The 4th EFCS Annual Tutorial

Ospedale Universitario di Cattinara, Strada di Fiume, Trieste

Handouts for lectures and workshops – I

I - Gynaecological cytopathology

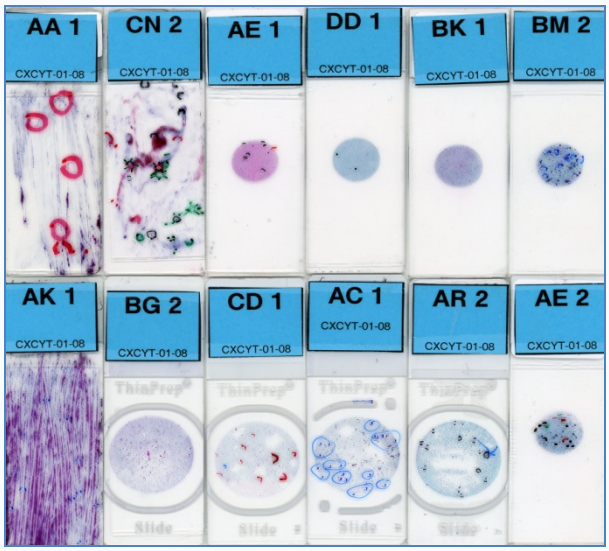
Gynecological cytology: technical aspects

Rietje Salet-van de Pol

Important in specimen processing is to obtain as much as possible well preserved cells for microscopically evaluation.

The quality of the smear depends on cell sampling, fixation and staining. For obtaining enough cervical material you are dependent on the cell sampler.

For cervical cytology two types of specimen are available: conventional smears and liquid based cytology (LBC).



Conventional, Thinprep and Surepath slides

In conventional cytology the cell sampler makes the smear and is responsible for the fixation of the cells. Reasons for unsatisfactory conventional smears can be obscuring blood or inflammatory cells, thick smears with overlapping cells, poor preservation of the cells due to late fixation and low cellularity.

In LBC the cell sampler immediately transferred the cellular material into a vial with fixative (fixating solution) which gives a better preservation of the cells. The laboratory is responsible for processing of the smear. LBC gives equally distribution of the cells in a thin cell layer of well preserved cells. The rate of unsatisfactory smears is lower. It reduces screening time due to better preservation and recognition of cells and reduction of screening area. Additional test (HPV) on same material is possible and it facilities automated screening.

For LBC two systems are FDA approved and are mostly used: Thinprep and Surepath.

In the Thinprep method (Hologic, Marlborough,USA) the transport vial contains a methanol-based fixative, the cell sample is rinsed in the vial. In the laboratory the cell suspension undergoes homogenization by spinning the vial. The cells are transferred to a transcyt filter by suction and the cellularity is controlled by pressure monitoring across the filter. The result is a 20 mm circle of cellular material.

The Surepath method (Becton Dickinson/Tripath, Burlington,USA) uses a ethanol-based fixative, the collection device head remains in the transport vial. In the laboratory homogenization takes place via syringing of the specimen, the passage through a sucrose density gradient column reduces blood and inflammatory cells. The final cell suspension occurs through gravity sedimentation on a glass slide. The slide coating allows only a single cell layer. The result is a 13 mm circle of cellular material.

Prior to staining spray fixatives, which contain alcohol and a waxy substance (carbowax), must be removed by two separate rinses of 95% ethanol (30 and 15 min.)

To visualize the cells and various cell components (modified) Papanicolaou stain is used. The modifications vary from staining times, use of tap or distilled water and progressive or regressive blue.

Papanicolaou stain (also **Papanicolaou's stain** and **Pap stain**) is a multichromatic staining and is a very reliable technique. The classic form of Pap stain involves five dyes in three solutions:

- A nuclear stain, haematoxylin, is used to stain cell nuclei. It is important to date haematoxylin when it is received in the laboratory, because the dye may oxidize overtime, especially in moist climates. The unmordanted haematein may be responsible for the yellow color imparted to glycogen.
- Orange G counter stain is an acidic dye. It stains keratin. The granules in eosinophylic, superficial cells are also stained. The original role of Orange G was to stain the small cells of keratinizing squamous cell carcinoma present in sputum.
- EA (Eosin Azure) counter stain, comprising of three dyes;
    - Eosin Y is an acid dye and stains the superficial epithelial squamous cells, nucleoli, cilia and red blood cells.
    - Light Green SF yellowish is an acid dye and stains the cytoplasm of metabolically active cells, intermediate squamous cells, parabasal and columnar cells, histiocytes, leukocytes, large- and small-cell undifferentiated carcinoma cells and cells deriving from adenocarcinoma green. This dye is now quite expensive and difficult to obtain, therefore some manufacturers are switching to Fast Green FCF, however it produces visually different results and is not considered satisfactory by some.
    - Bismarck brown Y stains nothing and in contemporary formulations it is often omitted.

EA has three separate formulations: EA-36 was originally formulated for staining gynecologic smears, EA-65 is a modification and was developed for staining thicker cell samples and EA-50 is a commercially prepared solution, it is supposed to be similar to EA 36.

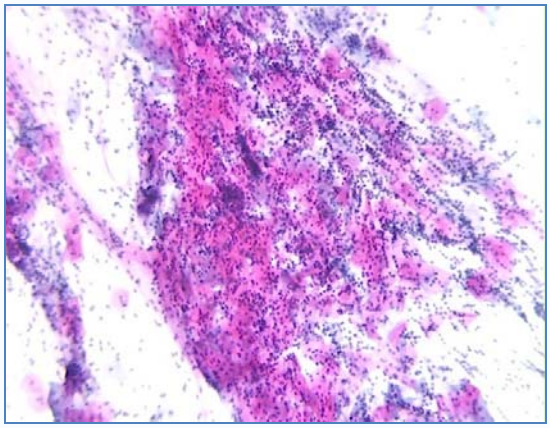
The dye should be filtered daily and stored in a dark well-sealed bottle when not in use to exclude as much light as possible.

When performed properly, the stained specimen should display hues from the entire spectrum: red, orange, yellow, green, blue, and violet. The chromatin patterns are well visible, the cells from borderline lesions are easier to interpret, the photomicrographs

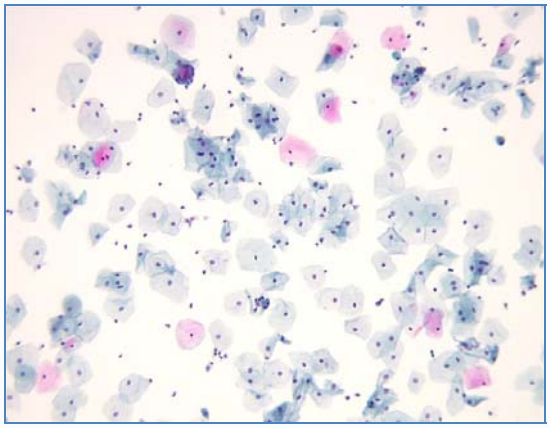
are better, and the stained cells are pretty. The staining results in very transparent cells, so even thicker specimens with overlapping cells can be interpreted.

On a well prepared specimen, the cell nuclei are crisp blue to black. Cells with high content of keratin are yellow, glycogen stains yellow as well. Superficial cells are orange to pink, and intermediate and parabasal cells are turquoise green to blue. Metaplastic cells often stain both green and pink.

Pap stain is not fully standardized. The selection of staining times for each solution, type of staining method used (progressive or regressive), use of tap or distilled water and PH of water and temperature differ from laboratory to laboratory. The progressive method is the most commonly used method in laboratories today. It is easier to control and provides more stability from day to day. Whichever modification is used it is advisable to standardize the staining method as much as possible to achieve reproducible results.



Conventional smear



Liquid based smear

Cover slipping should be performed under a well ventilated fume hood to avoid inhaling toxic vapors. A no.1 thinness coverslip is used to cover all the cells on the slide and because of its optical properties. The optimal refractive index of a glass slide is 1.515 ± 0.015, with a thickness range of 0.96-1.06 mm, this is best matched to a no. 1 thinness cover glass. Especially for photography the selection of glass slide and coverslip is critical. The refractive index of the mounting medium must be the same as that of the coverslip, the mounting medium should harden rapidly, may not dissolve the dye and it must stay clear after drying.

Destaining and restaining of the Pap stain is possible but gives only good results when the sample was properly fixed. First the coverslip is removed, next step is removal of the mounting medium, which may take several hours in xylene. The older the slide the more time it takes. To remove the nuclear dye the slide is placed in a dilute hydrochloric acid in an aqueous or an alcohol-based solution. Removal of hematoxylin may take 5 – 20 minutes. Prior to restaining the slide is then rinsed in running tap water for 5- 10 minutes.

Adequate conventional smears have a minimum of 10.000 to 12.000 squamous cells and in LBC the minimum is 5000 squamous cells. Cervical smears are satisfactory if cells are present from the transformation zone. In conventional smears and in LBC 10

well preserved endocervical or squamous metaplastic cells must be present , singly or in clusters.

Tips and troubleshooting

- It is important to introduce a microscopically control step after every staining procedure to anticipate on potential staining problems.
- The carbowax from spray fixatives must properly be removed before staining otherwise the dye cannot penetrate the cells.
- When slides are transferred from water to hematoxylin the glassy droplets of water on the slide must disappear otherwise the dye does not penetrate the nucleus.
- The hematoxylin has to be changed after 2000 slides or after 6-8 weeks.
- The PH of tap water following hematoxylin must be alkaline (PH 7.4).
- The PH of EA should be between 4.5 and 5.
- Alcohols must be changed regularly.
- Slides should not be left in alcohol solutions after OG and EA dyes because the stains are washed out of the cells in alcohol.
- Xylene has to be replaced when it becomes milky or contains small bubbles (water).
- To avoid cross-contamination solutions must be filtered after every use.
- Solutions should be kept covered when not in use and stored in a dark well-sealed bottle.
- When the cells have a hazy appearance there is a water contamination in dehydrating solutions or the spray fixative is not removed from the cells.

Nuclei too pale
Carbowax from spray fixative was not properly removed, time in hematoxylin was not adequate, PH of tap water following hematoxylin was not sufficiently alkaline or hematoxylin is exhausted.

Nuclei too dark
Overstaining in hematoxylin or excess dye not removed.

Cytoplasmic color not satisfactory
Sample air dried prior to fixation, excessive time in hematoxylin, inadequate rinsing of slides, PH of EA is not 4.5 – 5, inadequate staining times and exhausted dye.

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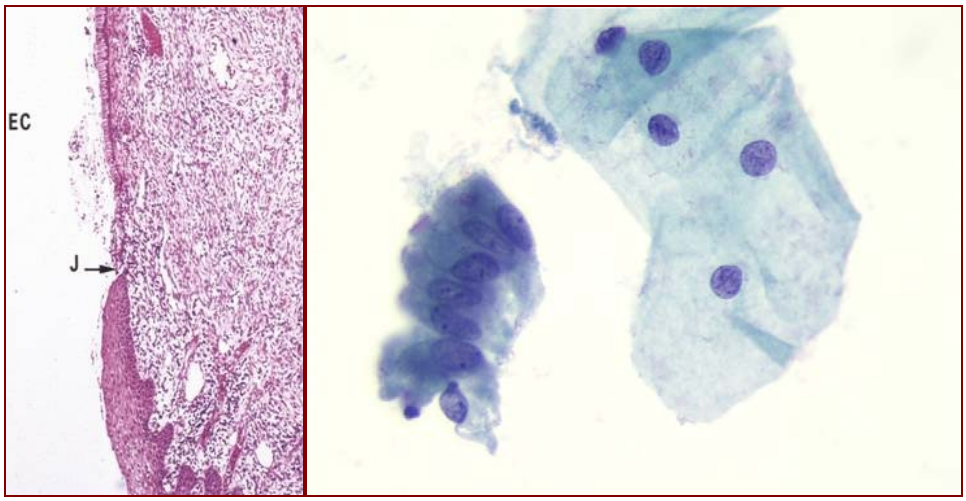
Non-neoplastic gynecological cytology

Rietje Salet-van de Pol

An adequate conventional smear contains 10.000 – 12.000 well-visualized well preserved squamous cells and a liquid based cytology circle contains at least 5000 squamous cells. According to the Bethesda criteria for adequacy: A specimen is considered "partially obscured" when 50% to 75% of the squamous epithelial cells cannot be visualized. Specimens with more than 75% of epithelial cells obscured are "unsatisfactory" (specify reason). For an adequate liquid based slide a minimum of 10 microscopic fields, usually at 40X (HP), should be assessed along a diameter that includes the center of the preparation and an average number of 4-9 (dependent on microscopic eyepiece and LBC method) well preserved squamous cells per field must be counted.

A satisfactory smear must contain cells from the transformation zone (squamocolumnar junction): in conventional smears and in liquid based smears at least 10 well preserved endocervical or squamous metaplastic cells must be present, singly or in clusters.

It is important to give a diagnose only on adequate and satisfactory smears, because several studies found that inadequate and unsatisfactory smears were more often from high risk patients and a significant number of these smears were followed by a severe lesion.



Squamocolumnar junction *Squamous and endocervical cells*

Normal smears

- Squamous cells: basal cells with scarce cytoplasm and large oval to round nuclei, parabasal round to oval cells with distinct cell borders and relatively large oval to round nuclei, intermediate polygonal cyanophylic cells with round to oval nuclei with finely granular chromatin and superficial polygonal cells with a small often pyknotic nucleus.

- Endocervical cells are tall mucus producing columnar cells with basally located nuclei, a large body of clear cytoplasm, ciliated cells may be present, cells are

arranged in strips or sheets with honeycomb pattern, nuclei are round to oval with finely evenly distributed chromatin, one or two small nuclei may be present.

- Endometrial cells can be present in first 10-12 days of menstrual cycle, are grouped cylindric cells in three dimensional clusters, cells are oval to round with scarce finely vacuolated cytoplasm, central or excentric nuclei, bone shaped or round to oval, nuclei have size of nucleus of intermediate squamous cells, sometimes small nucleoli.

Atrophic smears

Atrophic smears can be seen pre- and post menopausal and post partum. They are composed of basal and parabasal cells, cells are arranged in aggregates with indistinct cell borders, round to oval nuclei, relatively high n/c ratio and granular chromatin, nucleoli are not present.

Normally atrophic smears does not cause diagnostic problems. In cases of inflammation and atrophy nuclear chromatin can become coarsely granular and hyperchromatic and differentiation with dysplastic cells can be difficult.

Inflammatory conditions

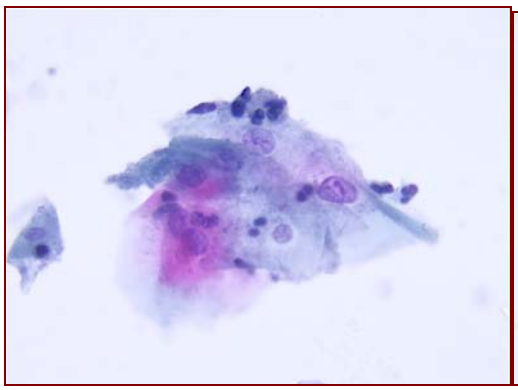
In inflammatory smears there are numerous polymorphonuclear leukocytes often with a large number of histiocytes, followed by lymphocytes and plasma cells if inflammation persists. Under the persistent effect of various microbiological infections and inflammatory reactions both squamous and columnar epithelial cells may undergo cell changes:

Cell changes in inflammatory conditions:

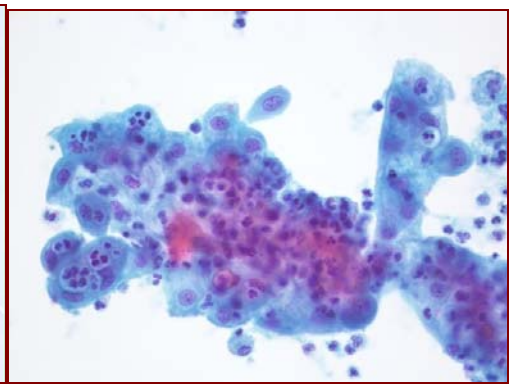
Enlargement and binucleation

Degenerative changes:

- irregular nuclear membranes (chromatin becomes blurry beaded along nuclear margins)
- hyperchromasia
- karyorrhexis and karyopyknosis
- vacuolization and included granulocytes
- degenerative color changes: pseudoeosinophilia
- perinuclear halo



Degenerative cell changes



Tissue repair

When there is a persistent irritation (infectious or non-infectious) the epithelial cells undergo morphologic changes:

- Tissue repair: cells in sheets with distinctive cell borders, nuclear enlargement, anisokaryosis, regular finely granular chromatin, chromocenters, prominent and multiple nucleoli, regular shaped nucleoli, abundant cytoplasm with granulocytes, normal mitosis, cellular cohesion and rarely solitary cells

- Metaplastic changes (replacement of simple columnar epithelium by a stratified squamous epithelium): mature and immature squamous metaplasia

- Endocervical columnar epithelium: columnar cell hyperplasia, polyp formation and tubal metaplasia

- Endometrial cells can show reactive, degenerative and metaplastic changes p.e. in association with chronic irritation due to an IUD.

- Tubal metaplastic cells are columnar cells that can show pseudostratification, can have hyperchromatic nuclei and increased n/c ratio, look for terminal bars and cilia

- Hyperkeratosis (anucleated squames) is an abnormal differentiation under influence of chronic stimulations such as prolaps of the uterus, inflammatory processes and as a reaction of hyperestrinism of long duration.

- Parakeratosis are relatively small superficial squamous cells, shape is rount to oval, polygonal or spindle-shaped. Nuclei are small and often pyknotic. (Beware of hyper- and parakeratosis overlying abnormal cell change. Advice is to make 2 smears, the first one to remove the keratosis.)

- Dyskeratosis is aypical keratinization often in the presence of chronic infections caused by human papillomavirus.

Chronic inflammation:

- *Follicular cervicitis*: lymphoid follicles in sub epithelial areas, cytologically composed of mature and reactive lymphoid cells and tangible body macrophages

- *Granulomatous cervicitis*: large aggregates of epithelioid cells, occasionally with Langhans-type multinucleated giant cells, may occur in presence of foreign bodies (IUD) or specific infections, such as tuberculosis

Specific infections:

- *Bacterial infection*: cells partly or complete covered by bacteria (bacilli and coccid organisms) and in background

- *Actinomyces*: belongs to the order of higher bacteria, occurs as dark woolly clumps with filamentous structures and is found in presence of intrauterine devices, vaginal pessaries and foreign bodies

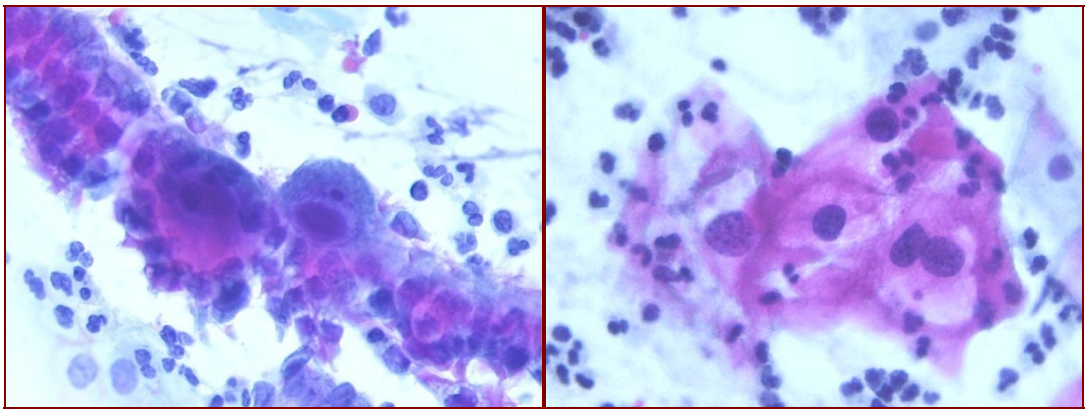
- *Fungal infections*: candida infections: numerous filamentous organisms revealing pseudo- and true hyphal and yeast forms, only yeast-budding forms occur, often fern-like arrangement of squamous cells with hyphen

- *Trichomonas infection*: protozoan organism, small round to oval pale grey-green structure with distinct faint nucleus, mostly eccentric with sometimes eosinophilic granules, occurrence of aggregates of leukocytes on squamous epithelial cells, occurs often together with leptotrichia

- **Viral infections**:
    - *Herpes Simplex Virus* (HSV): may effect squamous and endocervical cells, there is cytomegaly and karyomegaly, due to degenerative changes chromatin becomes finely divided, some against nuclear membranes (ground glass appearance), in the later stage of infection cells undergo the effects of viral replication and DNA integration and you can see multinucleation and molding, large and single eosinophylic intranuclear inclusions, usually round or oval with halo

    - *Cytomegalovirus* (CMV): belongs to the Herpes group, affects endocervical glands, cells are multinucleated and enlarged with round very large intranuclear acidophilic inclusion with a clear zone or halo around it given the cell an "owl's eye " appearance, intracytoplasmic inclusions also possible

    - *Human papillomavirus* (HPV): belongs to the family of Papovaviridae, from the more than 100 HPV types identified approximately 40 different types affect anogenital tract, some of them have oncogenic potential (a.o. 16,18,31,33,35), koilocyt is an excellent indicator: large clear-cut perinuclear halo, thickened cellular margin, loss of polygonal form with blunt or rounded corners, bi- and multinucleation, nuclei can be degenerated, pyknotic, chromatin finely divided, nuclear membrane can be wrinkled (raisin like), also dyskeratosis, parakeratosis and hyperkeratosis



Cytomegalovirus *Human papillomavirus*

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SIL and cancer; ASC-US, ASC-H, diagnostic pitfalls and look-alikes; glandular abnormalities

Giovanni Negri

Workshop cases

Each workshop case provides essential clinical data on the front and the diagnosis in accordance with TBS 2001 on the rear of the package. In some cases histology sections are also included.

Conventional smears and LBC: similarities and differences

The morphological differences between conventional Pap smears (CP) and liquid-based cytology (LBC) are a consequence of the sampling, fixation and slide preparation peculiarities of LBC. LBC shows in most cases a better sample quality than CP with potential reduction of repeats for inadequate tests (1,2). The possible advantages and cost-effectiveness of LBC must however be evaluated in the local context, particularly depending on the quality of a pre-existing CP. In fact, implementation of LBC is likely to be particularly successful when the quality of preexisting CP is poor (3). Liquid-based techniques also allow the use of ancillary techniques such as HPV-testing or immunocytochemistry, which in some laboratories may be an additional reason for converting to LBC .

Morphologically there are some minor differences between CP and LBC, which must be taken in account when moving to LBC and that may cause a temporary increase of borderline reports. Although the basic cytological criteria are the same, the loss of topographic distribution of cells may cause some perplexity at first, as well as the high nuclear detail that may raise suspicion of dysplasia in reactive changes, particularly in metaplastic cells. On the other hand, the reduction of brushing and smearing artefacts as well as inflammatory exudates may allow a better evaluation of the architecture of cell sheets, which may be useful particularly in glandular aggregates (4).

Squamous and glandular lesions, diagnostic pitfalls and look alikes

The European guidelines (5) recommend the use of The Bethesda System 2001 (TBS) to classify cervicovaginal findings or, at least, the use of a terminology that may be easily translated into TBS. TBS and most other classification systems that are used in Europe divide precancerous squamous lesions in two categories, including HPV-induced changes and mild dysplasia in "low grade intraepithelial lesions", whereas moderate and severe dysplasia are included together in "high grade intraepithelial lesions". More substantial differences between classification systems may be found in the terminology of glandular findings (6), which in part reflect the difficulties in the interpretation of both histology and biology of these lesions.

Basically, in cervicovaginal cytology one has to distinguish three main cell patterns: large cells, small cells and glandular epithelia. If the cells are abnormal, each of these patterns calls for a limited number of differential diagnoses.

Squamous cell lesions of the cervix

Atypical large cells may be found particularly in LSIL, invasive cancer, tissue repair and reactive changes. Most cases with abnormal large cells include superficial squamous epithelia with nuclear enlargement and hyperchromasia. Some cases may also show distinct perinuclear halos (koilocytosis). When no tumor diathesis or nucleoli are evident, one may classify these lesions as LSIL.

Rule out: invasive cancer (high grade nuclear atypia, nucleoli, tumor diathesis), tissue repair (sheets of epithelia with evident nucleoli, no isolated cells, no atypia, no diathesis), reactive changes (no hyperchromasia, nuclear enlargement < 2x). Borderline changes, as may be seen in some inflammatory smears may be classified as ASC-US according to TBS.

Atypical small cells may be found particularly in HSIL, invasive cancer, immature metaplasia and endometrial cells. HSIL typically show squamous cells with definite hyperchromasia, variability of nuclear size and shape, abnormal n/c ratio and indentations of the nuclear membrane. Conventional smears may show a typical "Indian filing", which is lost in LBCs. When small cells show high grade nuclear atypia and nucleoli or tumor diathesis is present, an invasive cancer should be ruled out. Reactive changes on metaplastic cells usually lack hyperchromasia and show a modest nuclear enlargement. HSIL may occasionally mimic a glandular lesion, the typical criteria for AIS (sheets with crowded, elongated nuclei and feathering), however, are usually not fulfilled. Endometrial cells may mimic a HSIL, but are mostly arranged in typical three-dimensional clusters and often show some degenerative changes. Borderline changes, particularly in smears with atypical metaplastic cells, may be classified as ASC-H according to the TBS.

Glandular lesions of the cervix

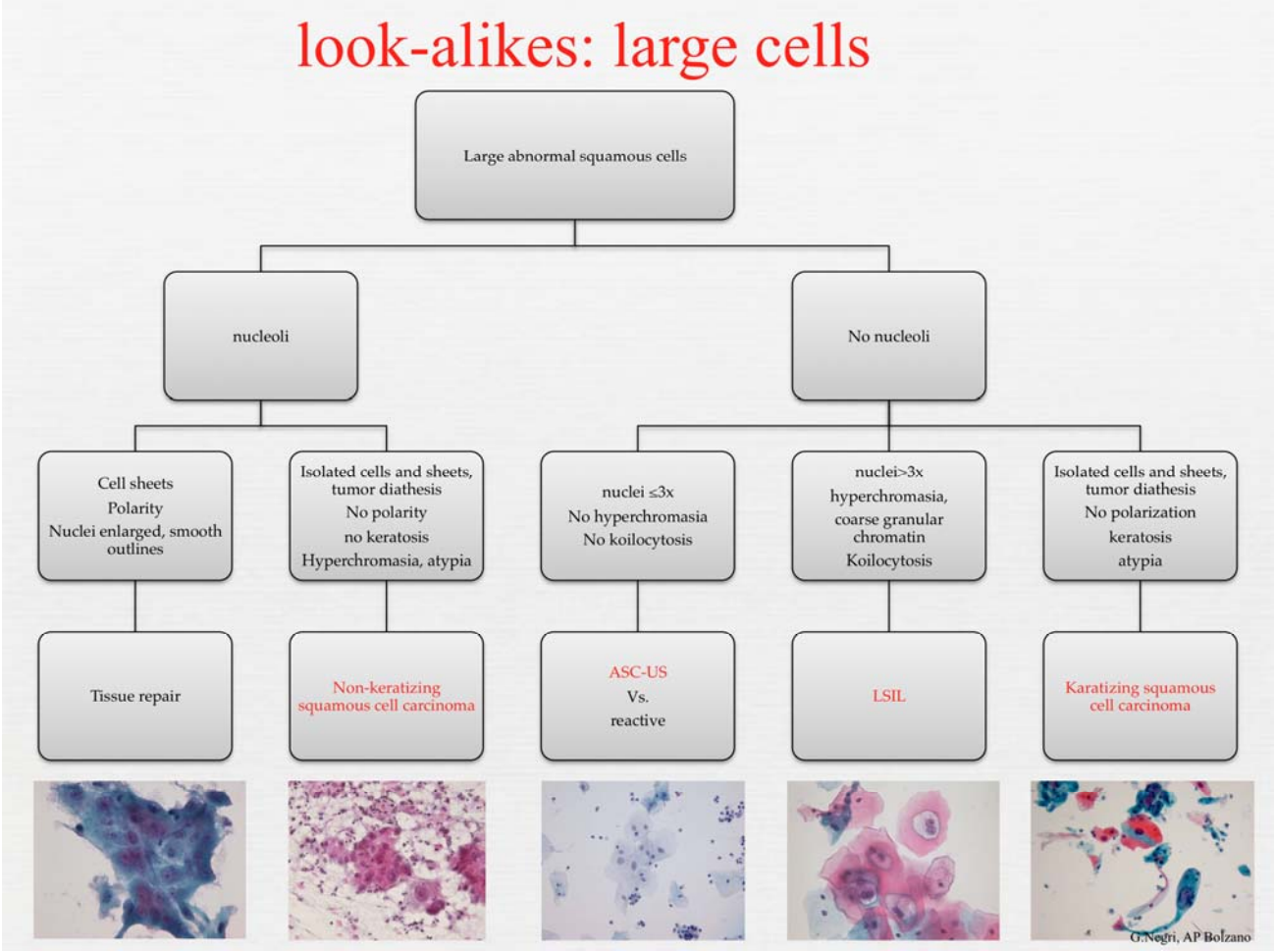
Atypical glandular cells may be found in adenocarcinoma in situ (AIS), endocervical adenocarcinoma, endometrial adenocarcinoma and metastasis of extrauterine malignancy.

Particularly in LBC specimens, AIS is often characterized by a high cellularity. Already at screening magnification, several hyperchromatic sheets are mostly readily evident. At higher magnification, the sheets consist of abnormally pseudostratified cylindrical cells with crowded nuclei which are enlarged, elongated and often hyperchromatic. The cytoplasm is typically reduced due to the nuclear enlargement, and nucleoli are mostly inconspicuous or small. Feathering is often observed at the periphery of the strips and pseudorosettes may be also present. Since cell overlapping is reduced in LBC, the presence of brushing artefact-like features at screening magnification in liquid-based samples always raises suspicious of an endocervical neoplasia which should be carefully ruled out. Tumor diathesis is not a feature of AIS, and its presence strongly indicates the possibility of infiltration, as well as the loss of cell cohesion and the evidence of highly atypical, polymorphic cells. Since in about 50% of endocervical lesions a SIL may coexist, atypical squamous cells are often found. Borderline findings that are suspicious but not definite for glandular neoplasia may be classified as AGC according to TBS.

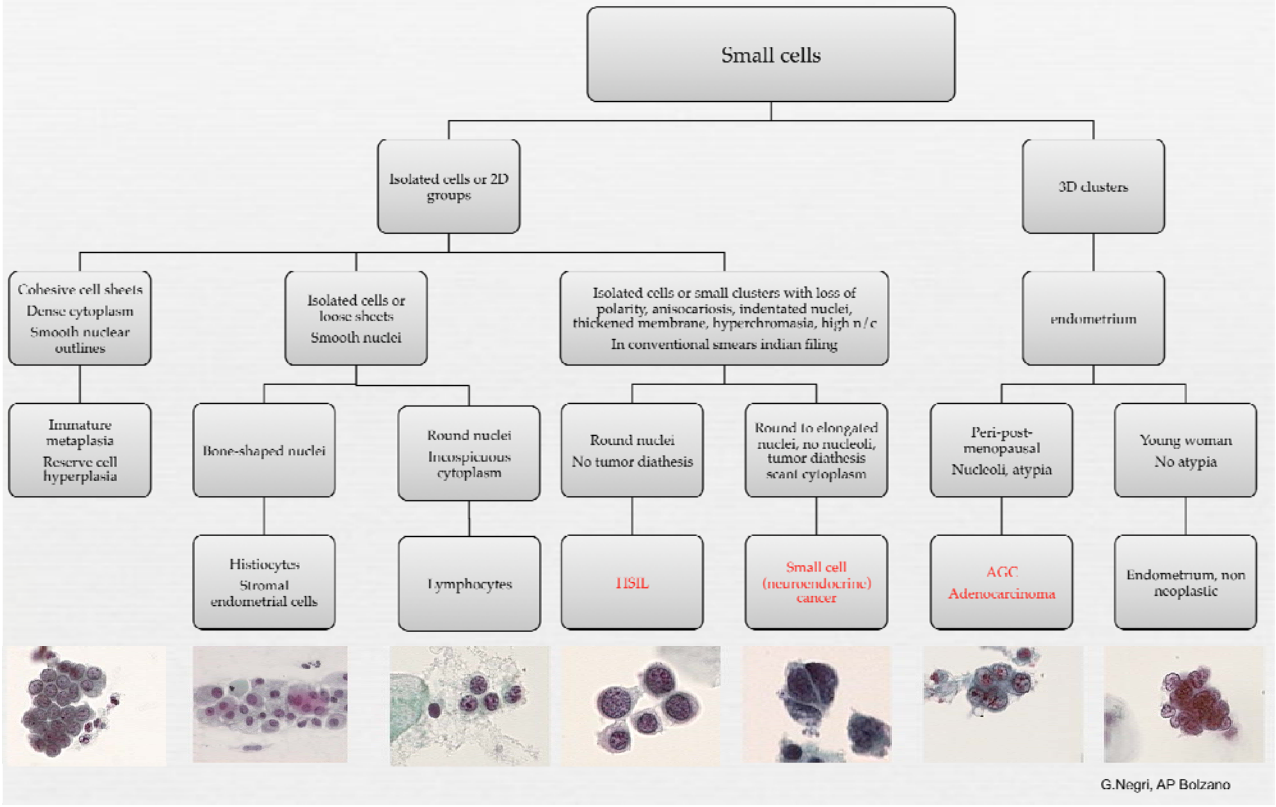
Rule out: reactive changes of endocervical cells (mostly few abnormal sheets, round

nuclei, no nuclear crowding, often macronucleoli in the absence of tumor diathesis), HSIL with pseudoglandular clusters (no feathering, mostly round or only slightly elongated nuclei).

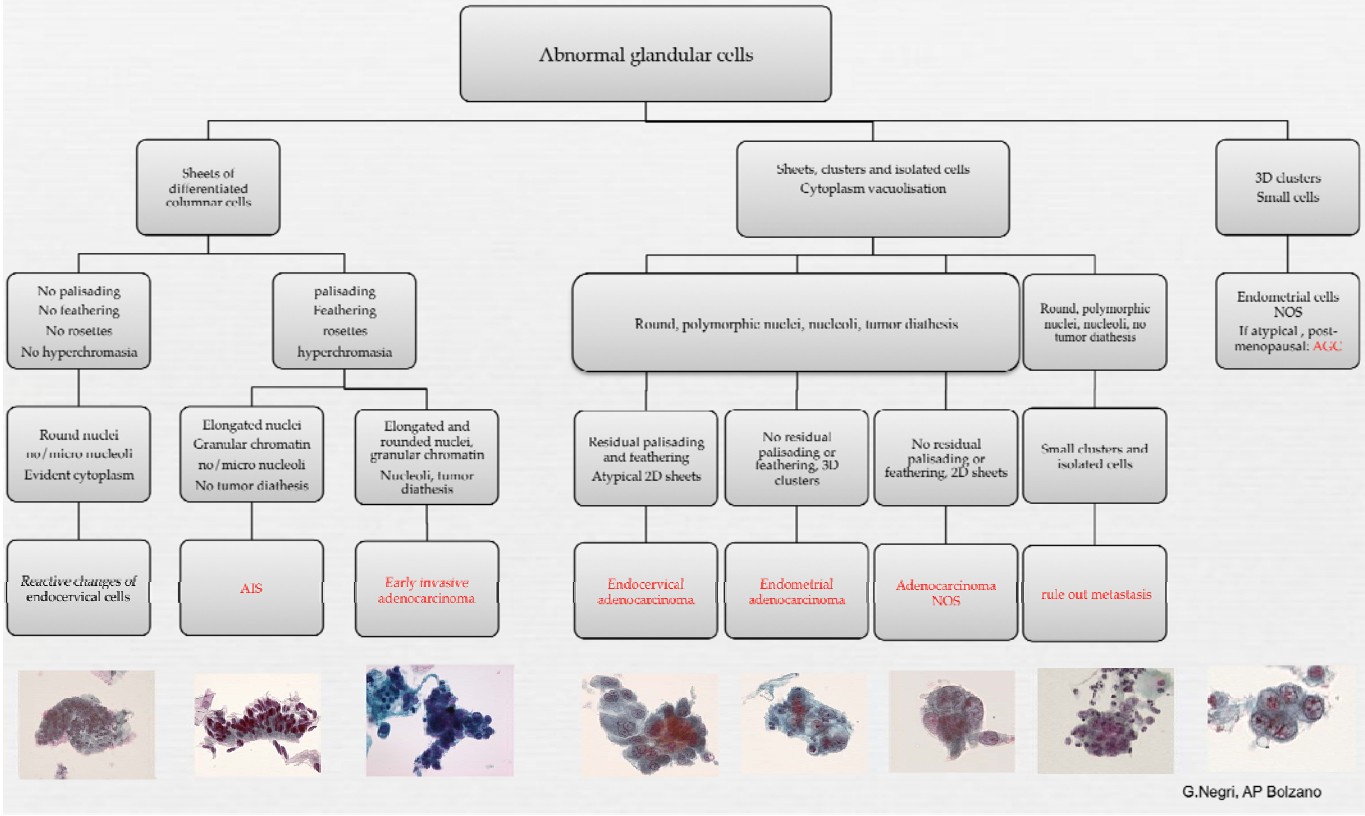
Look-alike



look-alikes: small cells



look-alikes: glandular cells



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Invasive cervical cancer audit; EU guidelines for quality assurance

Amanda Herbert

Workshop cases

Each workshop case provides a brief case history on the front of the packet, slides that include conventional slides taken before or at the time of diagnosis and in some cases histology sections. Examine the slides and make up your own mind as to how they should have been reported. A summary of the screening history and slide review is given on the back.

European guidelines for quality assurance in cervical cancer screening

The European guidelines for quality assurance in cervical cancer screening recommend:

> "Rescreening of smears from patients with negative or low-grade test results less than 3-5 years before the diagnosis of invasive cancer forms an important part of quality control but should be taken in the context of all components of the screening history, including cytological screening errors, sampling errors, non-compliance with follow-up recommendations, incomplete treatment and whether or not the cancer was screen-detected."(1)

Aims of invasive cervical cancer audit

Cervical cancer audit is primarily designed to identify areas where procedures have gone wrong, not necessarily through any one's fault, and to identify where local or national procedures could be improved. Cancer audit demonstrates the importance of these seemingly demanding quality assurance recommendations. Paradoxically, cancer audit demonstrates the effectiveness of screening as a process, since many of the cases might have been prevented if the guidelines had been followed.

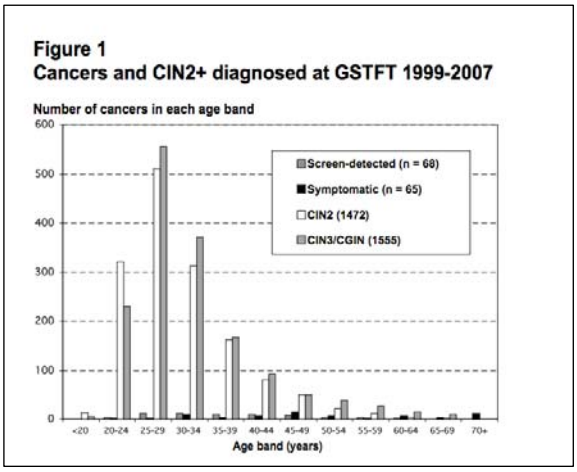
In order to place invasive cancers in the context of laboratory performance, it helps to consider them along with cases of high-grade CIN detected and treated during the same period of time: Figures 1 and 2 are taken from an audit at Guy's & St Thomas' (2), using a population-based audit in Southampton as a baseline (3,4). CIN3 was 10-fold more frequent than cancer (133 cancers, 1503 CIN3, 52 CGIN in 9 years: Figure 1). This ratio and age distribution of invasive/in situ cancer is similar to England (http://www.statistics.gov.uk/StatBase/Product.asp?vlnk=7720).

Unlike other national audits (5,6), which assessed the relative risks of cancer in screened and unscreened women, our audits aim to identify reasons why cancers had not been prevented in individual women and did not include control cases.

The relevance of screen-detected cancers in asymptomatic women

We recorded whether the cancers were found on investigation of symptoms (symptomatic cancers) or investigation of abnormal cytology (screen-detected cancers).

Screen-detected cancers were significantly more likely to be FIGO stage IA or IB1 (99%) compared with symptomatic cancers (42%).



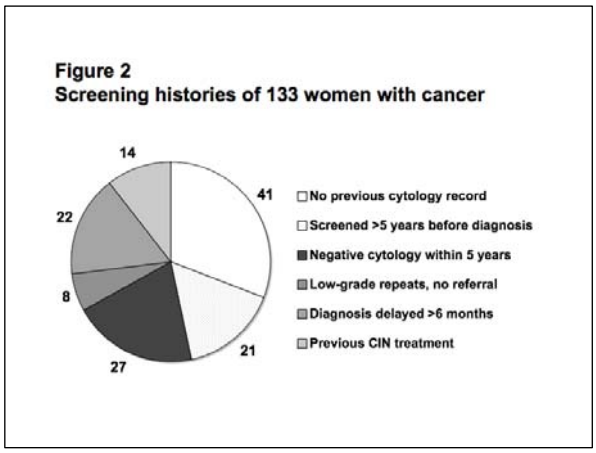
Women who had not been screened within 5 years

Since 1988 all women in England aged 20-64 years (from age 25 years since 2004) have been personally invited for cytology screening tests without charge every 3-5 years; and yet in both audits (2,3,4), about half of the women with cancer had not been screened within five years (Figure 2).

These women could be divided into those who had never been screened (who represent around 1 % of women in the UK) and those who are overdue for tests.

- Most had symptomatic cancers
- Some in their early 30s had been screened when younger, in their early 20s, and had not responded to reminders
- Around 10% were known to have had previous cytological abnormalities: previous treatment of CIN or low-grade cytology not followed up.

These cases justify the recommendations in the guidelines for cytological surveillance of previous abnormalities and reminder letters for normal recall.



Interval cancers (women screened within 0.5-5.0 years before diagnosis)

Why were more than half of cancers developing in women screened within 5 years (described as 'interval cancers' in our audits)? How could compliance with guidelines have avoided those cancers? What had we done wrong and what are the reasons for women developing cancers in well-screened populations?

- The higher the screening coverage, the greater will be the percentage of cancers that are in previously screened women. If all women were screened, all cancers would be 'interval cancers' even though there would be very few of them.
- In Southampton, there was a significant trend towards interval cancers as screening coverage and quality control improved and incidence fell (4).
- Half of invasive cancers were detected by the test in asymptomatic women. Of these nearly two-thirds were microinvasive (stage IA).
- The most clinically important group of women with interval cancers, which were most likely to be symptomatic rather than screen-detected, were those who had previous negative cytology.

Women with previous negative cytology within 5 years

Screening is not perfect and cancer may develop in women previously screened as negative. In the Guy's & St Thomas' audit (2):

- About 10% of women with cancer had at least two negative tests within 10 years; similar to other audits (6,7).
- Others had been screened infrequently, were overdue for 3-year recall or only had one previous test.
- About 60% of slides were confirmed as negative on review; about 20% showed high-grade dyskaryosis and 33% showed borderline changes (often ASC-H or AGC).
- Most had very few abnormal cells, known to be at risk as false negatives (8).
- Although 49 (37%) had previous negative cytology within 5 years, almost half of these also had abnormal cytology; and these 49 were among more than 30,000 reported as negative each year.

These cases show the importance of accurate screening, which is improved by procedures recommended in the guidelines such as rapid review of negative and inadequate tests, calculating and comparing screeners' sensitivity, comparing abnormal reporting rates between laboratories and reviewing previous negative cytology in women with cancer and high-grade CIN; in order to find out what types of abnormalities may be missed and use them for teaching. Accuracy of screening could also be improved by introducing automated screening (9), especially if used for quality control alongside careful routine screening.

Women with previous repeats for low-grade cytology

The NHSCSP guidelines recommend colposcopy on the first or second occurrence of mild dyskaryosis and third borderline result; or on the first borderline result if 'high-grade dyskaryosis is not excluded' (i.e. ASC-H). Three annual negative smears are recommended before return to normal recall (10).

- There were very few women in this category

- None had three negative repeats after a low-grade cytology report.
- Several had severe dyskaryosis on repeat cytology and were found to have stage IA1 squamous cell carcinomas.
- Most were overdue for follow-up or had not had repeat smears

Review of low-grade slides showed that one-third had been under-called, one-third would better have been reported as ASC-H or AGC with a recommendation for colposcopy while one-third were confirmed as ASC-US or LSIL.

Low-grade cytology is common and most of it regresses spontaneously, but follow-up is needed to identify the minority that either progress or were under-called on cytology.

Referral on first occurrence of mild dyskaryosis, ASC-H and AGC, HPV triage, comparing low-grade rates between laboratories and monitoring their outcome at colposcopy all help avoid these cases.

Even if referred for colposcopy, women with low-grade changes need follow-up to find the minority of lesions that persist or progress; as recommended in all the guidelines.

Women referred for colposcopy more than 6 months before diagnosis

A significant proportion of women in both audits had been recommended for colposcopy during the preceding 5 years, but had either not had colposcopy (within 6 months) or had colposcopy and cancer was not detected or prevented. Reasons for delay were related to patient compliance or management:

- Patients refused treatment or did not attend
- Waiting list delays between cytology and biopsy or biopsy and treatment
- Failures in 'failsafe' of women who changed address

Delays are now avoided by direct referral from the laboratory, waiting times being monitored and controlled and 'see-and-treat' protocols (although the risks of over-treatment must also be considered).

We concluded that prompt referral for high-grade dyskaryosis is an important recommendation, especially for severe dyskaryosis (the referral diagnosis in most of these cases).

Women who had previous treatment of CIN

A similar percentage of cancers (10-12%) in both audits were in women who had previous treatment of CIN, which was usually (but not always) CIN3.

- Most had high-grade cytology or biopsy after CIN treatment
- Some had defaulted from treatment or follow-up
- Rare to have had regular negative follow-up smears after treatment

Women with cancer following CIN treatment were significantly more likely to have initially been treated when older (35-64 years) compared with routine practice (20-35 years).

These cases were rare: 16 in the Guy's & St Thomas' audit compared with 3027 (0.5%) treated for CIN2+ (Figure 1); the percentage would be higher if women with CIN3 aged 35-64 years were taken as the denominator.

The results support screening programmes that start in younger age groups and show the importance of follow-up and treatment of any persistent high-grade abnormalities.

Summary

Despite all these errors and omissions most cancers in previously screened women were screen-detected stage 1A1 or 1B1 cancers in younger age groups; and CIN3 was 10-fold more frequent than invasive cancer.

Invasive cervical cancer audit, which is recommended by the NHSCSP for all cases of invasive cancer (11), shows the importance of accurate cytology, compliance with recommendations for repeat tests and investigation, effective failsafe procedures (all recommended in the guidelines) and, perhaps as important as anything, advice to women that regular cytological screening prevents the vast majority of cancers, especially the ones that kill.

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