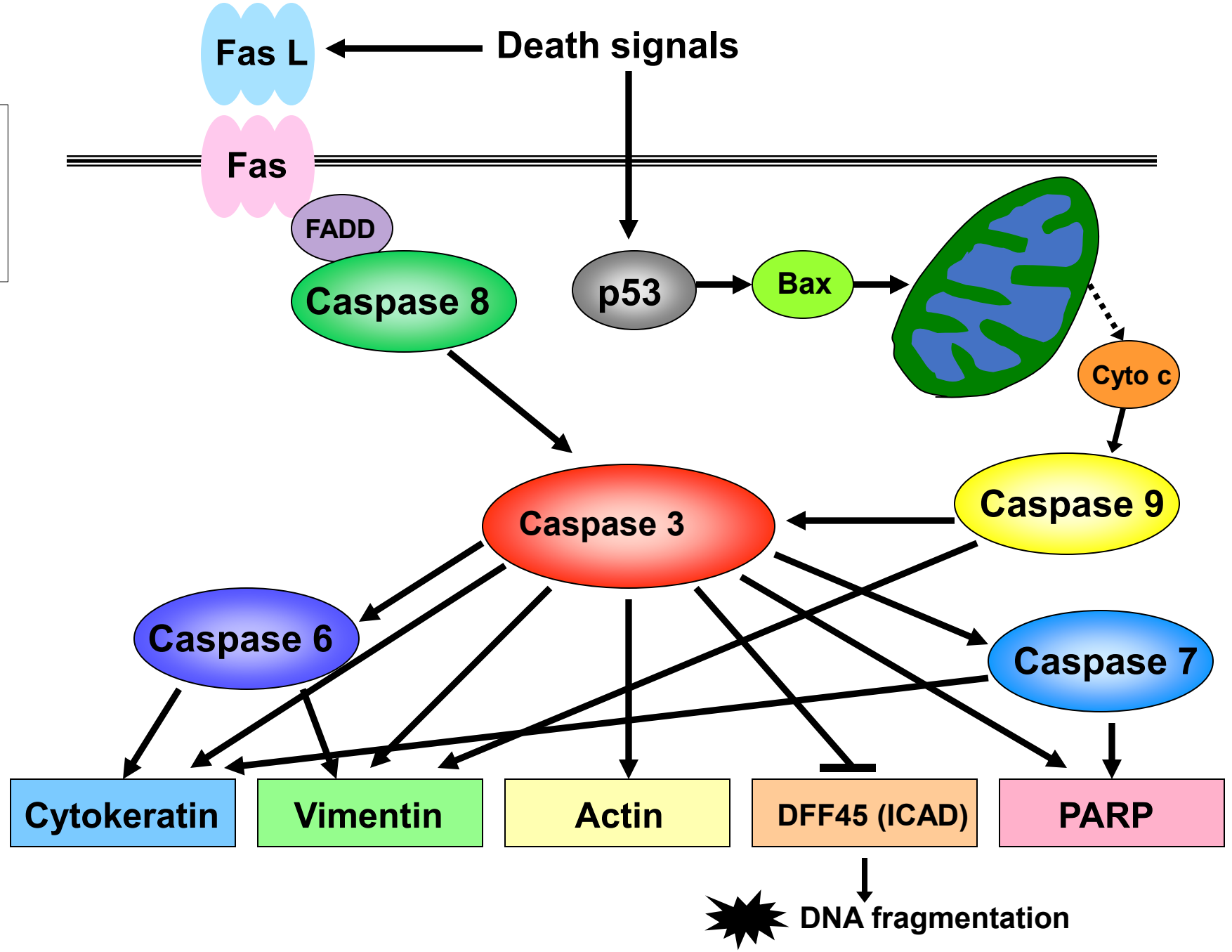


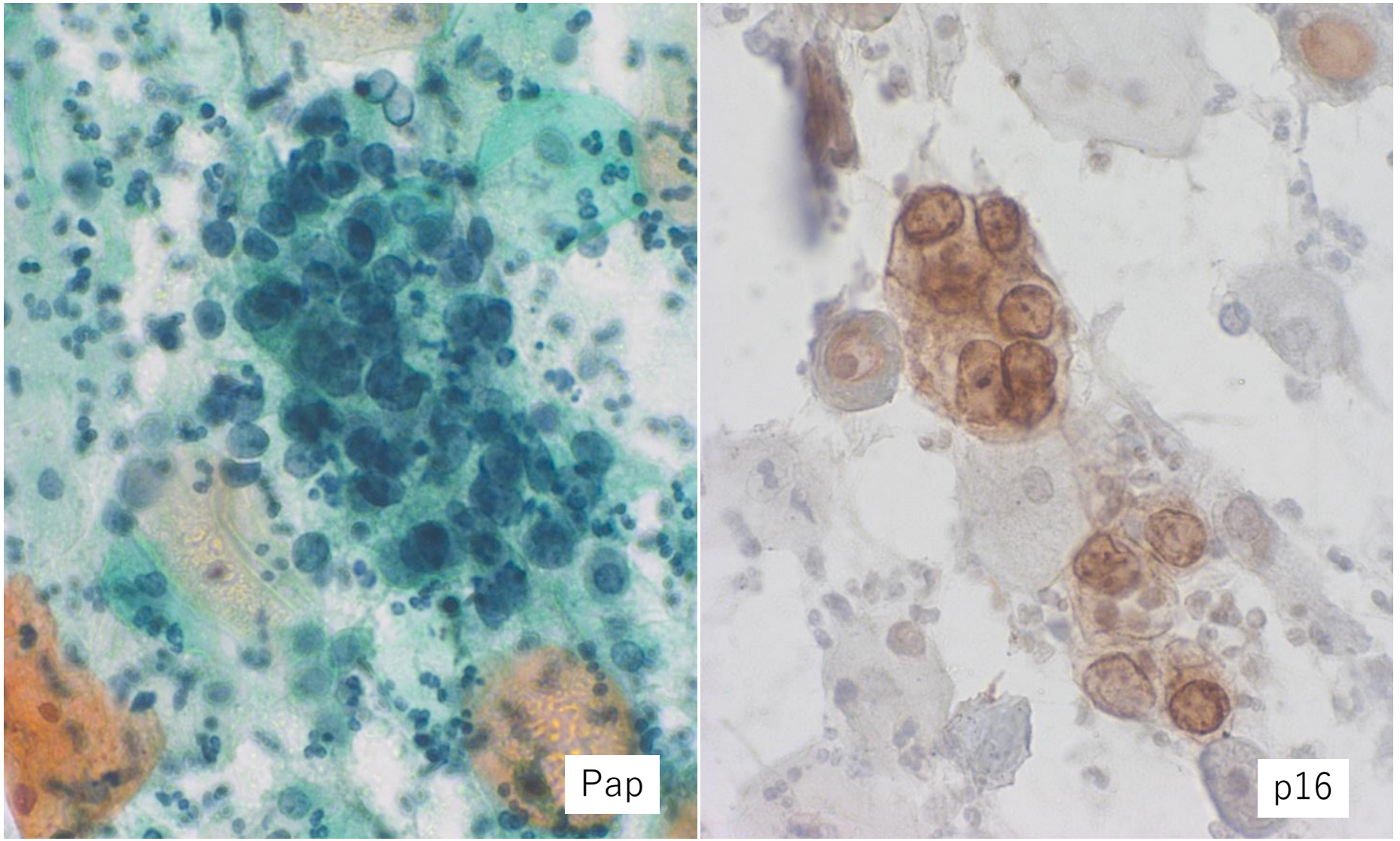
Apoptosis in pleural malignant mesothelioma seen in a male patient aged 60’s. Apoptotic cells are consistently visualized by immunostaining for cleaved caspase 3 (left) and cleaved cytokeratin 18 (right). The cytoplasm and nuclei are stained brown.



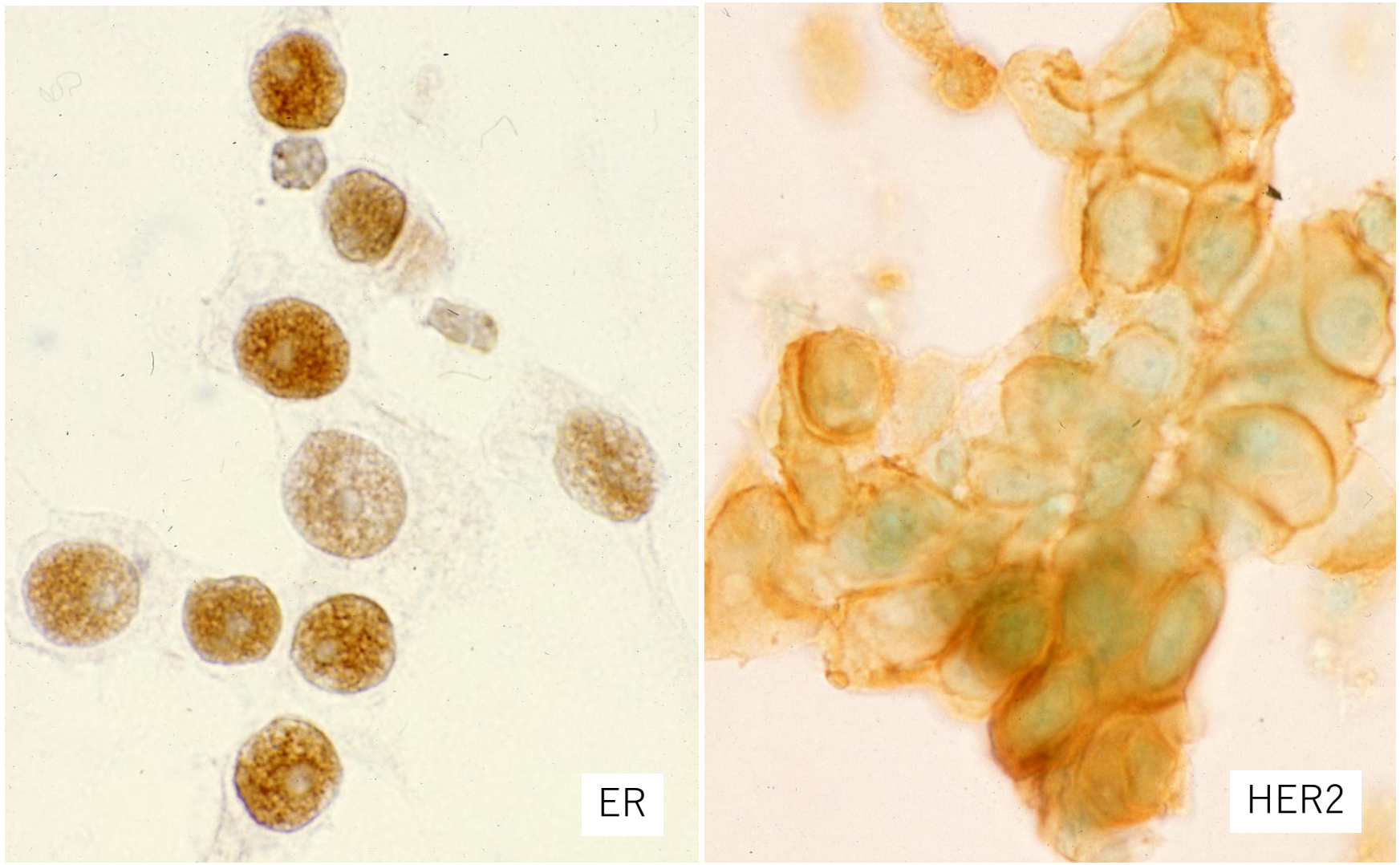
Apoptosis in squamous cell carcinoma of the uterine cervix seen in a female patient aged 40's. Bizarre squamous cancer cells are seen in the cervical smear (left). Re-staining method discloses an apoptotic small-sized fragmented cell immunoreactive for cleaved caspase 3 (right). After radiotherapy, the number of cleaved caspase-3 immunoreactive cells increases.

Cascade of apoptosis

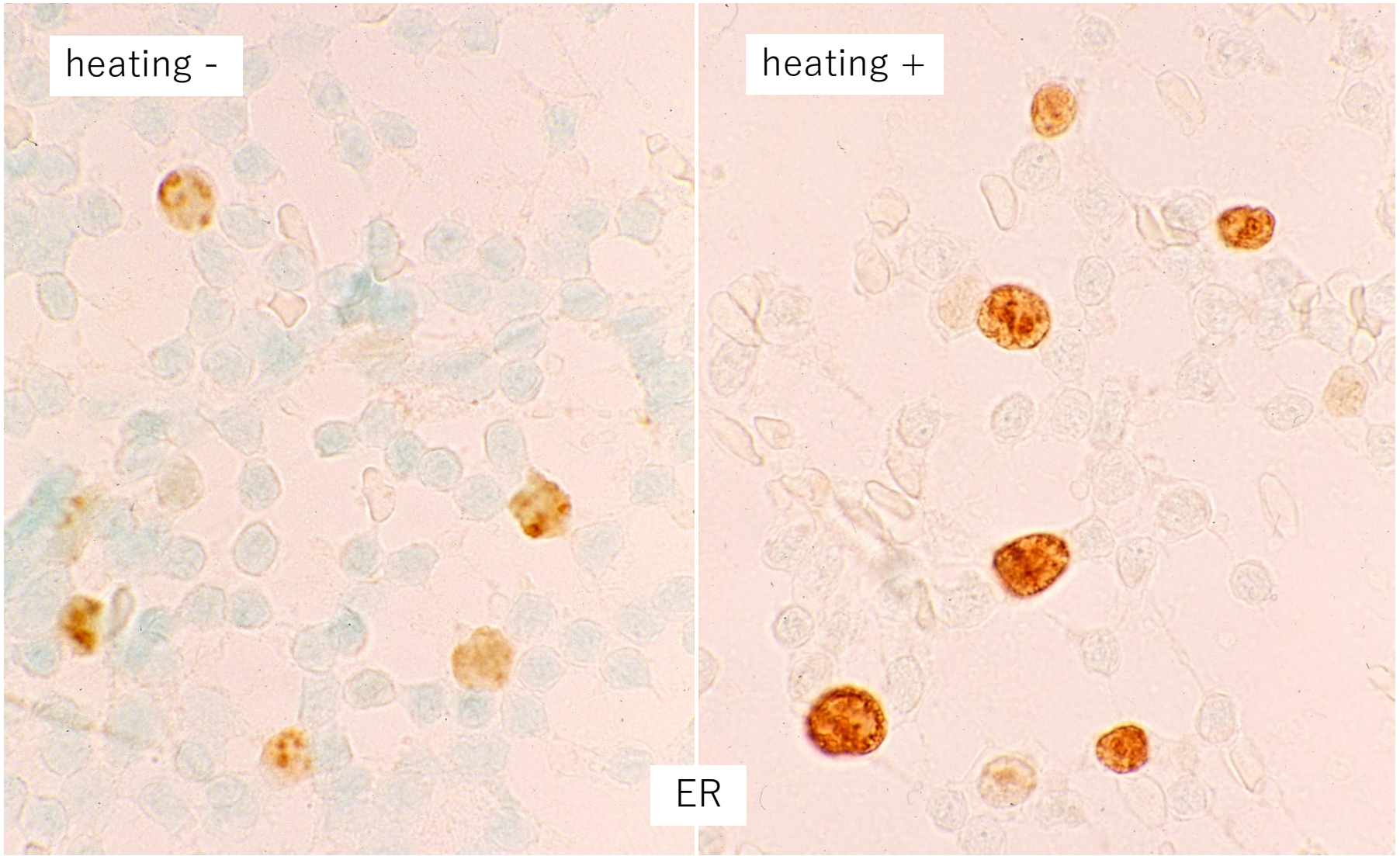




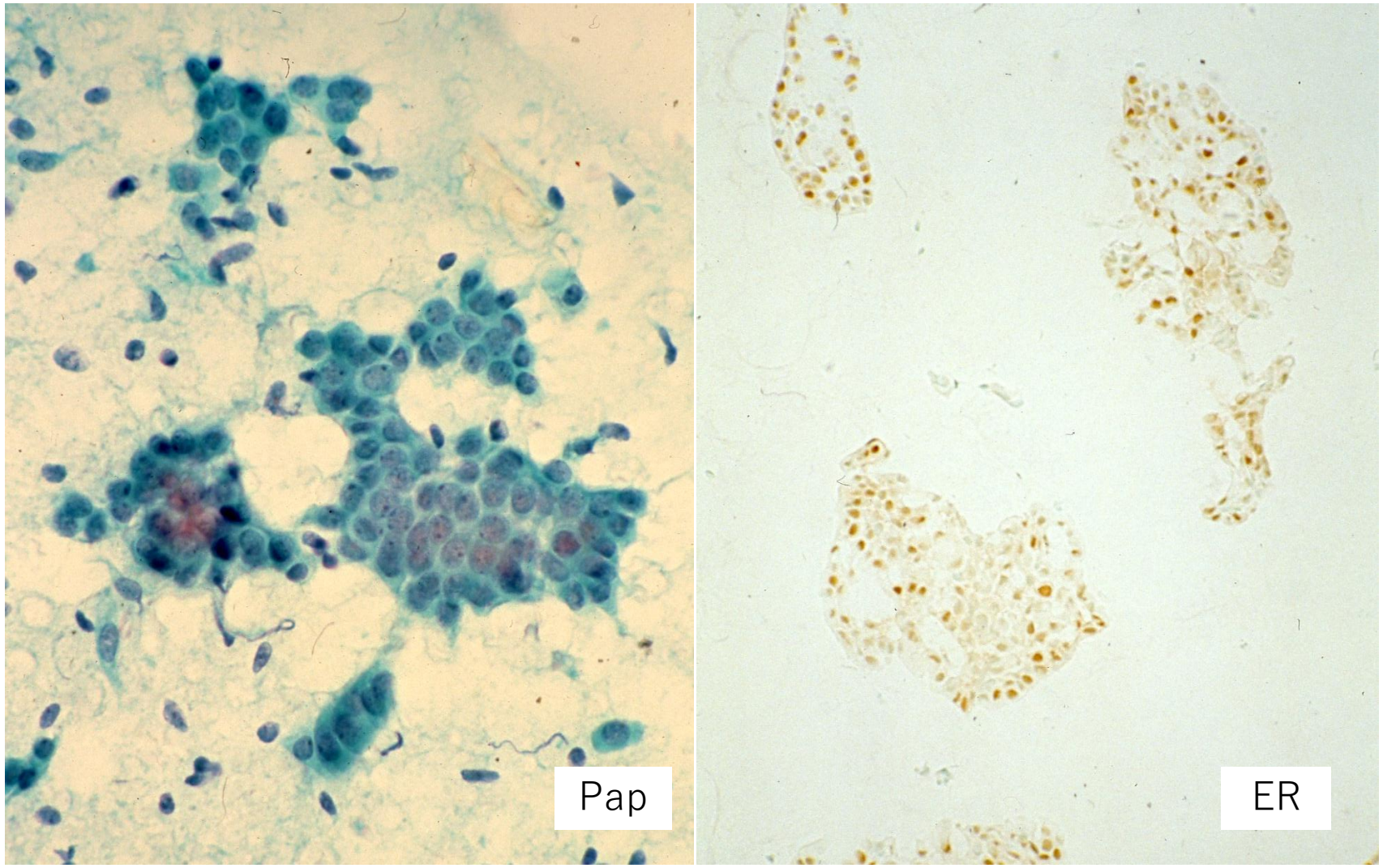
p16 expression in severe dysplasia (HSIL) in the cervical smear sampled from a postmenopausal lady. p16 expression is useful for the differential diagnosis between HPV-related intraepithelial neoplasia and senile colpitis. Senile colpitis reveals parabasal cells with nuclear enlargement without p16 expression.



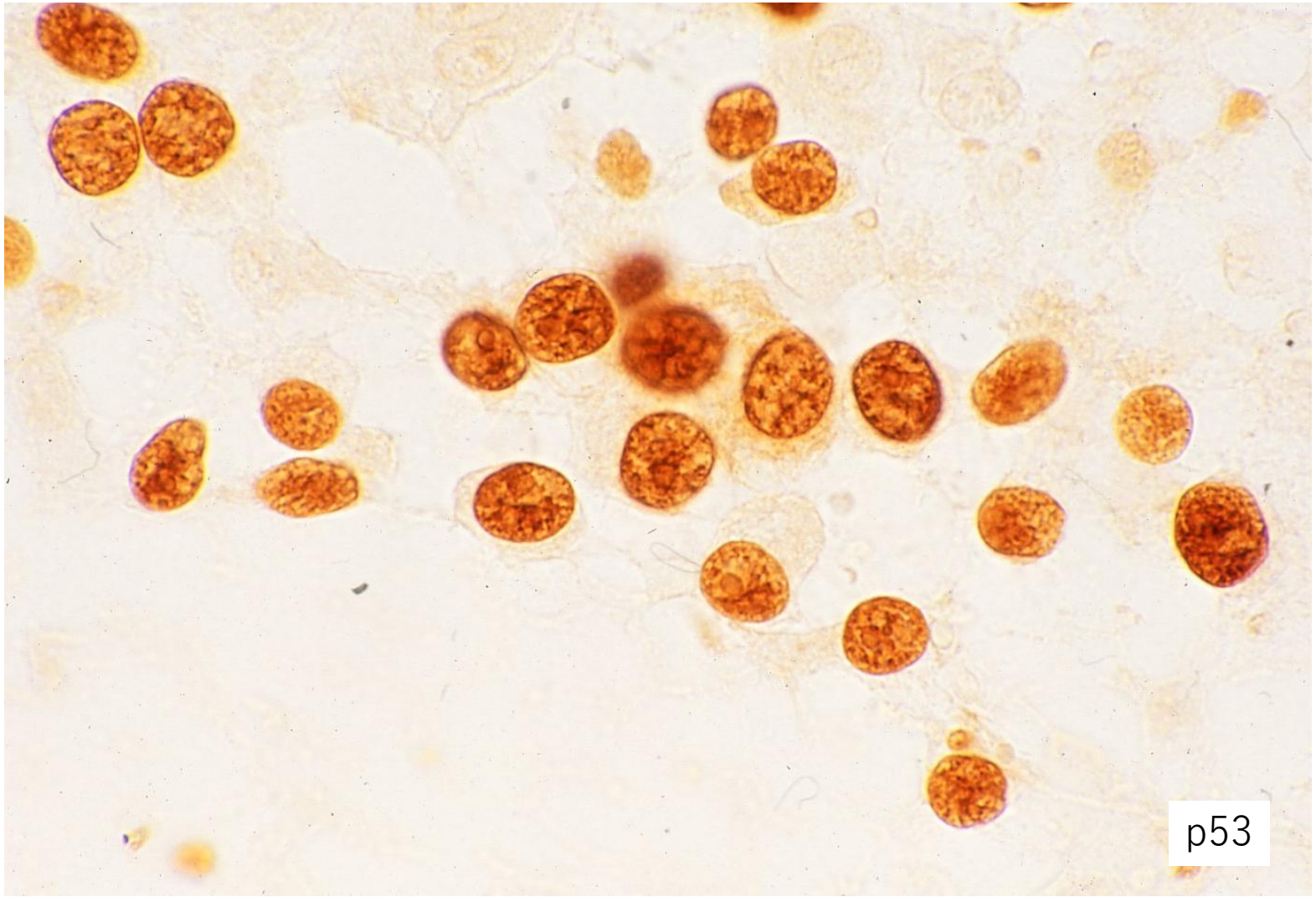
Breast cancer (invasive ductal carcinoma) aspirated from a female patient aged 40’s. In addition to the nuclear expression of ER (left), plasma membrane overexpression of HER2 is detected, categorizing the tumor as luminal HER2 type.



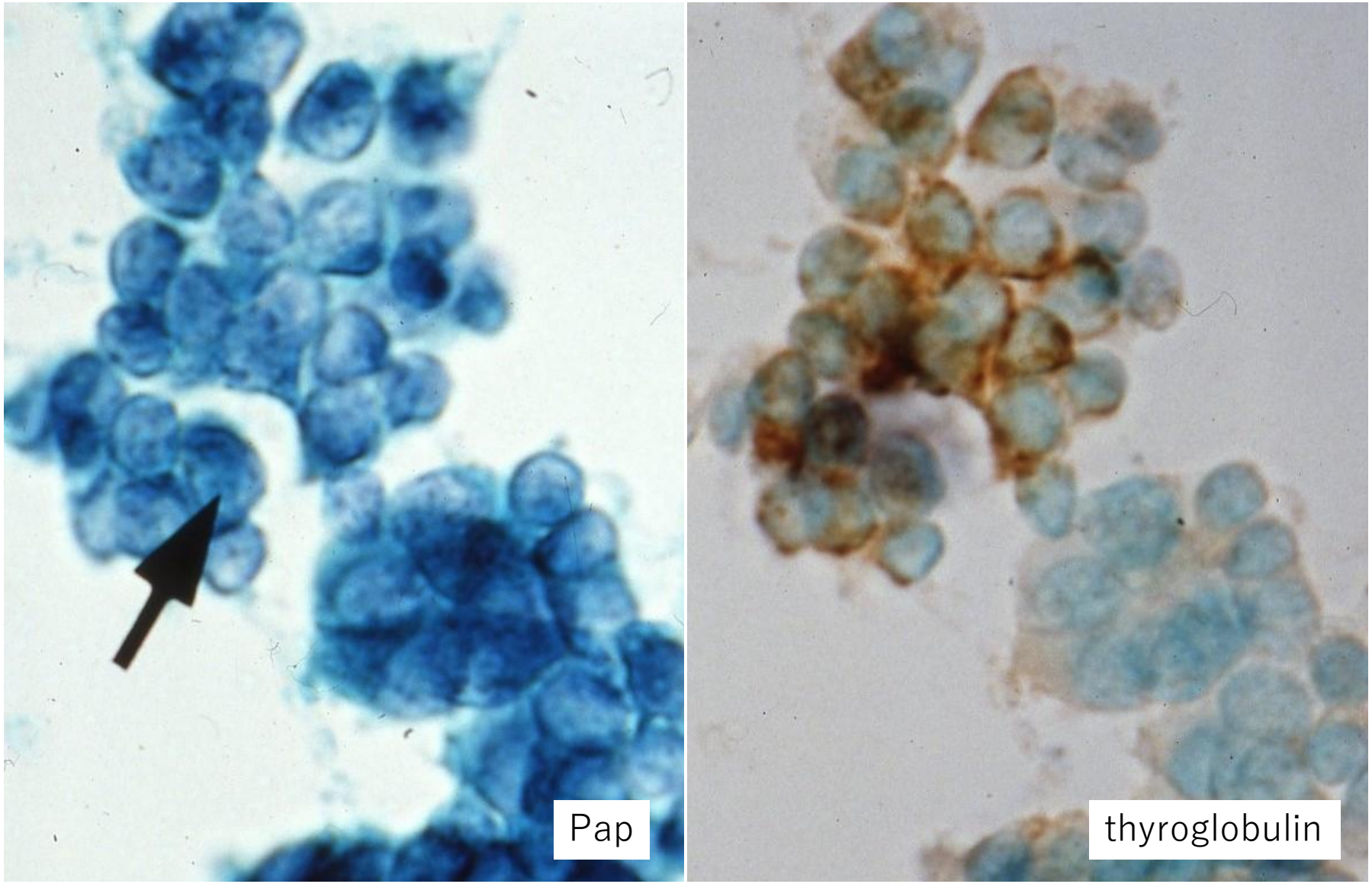
Detection of ER in alcohol-fixed cytology specimens of aspirated breast cancer (invasive ductal carcinoma) before (left) and after (right) heat-induced epitope retrieval. Heating pretreatment is effective for detecting the ER antigenicity. The use of silane-coated glass slides is essential for avoiding the detachment of the cells.



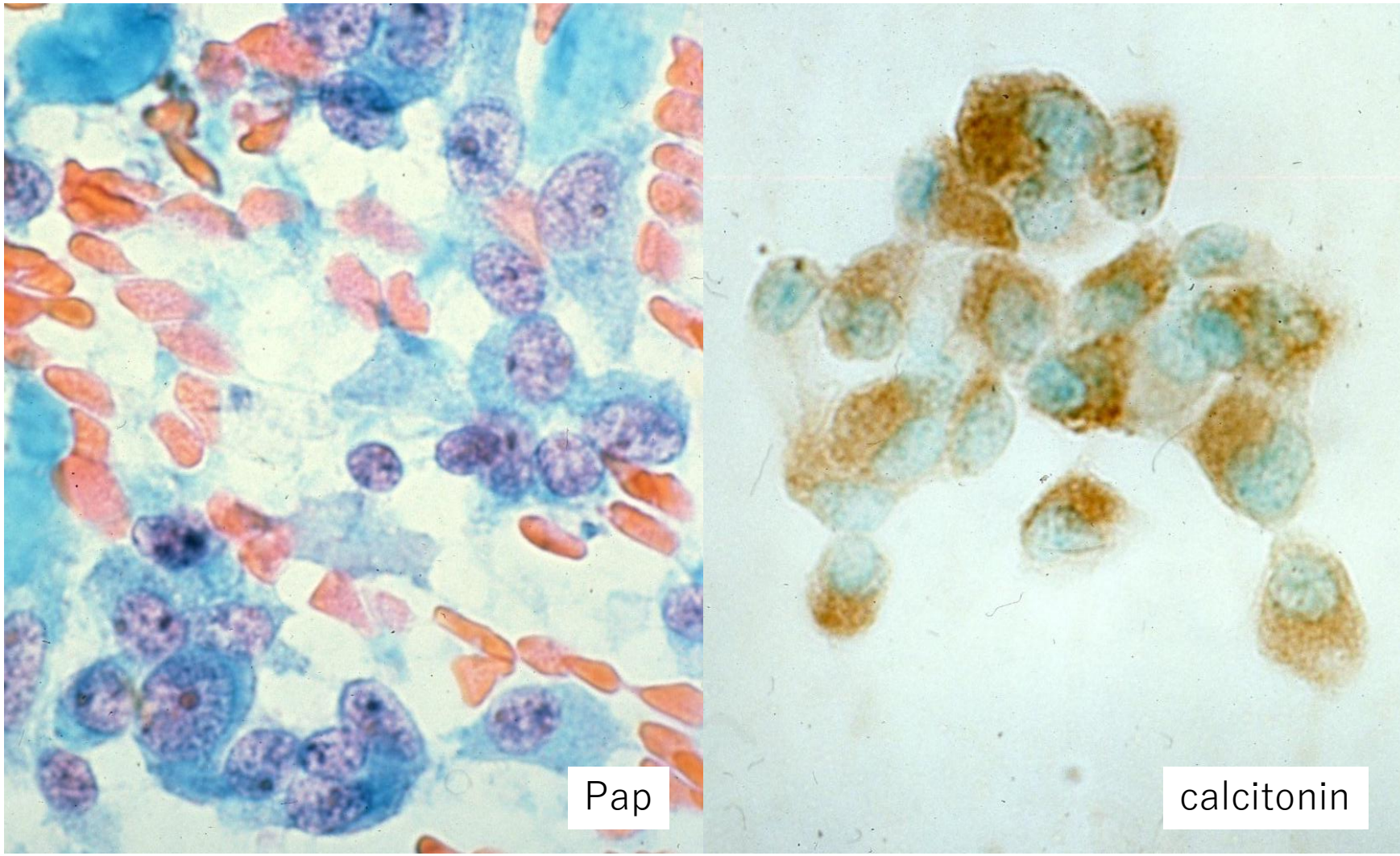
ER expression in fibroadenoma of the breast aspirated from a female patient aged 20's. Typical cytologic features of fibroadenoma are seen in the Pap smear (left). ER is consistently expressed in the nuclei (right).



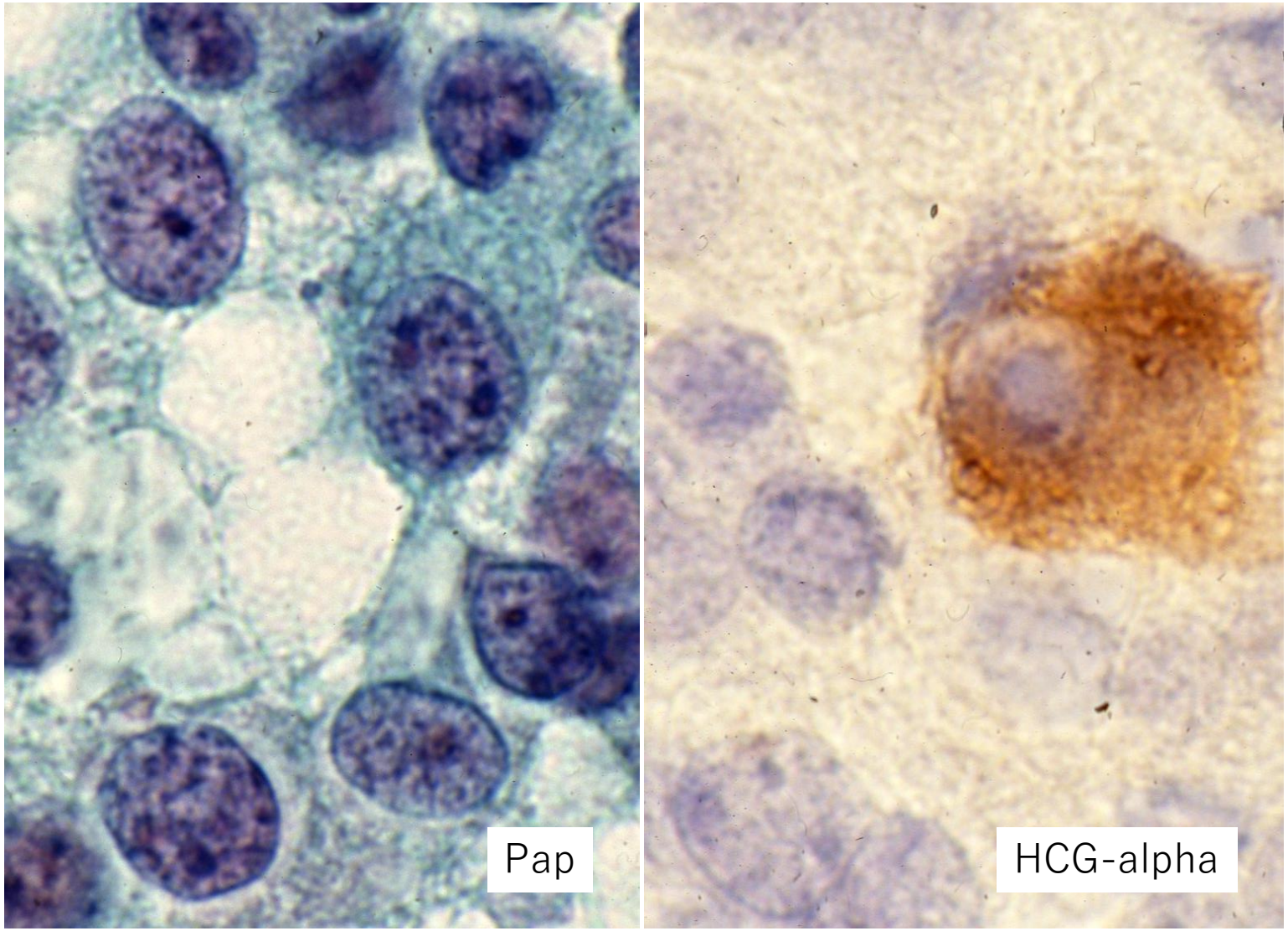
p53 expression in the nuclei of lung adenocarcinoma in the scraping cytology specimen. Expression of mutated p53 proteins is indicated based on the diffuse nuclear expression.



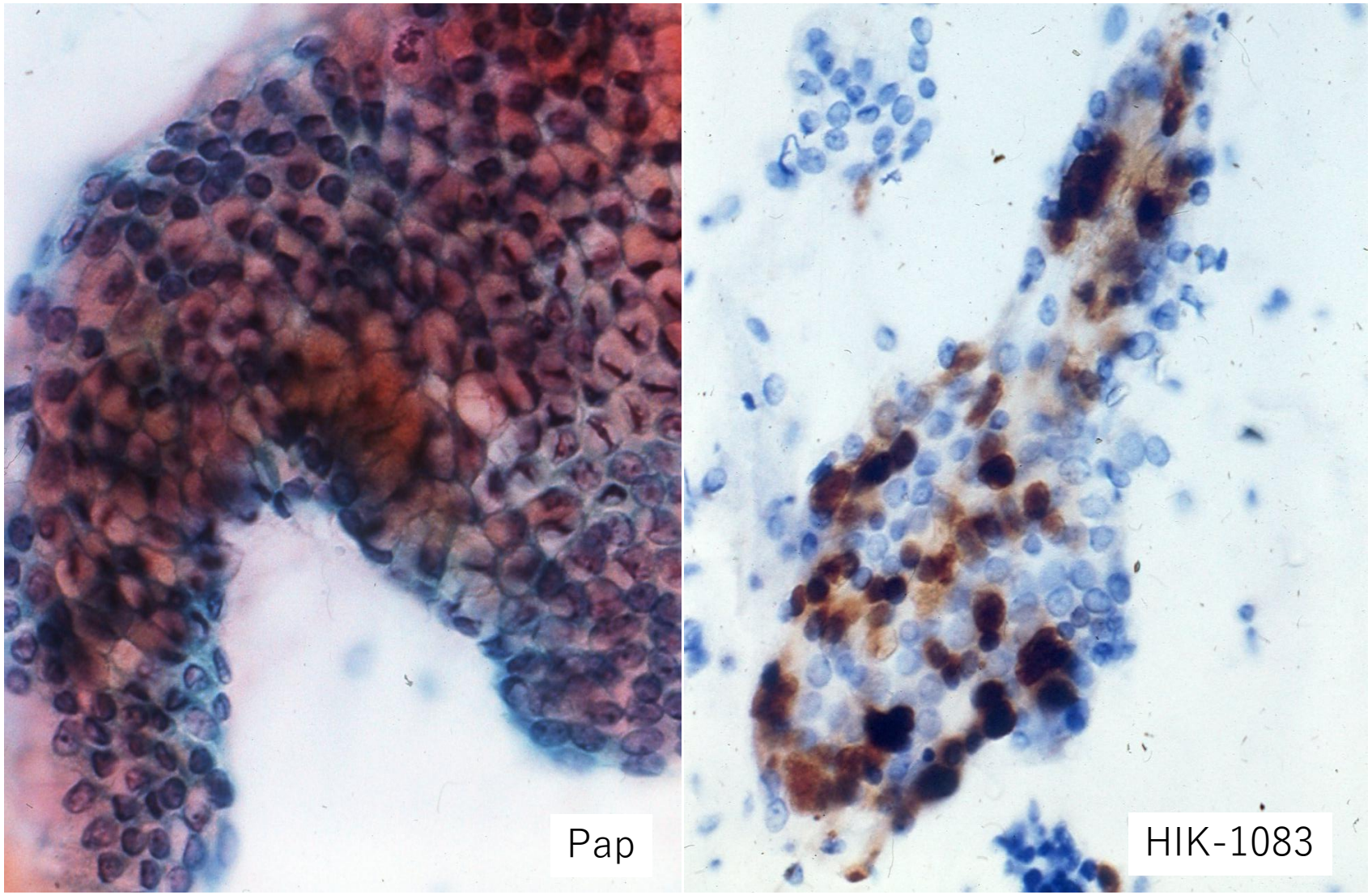
Thyroglobulin expression in papillary thyroid carcinoma, aspirated from a female patient aged 40’s. Intranuclear cytoplasmic inclusion is focally seen in papillary clusters of cancer cells (arrow, left: Pap). Re-staining method demonstrates cytoplasmic immunoreactivity of thyroglobulin (right).



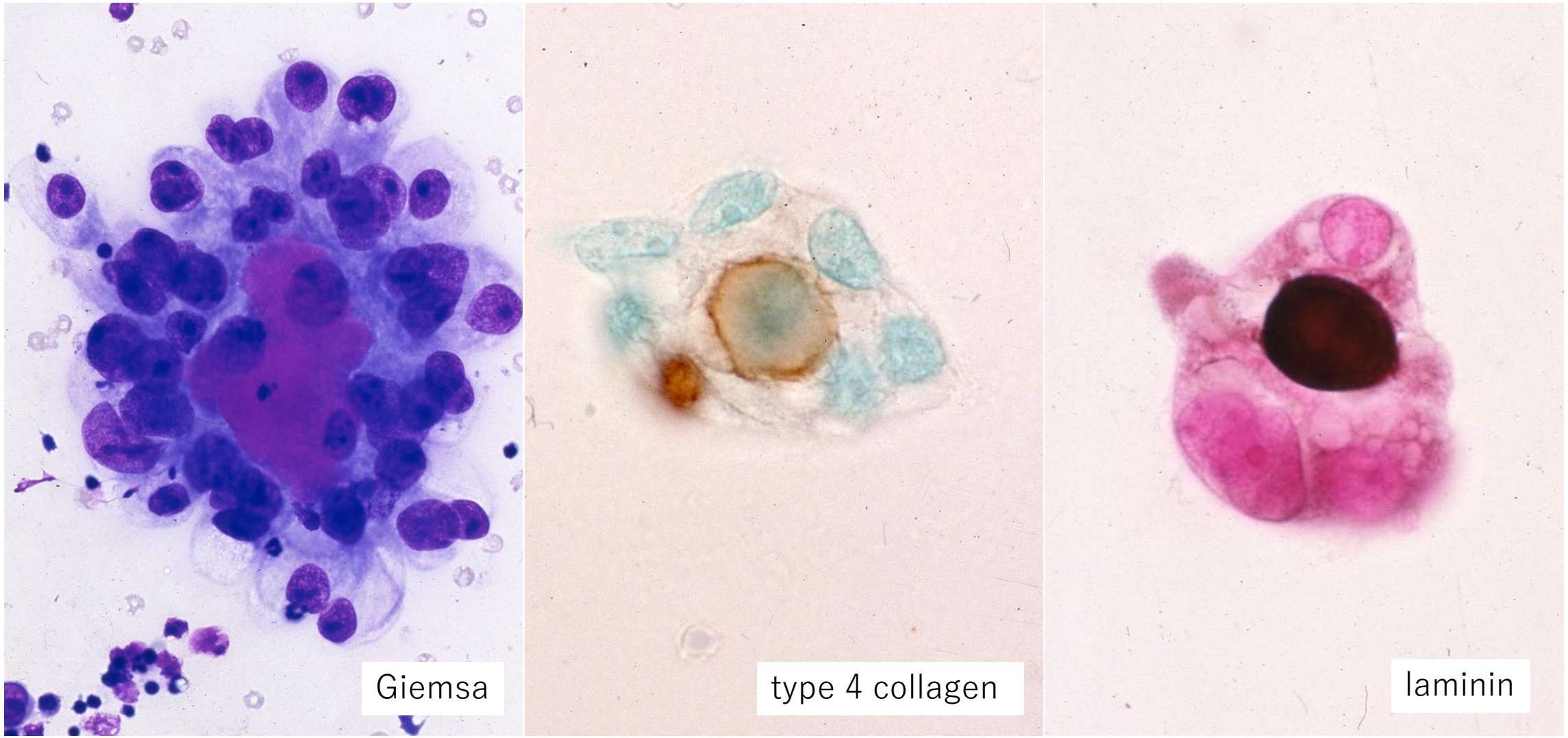
Calcitonin expression in medullary thyroid carcinoma, aspirated from a male patient aged 70's. Amyloid component is seen among the loosely attached polygonal tumor cells. The cytoplasm is granularly positive for calcitonin (right).



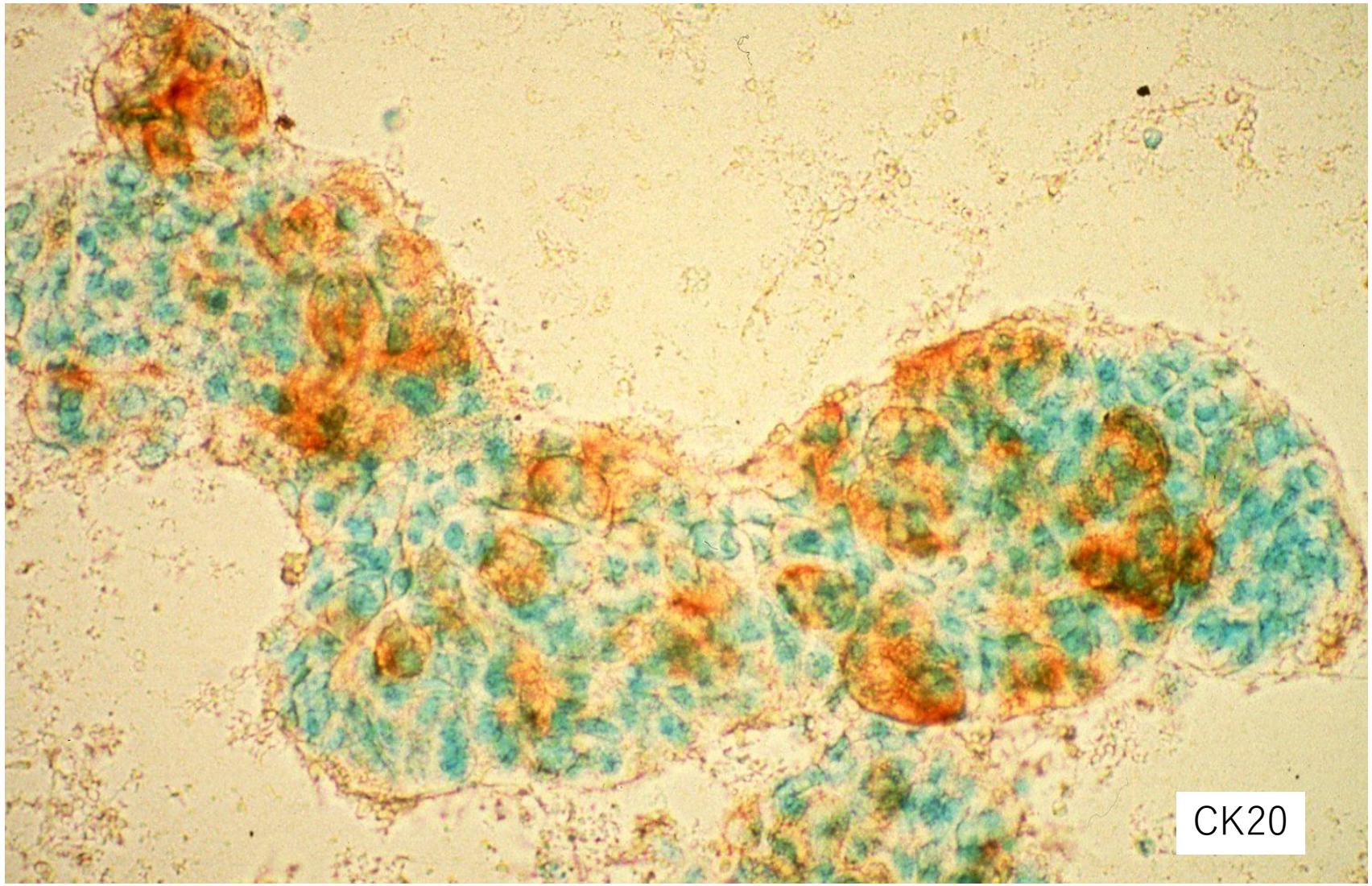
Expression of the alpha-subunit of HCG in bronchial carcinoid tumor.
Stamp smear from the surgical specimen of a female patient aged 40's.
The carcinoid tumor cells possess round nuclei and granular cytoplasm (left). HCG-alpha is expressed in some of the tumor cells (right).



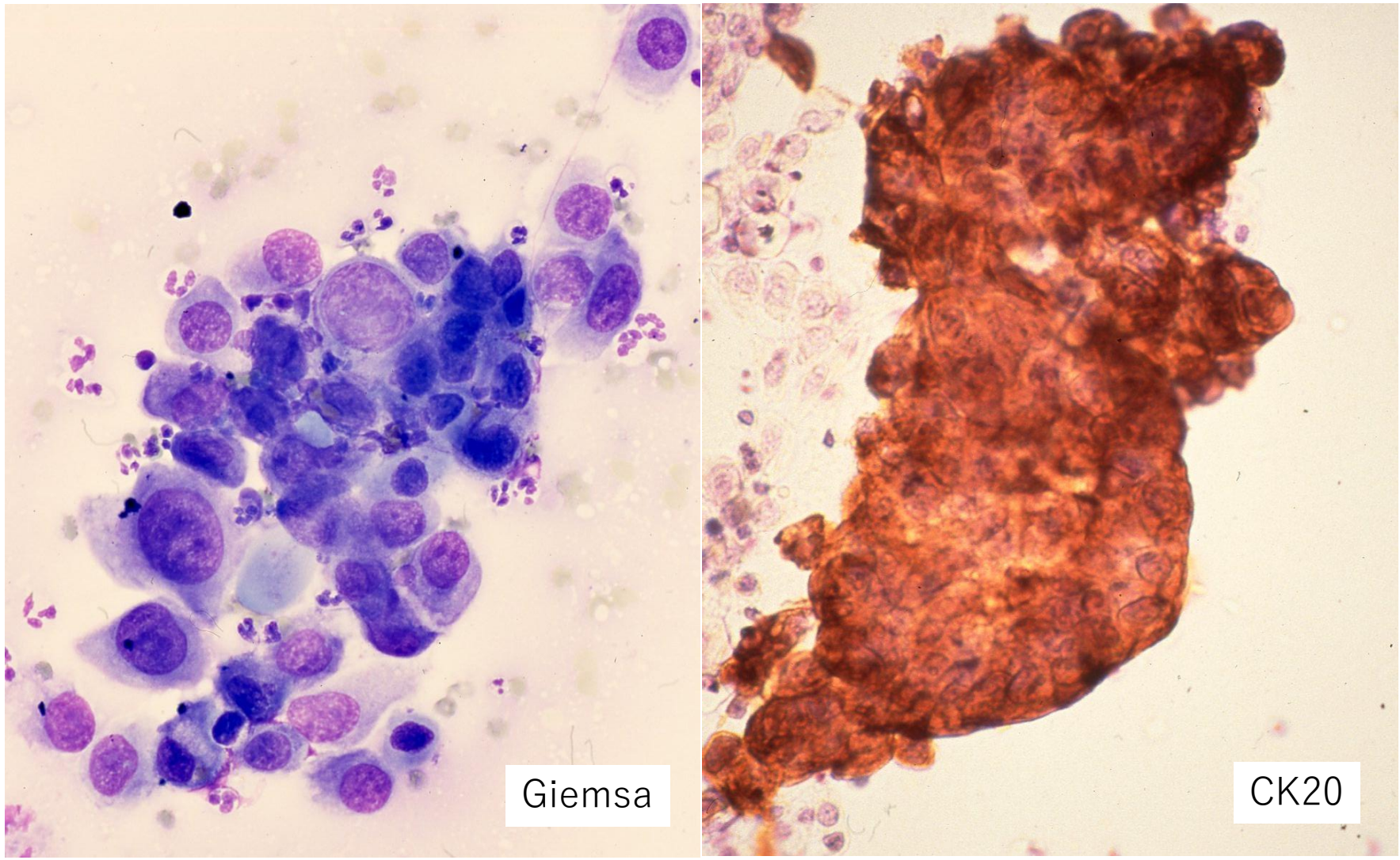
HIK-1083 immunoreactivity seen in adenoma malignum (minimal deviation adenocarcinoma of the uterine cervix). Stamp smear cytology of the surgical specimen of a 35 y-o female patient. Bland mucin-containing cells are clustered (left). The mucin component is immunostained for HIK-1083, a marker of gastric pyloric gland mucin (right).



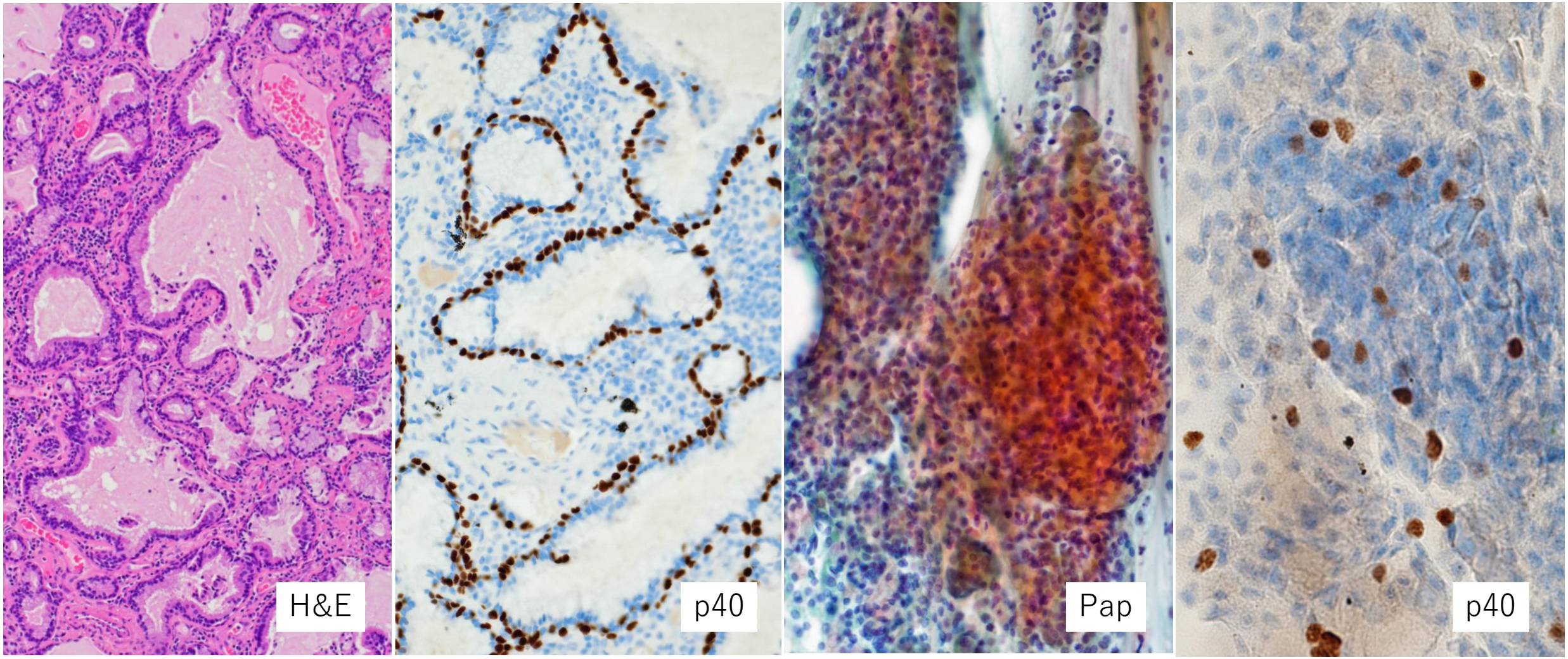
Type 4 collagen immunoreactivity in a raspberry body of clear cell carcinoma of the ovary. The ascites fluid from a female patient aged 50's. A metachromatic core is surrounded by atypical clear cells, forming the raspberry body (left: Giemsa). The core is immunoreactive for type 4 collagen (center) and laminin (right).



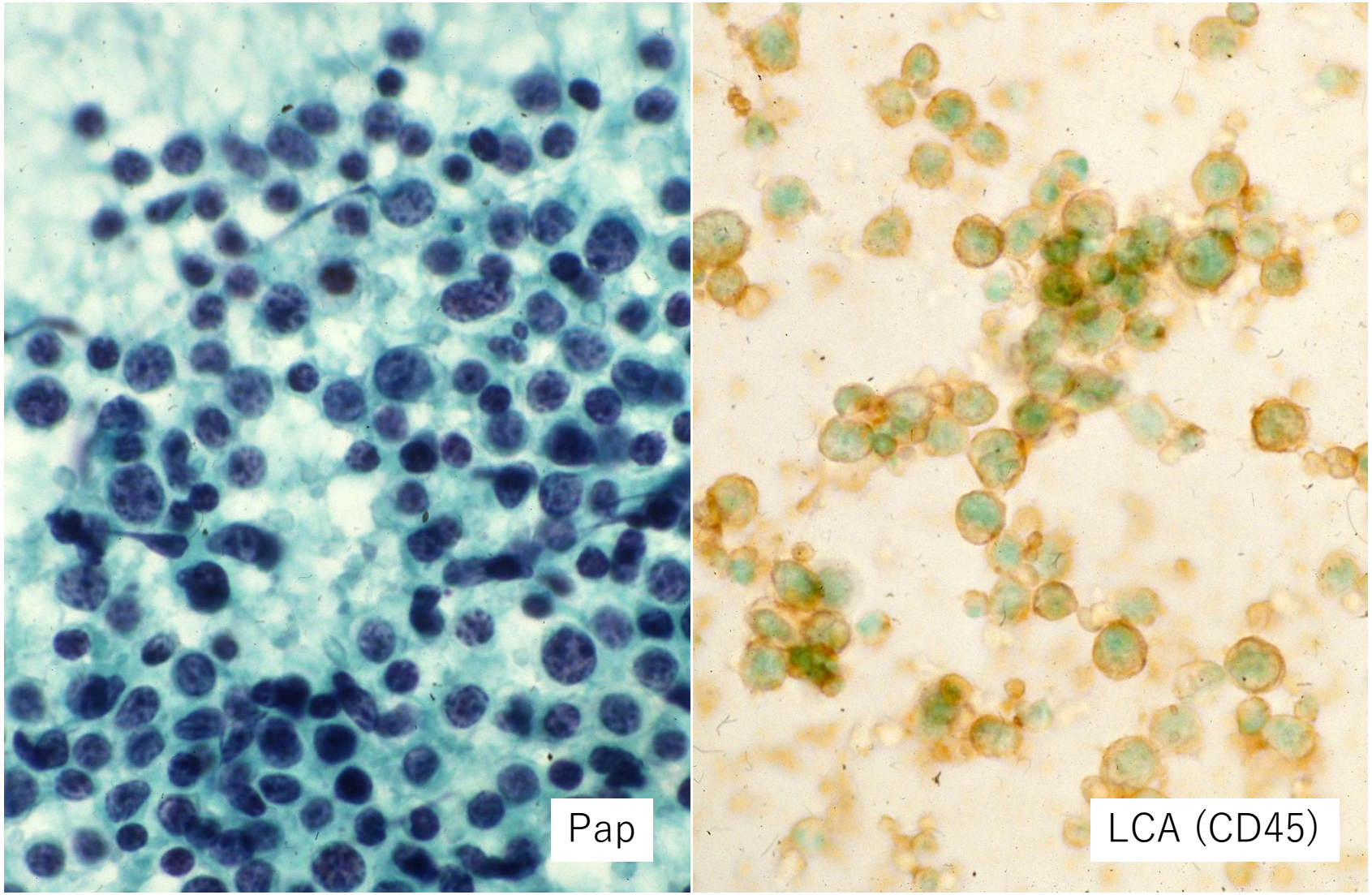
CK20 expression in mucinous adenocarcinoma of the ovary, disseminated onto the peritoneum of a female patient aged 60's. A clustered cancer cells with mucin production are immunoreactive for CK20.



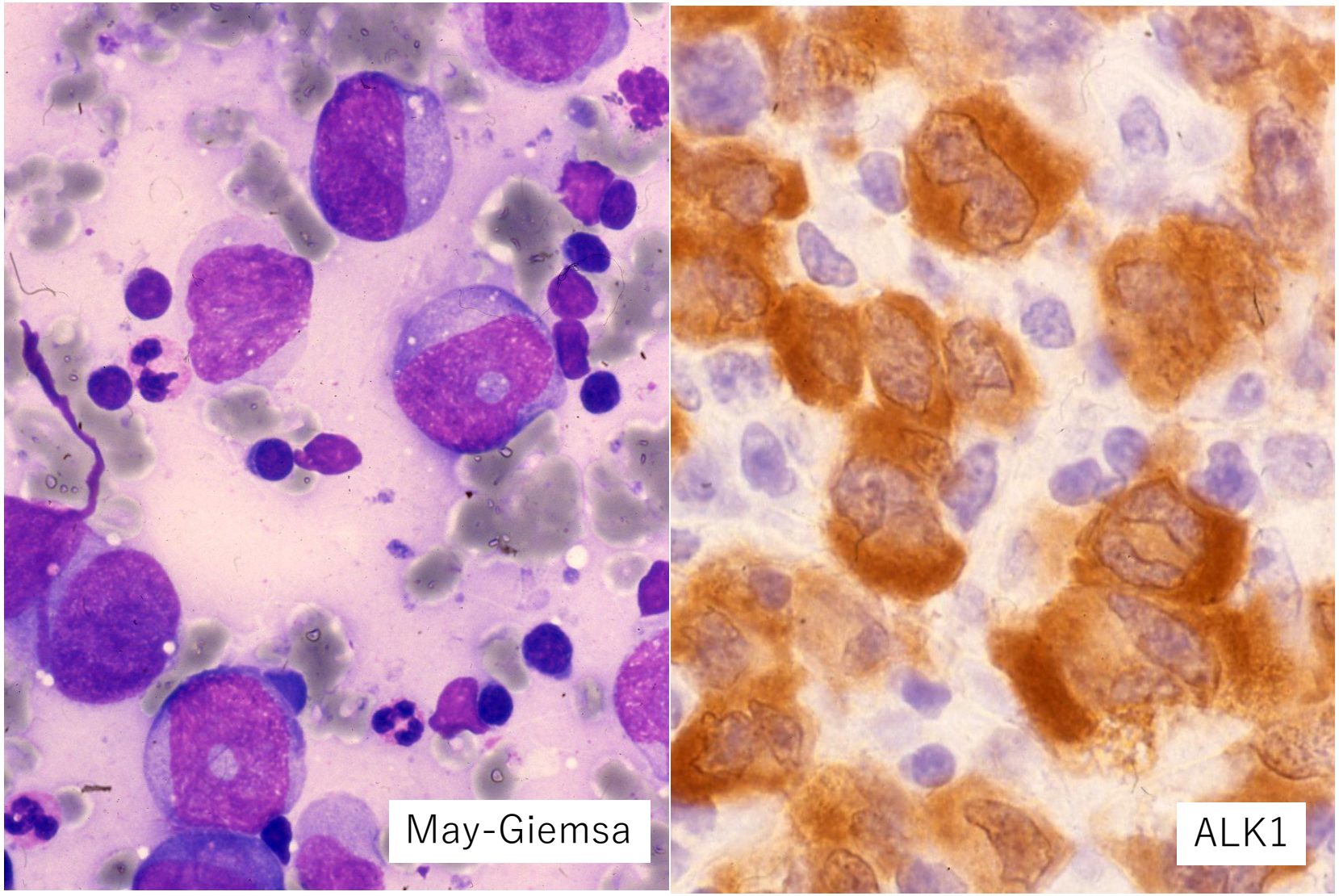
CK20 expression in colonic adenocarcinoma seen in a 73 y-o male patient. Ascites cytology demonstrates peritoneal dissemination of pleomorphic atypical cells (left: Giemsa). The colon cancer cells strongly express CK20.



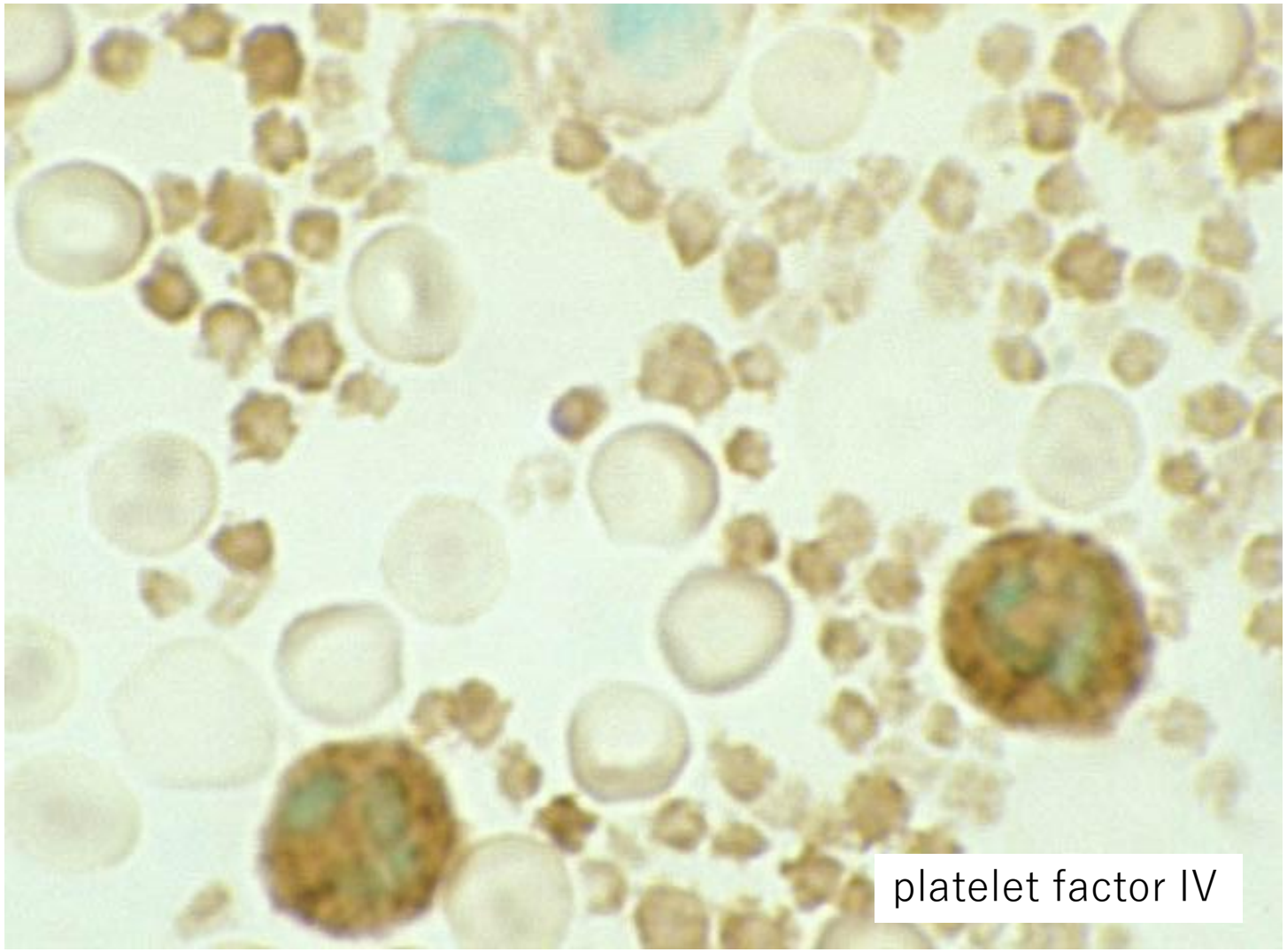
Bronchiolar adenoma, peripheral type, seen in a 70 y-o male patient. The 7 mm-sized peripheral lung nodule microscopically consists of bland ciliated cells, mucous cells and basal cells with a lepidic growth pattern. Stamp cytology of the lung lesion shows clusters of bland epithelial cells. The presence of p40-immunoreactive basal cells (the two-celled pattern) indicates benignancy.



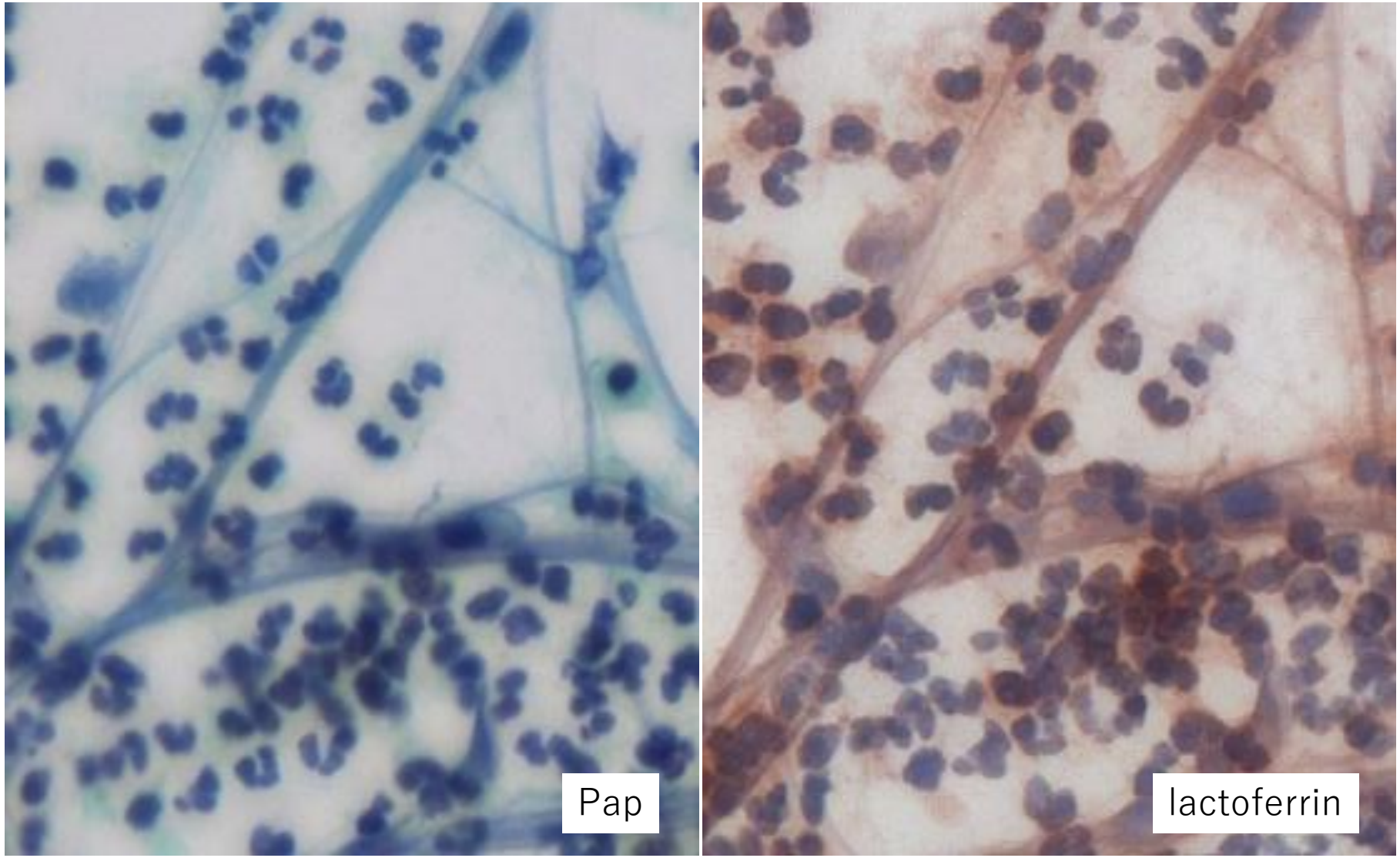
CD45 (leukocyte common antigen: LCA) expression on the surface of malignant lymphoma. Touch smear cytology of the lymph node biopsied from a male patient aged 40's reveals the growth of atypical medium- to large-sized lymphocytes (left). CD45 is expressed on the plasma membrane of the lymphoma cells (right).



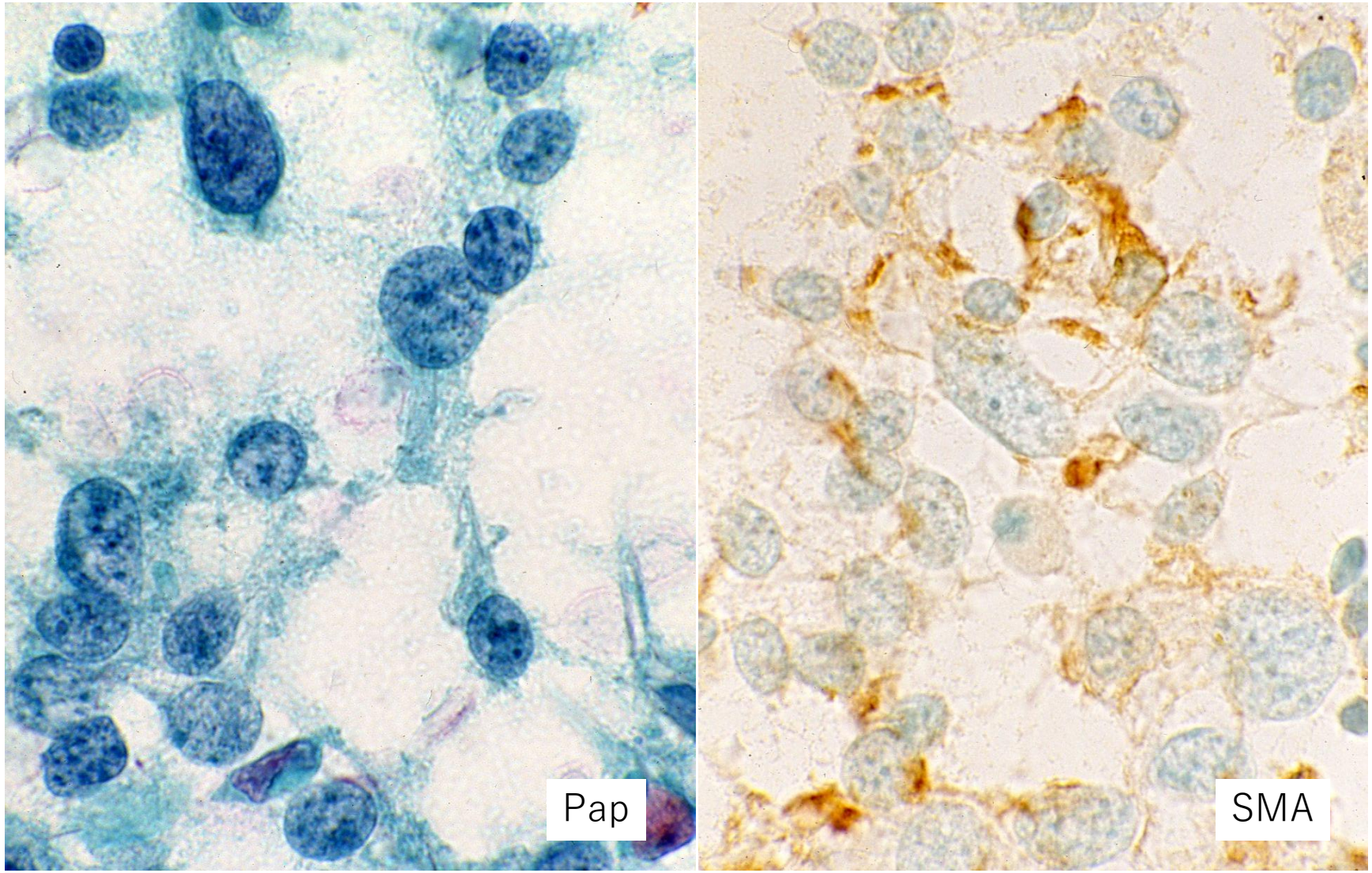
ALK-1 expression in large cell anaplastic (Ki-1) lymphoma. Touch smear cytology of the lymph node biopsied from a male patient aged 30's reveals the growth of atypical large Hodgkin-like lymphoma cells with prominent nucleoli (left). ALK-1 is diffusely expressed in the cytoplasm of the Ki-1 lymphoma cells (right).



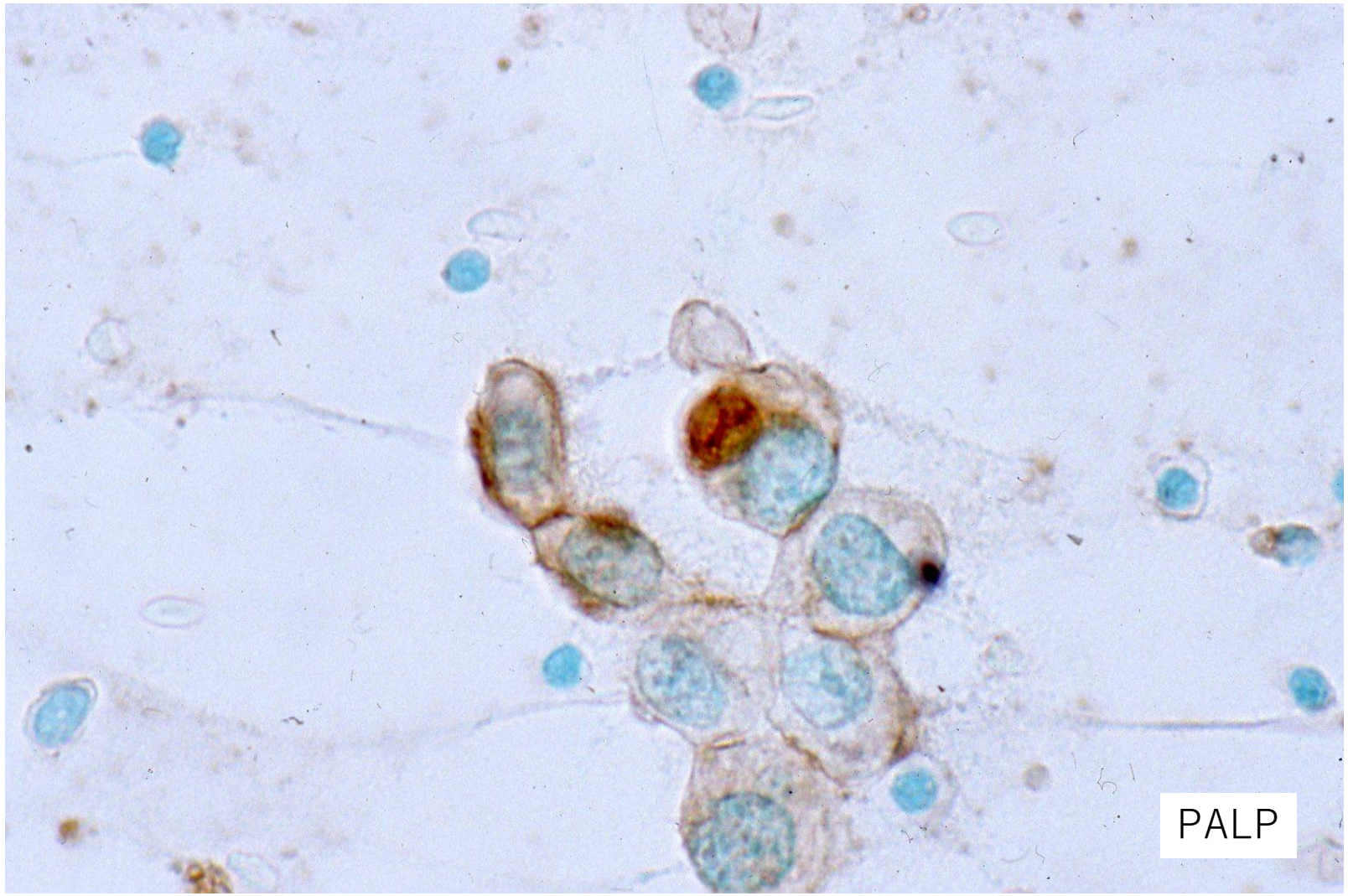
Platelet factor IV expression in platelets in the smeared buffy coat of the peripheral blood. Granular cytoplasmic positivity of platelet factor IV is demonstrated in the platelets. Neutrophils reveal endogenous peroxidase activity.



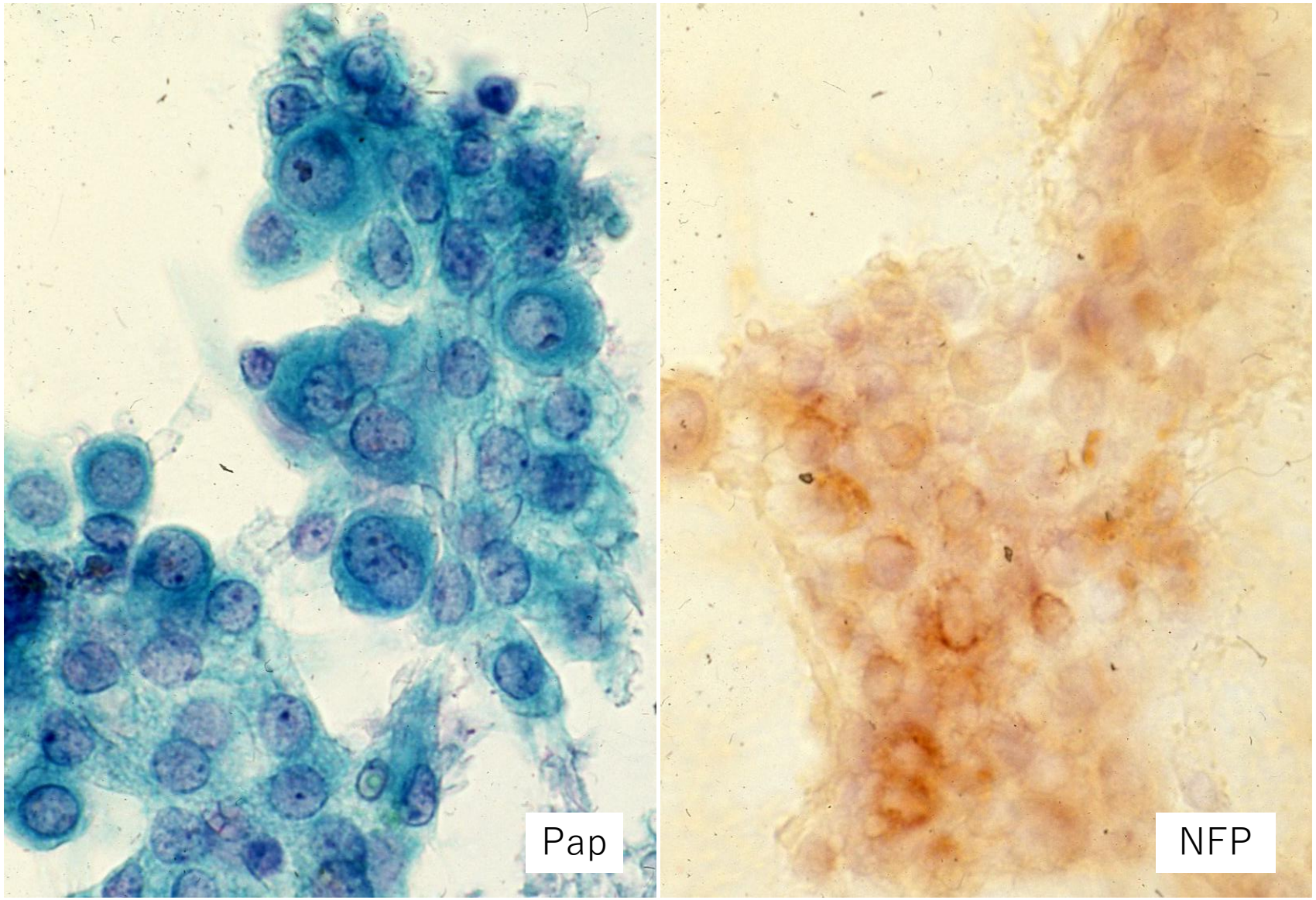
Lactoferrin immunoreactivity on neutrophil extracellular traps (NETs) seen in breast abscess. NETs, spiderweb-like filamentous structures, are formed through cell death of neutrophils at the site of acute inflammation, and can consistently be visualized with immunostaining for lactoferrin. Lactoferrin is a normal constituent of neutrophil granules.



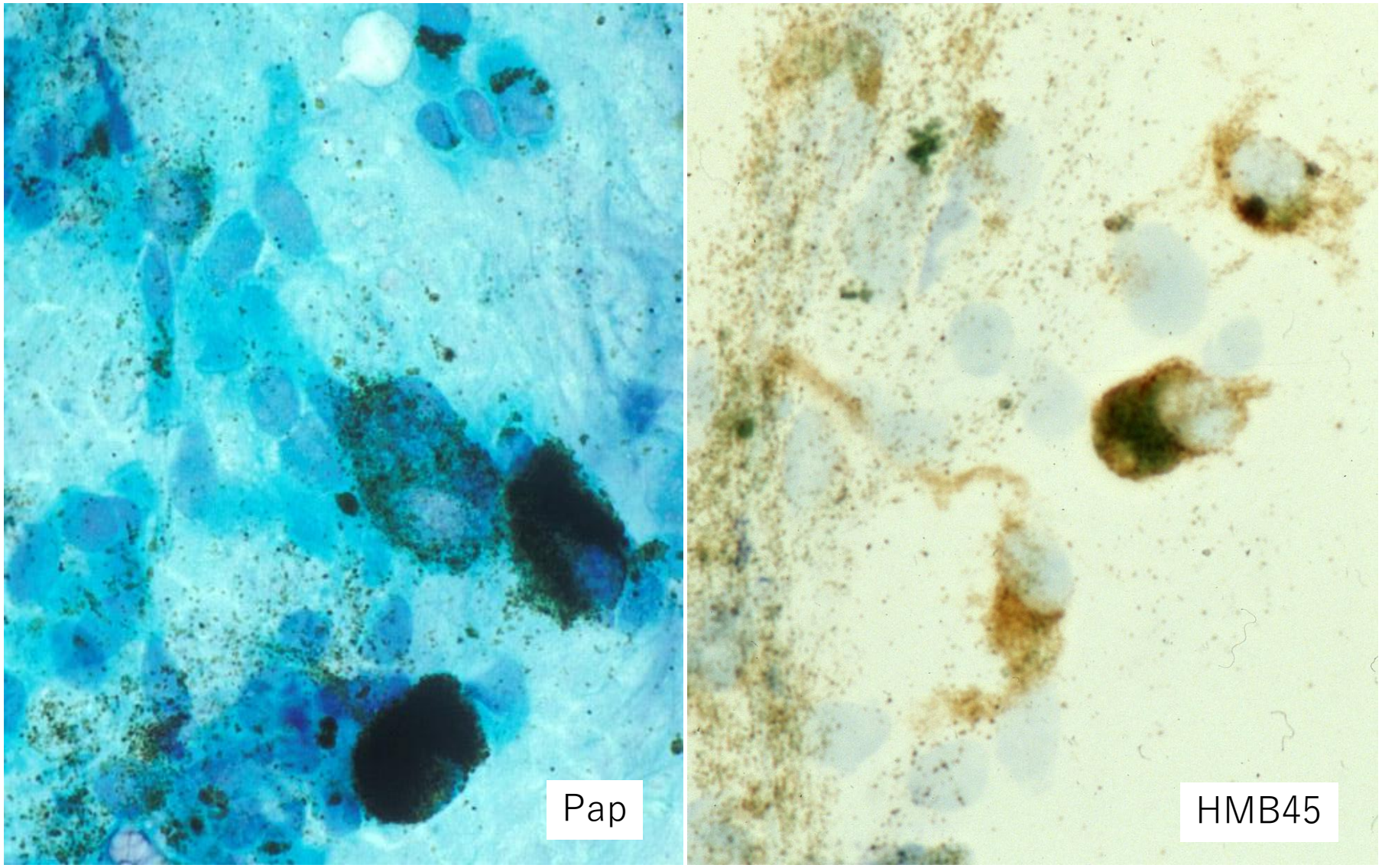
SMA expression in leiomyosarcoma of the soft tissue of the thigh aspirated from a male patient aged 50’s. The cytoplasm of atypical spindle cells is immunoreactive for SMA.



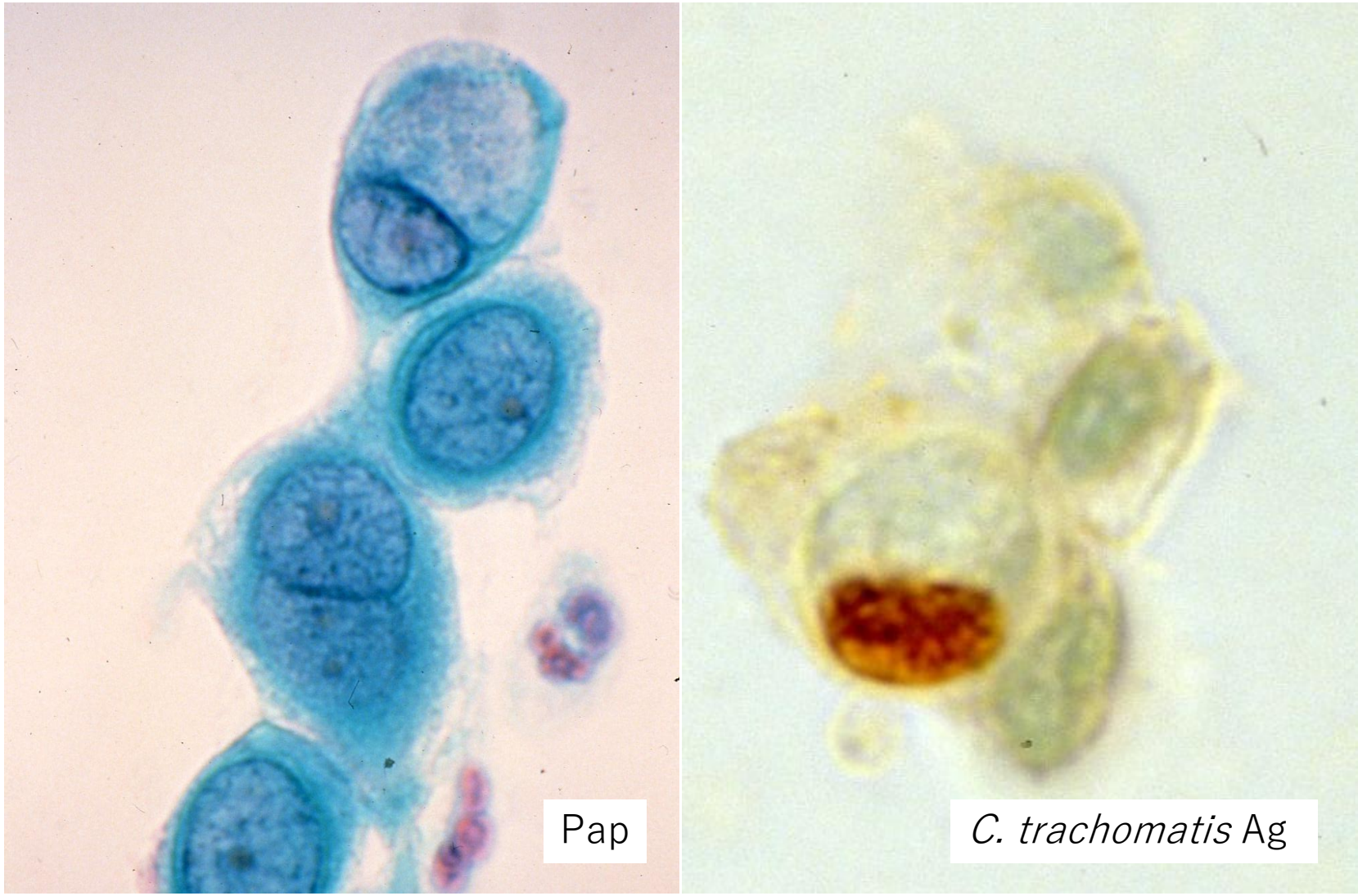
Placental alkaline phosphatase (PALP) expression in testicular seminoma. Touch smear preparation sampled from a male patient aged 30’s contains a cluster of large atypical cells. The plasma membrane and the Golgi area of the seminoma cells are labeled for PALP.



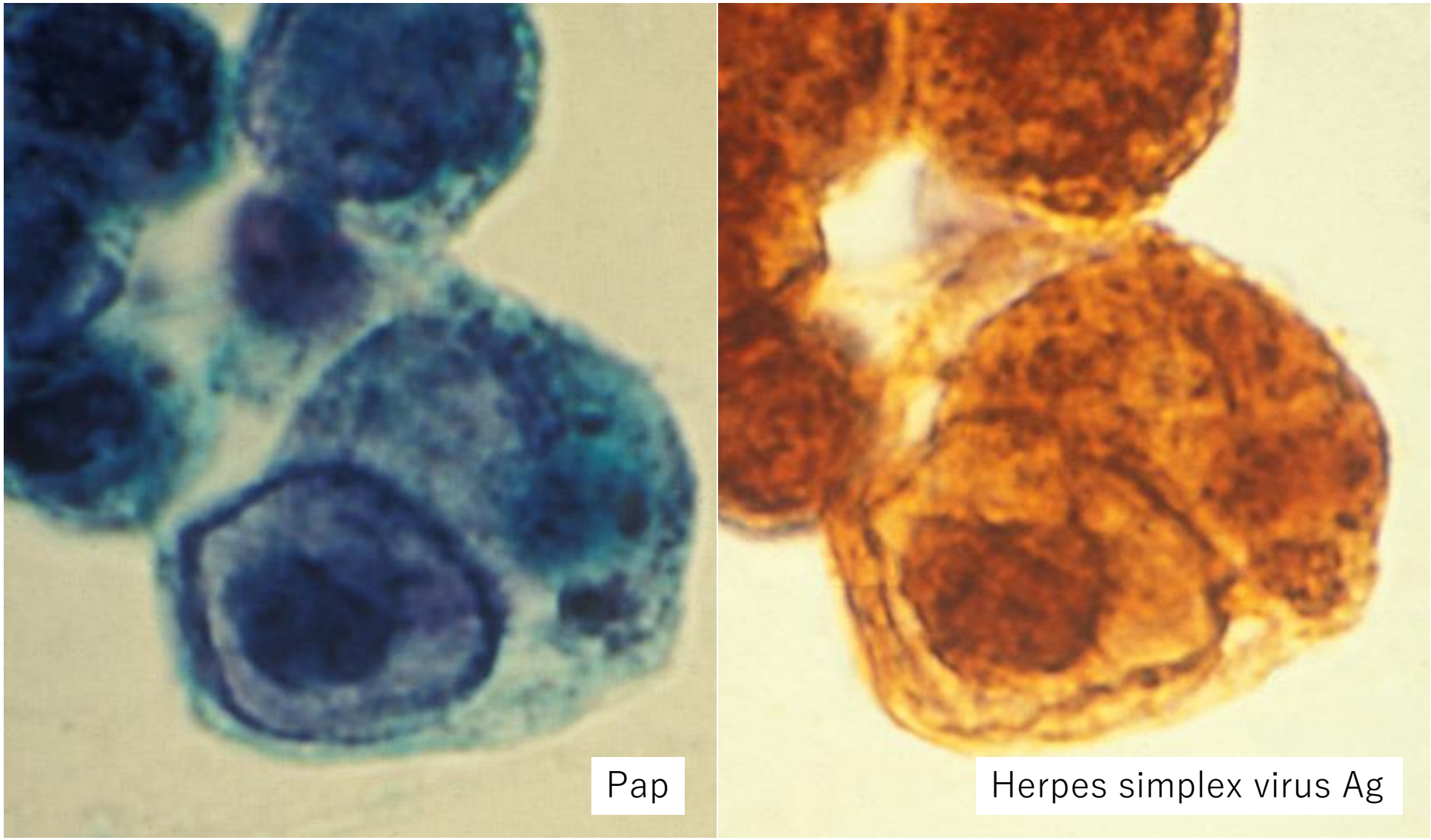
Ewing tumor/PNET (primitive neuroectodermal tumor) disseminated into the ascites fluid of a male patient aged 20's. Small round tumor cells are clustered (left). A good number of the tumor cells express neurofilament protein (NFP), indicating neuroectodermal differentiation (right).



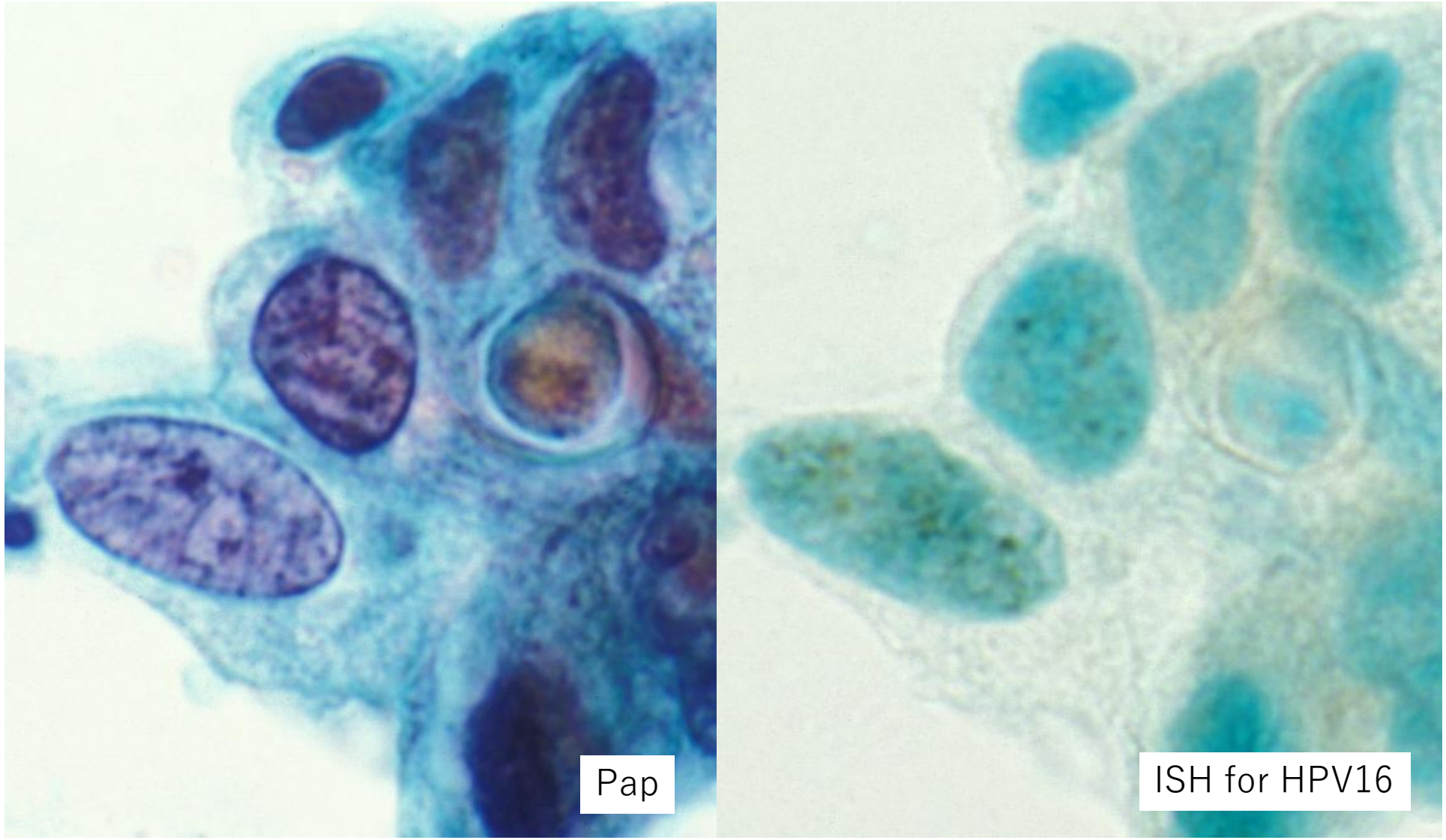
HMB45 expression in malignant melanoma cells seen in the black-colored nasal discharge. Melanin-containing tumor cells are observed (left: Pap). The cytoplasm of the nasal melanoma cells is positively labeled for HMB45 (right).



Cervical smear of chlamydial cervicitis seen in a female patient aged 20's. Nevular inclusion body is formed in the cytoplasm of the cervical columnar cell (left). The cytoplasmic inclusion is immunoreactive for *Chlamydia trachomatis* antigen, confirming the cytodiagnosis of chlamydiosis.



Herpetic vulvitis seen in a 25 y-o female patient. Intranuclear and cytoplasmic viral inclusions are discerned in the Pap smear preparation (left). Re-staining method discloses the positivity of Herpes simplex virus antigens in the same cells (right).



Severe dysplasia (HSIL) in the cervical smear preparation sampled from a female patient aged 30's (left: Pap). Re-staining was performed after the cell transfer onto the Silane-coated glass slide. *In situ* hybridization (ISH) for HPV type 16 genome demonstrates dotted signals in the nuclei of the dyskaryotic cells (right). Dots represent the integration of the viral genome into the host genomic DNA.