Hyperchromatic crowded cell groups in gynaecological liquid-based cytology samples

E. CROLL*, D. N. RANA† and L. J. WALTON‡

*Cytology Department, Pennine Acute Hospitals NHS Trust, Royal Oldham Hospital, Oldham OL1 2JH; †Manchester Cytology Centre, Manchester Royal Infirmary, Manchester M13 9WW; and ‡Liverpool John Moores University, Liverpool L3 3AF, UK

Accepted: 30 July 2010

ABSTRACT

Cervical cytology screening is a complex and demanding procedure. Correct diagnosis depends on accurate interpretation of cells, including dense clusters known as hyperchromatic crowded cell groups (HCCG). These groups are frequently encountered in liquid-based cytology (LBC) samples and can be difficult to identify as a specific cell type. Although usually benign and thus often overlooked, they may occasionally represent severely abnormal cells. Thus, their correct interpretation is vital for accurate reporting. Such groups are responsible for false-positive and false-negative reporting and have been implicated in cases of missed dyskaryosis and cervical cancer. Normal and abnormal cells of both squamous and glandular origin, together with non-epithelial elements, may present as HCCG and this review uses the authors' experience with SurePath to describe the morphological criteria used to evaluate them when screening. Despite the introduction of semi-automated screening systems for LBC, there is currently no complete replacement for human interpretation of cell morphology in cytology screening.

KEY WORDS: Cytology.
Microscopy.
Cervical smears.

Introduction

The objective of cervical screening is detection and identification of abnormal cells from the uterine cervix and occasionally other parts of the gynaecological tract. Cells are stained by the Papanicolaou technique, involving a combination of dyes including haematoxylin. A diagnostic problem encountered with many routine samples is the presence of dense clusters of darkly staining cells, easily seen with a x10 microscope objective but sometimes difficult to identify as a specific cell type.[1] These tissue fragments or microbiopsies were first termed hyperchromatic crowded cell groups (HCCG) in the 1990s.[2] Their presence was first described in conventional smear preparations, but since the introduction of liquid-based cytology (LBC) their appearance has become more significant as they now 'stand out' from the background monolayer of cells. Hyperchromatic crowded cell groups usually represent benign entities (e.g., squamous atrophy or metaplasia, normal endocervical and endometrial cells). However, occasionally they may be composed of severely abnormal cells (e.g., high-grade cervical intraepithelial neoplasia [CIN], squamous cell carcinoma [SCC] and glandular neoplasia of cervical [CGIN] and non-cervical origin). Their precise classification is therefore vital for correct diagnosis. Owing to their frequent presence and the fact that they are usually benign, there is a tendency to 'overlook' HCCG and assume they are of no clinical significance. However, they occasionally represent a serious abnormality and all HCCG warrant careful scrutiny in order to avoid misdiagnosis.

Definition of hyperchromatic crowded cell groups

Hyperchromatic crowded cell groups are three-dimensional clusters of crowded cells with hyperchromatic nuclei, which remain intact throughout the sampling process due to preservation of intercellular connections. Such interconnections are in the form of specific areas of attachment, first termed nodes of Bizzozero but now known as desmosomes,[3] where plasma membranes of adjacent cells form a continuous link. Desmosomes are linked to intermediate filaments and together form a cytoskeleton within cells.[4] Adhesion molecules such as E-cadherin[5] are needed for cells to remain tightly associated and maintain tissue architecture.

Hyperchromatic crowded cell groups usually represent benign cells of various types. These may be squamous, sampled directly from the ectocervix, but are more often endocervical or endometrial in origin. The sampling device (Cervex broom) used to obtain cells from the cervix may explain the appearance of these groups in routine LBC preparations. The broom reaches high into the endocervical canal and may directly sample both endocervical cells from the canal and endometrial cells from the lower part of the uterus. This is illustrated in Figure 1.

Hyperchromatic crowded cell groups in women with previous cervical abnormalities treated by surgical loop excision, cone biopsy or trachelectomy can present a particular diagnostic problem. Surgery facilitates direct endometrial sampling by shortening the length of the residual endocervical canal. Misinterpretation of HCCG from the lower uterine segment (LUS) has been found to be a cause of false-positive reporting in follow-up samples from

Correspondence to: Elizabeth Croll
Email: liz.croll@pat.nhs.uk

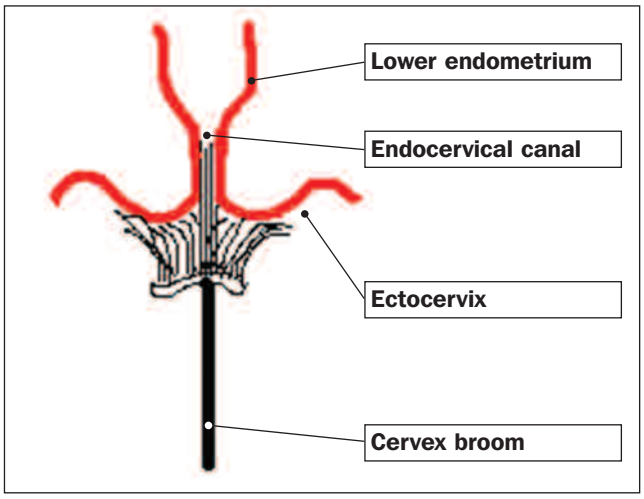

Fig. 1. Diagram illustrating the position of the Cervex broom within the uterine cervix during the sampling procedure.

patients with previous invasive disease,[6] particularly endocervical adenocarcinoma where the presence of crowded glandular cells raises the suspicion of recurrence. Use of the endocervical brush to sample the cervix will also lead to direct sampling of these cells. Clusters of endometrial cells shed due to hormonal influences or physical trauma such as the presence of an intrauterine contraceptive device (IUCD) may also appear. Thus, clinical information including previous smear history and treatment, last menstrual period (LMP), details of any hormonal treatment such as hormonal replacement therapy (HRT) and presence of IUCD is vital to assist in interpretation of HCCG and allow accurate reporting of cytology samples.

Occasionally, HCCG may comprise abnormal cells of squamous or glandular origin. As mentioned previously, these are apparent in LBC preparations due to direct sampling from the cervix or, in the case of certain glandular abnormalities, exfoliation from other parts of the gynaecological tract.

The two National Health Service Cervical Screening Programme (NHSCSP)-approved LBC preparation methods ensure that HCCG are apparent on screening. For SurePath slides, preparation involves centrifugation of samples through a polysaccharide density gradient to remove obscuring inflammatory cells and debris. This produces a cleaner preparation and less obscuring of cells when viewed microscopically. The relatively dense HCCG will, therefore, remain in the sample following this process and be part of the cellular component of slides, which are prepared by gravity sedimentation of the sample. For ThinPrep slides, the process involves membrane filtration of the sample, again to remove obscuring inflammatory cells and debris. Larger cells and cell groups, including HCCG, cannot pass through the filter but are held there. Once saturated with cells, this filter is brought into contact with a microscope slide and the cells transfer to the slide. Therefore, HCCG present on the filter will be present on the prepared slide.

Significance

Hyperchromatic crowded cell groups can cause interpretative errors and are responsible for both false-positive and false-negative reports. Potential implications for patients subject to misdiagnosis, although relatively uncommon, are considerable. False-positive reports lead to considerable anxiety and unnecessary treatment of healthy women, including invasive surgical procedures, with resulting potential implications for fertility. False-negative reports may lead to progression of untreated disease and potentially life-threatening consequences.

As stated previously, HCCG are usually benign and have a tendency to be disregarded. However, if they are composed of abnormal cells this abnormality will be high-grade, which, if missed, may have serious implications for the patient. One study found that out of 12 high-grade dyskaryosis cases missed on primary screen, five contained the abnormal cells presenting as "cohesive microbiopsies".[7] The conclusion drawn was that the abnormality was missed due to misinterpretation of the groups as benign. This echoed findings of an earlier paper[8] which examined previous negative smears from women who had developed cervical cancer. The authors identified this same

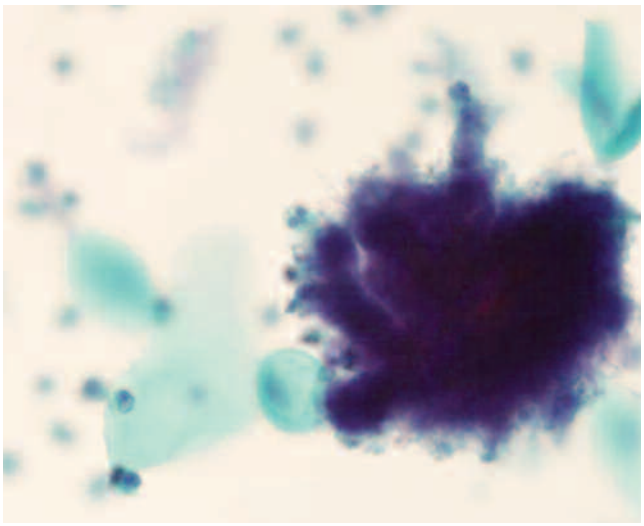

Fig. 2. Bacteria forming a hyperchromatic crowded cell group (original magnification x400).

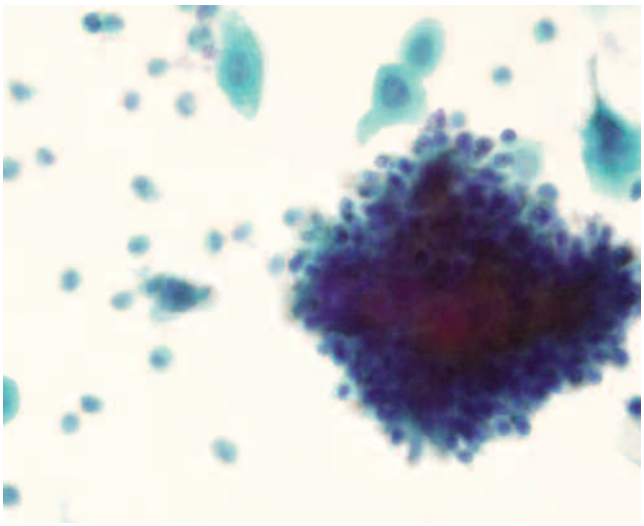

Fig. 3. A dense cluster of neutrophils (original magnification x400

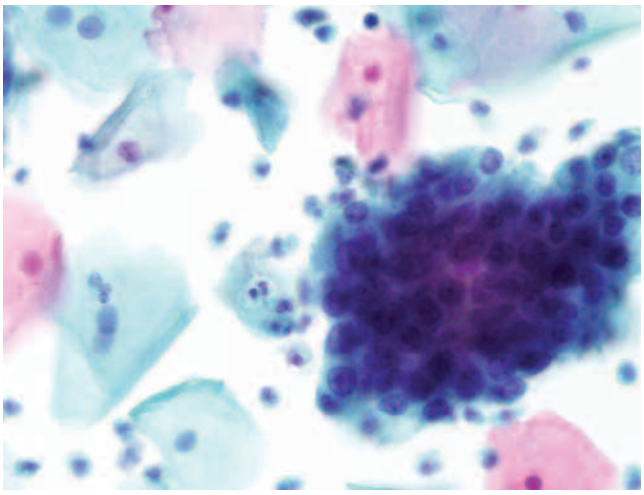

Fig. 4. Hyperchromatic crowded cell group of metaplastic cells showing round, evenly spaced cells (original magnification x400)

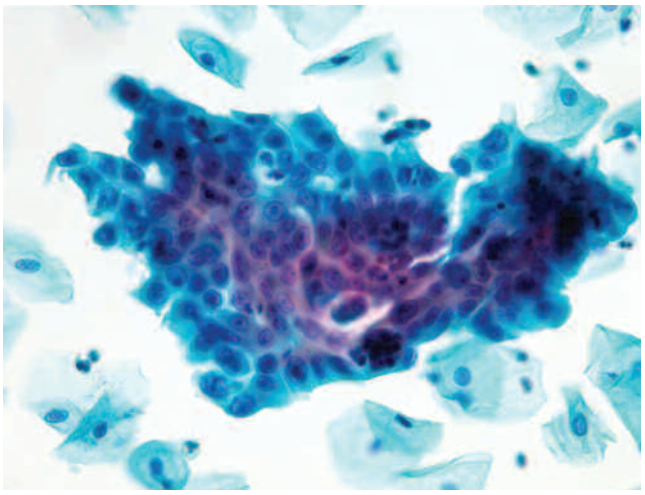

Fig. 5. Repair cells showing prominent nucleoli (original magnification x400; image provided by Dr. D. N. Rana).

presentation of dyskaryotic cells in 24 out of 92 (26%) smears falsely reported as negative.

Missed cases of cervical adenocarcinoma have also been linked to misinterpretation of HCCG.[9,10] Hyperchromatic crowded cell groups have been implicated in cases of litigation for such errors.[11,12] It has been stated that DeMay, who was responsible for the term hyperchromatic crowded cell groups, first became aware of their potential significance when examining past 'negative' cervical samples from such a case.[13] A study investigating the effectiveness of rapid review as a quality control measure for negative slides also implicated abnormal HCCG in cases of missed dyskaryosis.[14]

The current NHSCSP audit of invasive cervical cancers has specified microbiopsies as a potential cause of missed dyskaryosis and has listed several benign forms of HCCG as causes of false-positive reports.[15] Such groups are also listed as problems in interpretation of dyskaryosis.[16] Hyperchromatic crowded cell groups cause diagnostic uncertainty, with one study suggesting they are responsible for over 60% of 'inconclusive – possible high-grade abnormality' reports.[17] This study found that 74% of cases with inconclusive reports due to HCCG had high-grade cervical disease on biopsy. However, patients with negative colposcopy/biopsy are a problem both in cytological terms and in clinical management. There remains a small proportion of patients with HCCG who cannot be definitively categorised, and who must be carefully managed. This will involve discussion at multidisciplinary team meetings and possibly use of high-risk human papillomavirus (HPV) testing, which may be helpful in such cases.

Hyperchromatic crowded cell groups are a commonly found component of routine LBC samples. One study identified them in nearly 80% of slides, with over 95% of these being benign and the majority composed of endocervical cells.[13] The study also found abnormalities only in slides containing HCCG and concluded their presence indicated correct transformation zone sampling with the associated increased chance of sampling any cervical abnormality present. However, the report acknowledged that HCCG occasionally may represent the abnormal cells. They are therefore an important factor in cervical cytology, the significance of which should not be underestimated.

It is important when screening to spend time studying

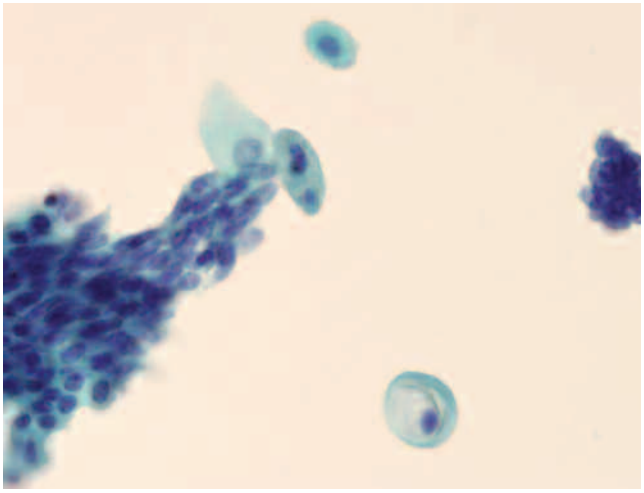

Fig. 6. Hyperchromatic crowded cell groups in atrophy. The cluster of bare nuclei on the right closely resemble those in the syncytial sheet on the left (original magnification x400).

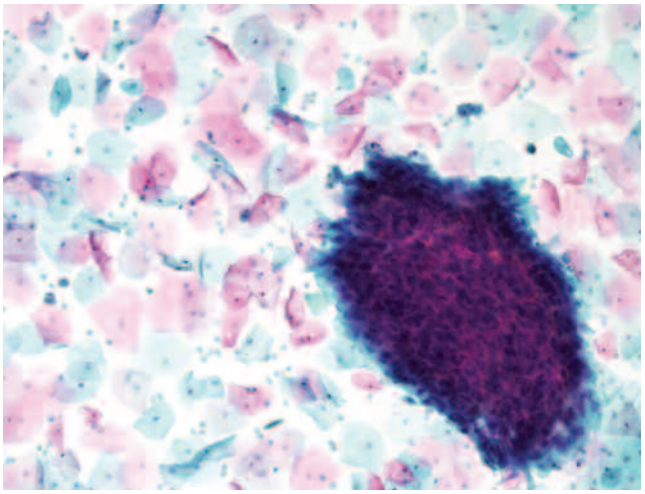

Fig. 7. Severe squamous dyskaryosis presenting as an HCCG (original magnification x100).

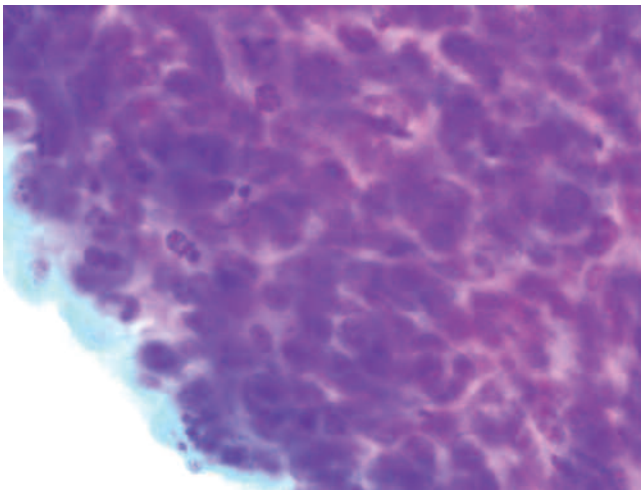

Fig. 8. Severe squamous dyskaryosis showing mitotic figures within the HCCG (original magnification x400).

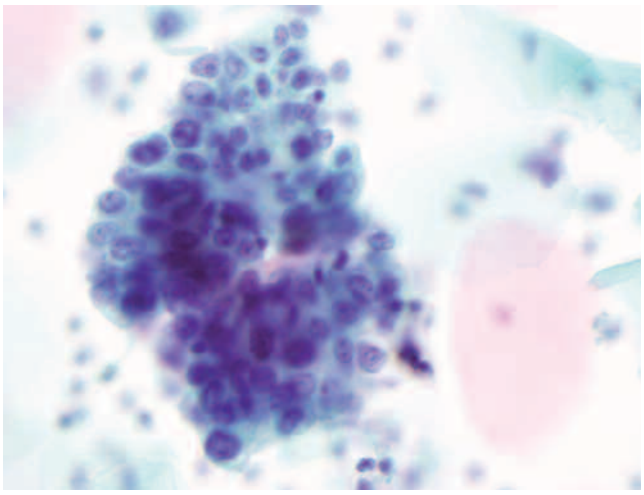

Fig. 9. Severe squamous dyskaryosis involving endocervical glands. The nuclei have smooth outlines with prominent nucleoli (original magnification x400).

them and attempting to identify the exact nature of the groups. As the authors' experience is with SurePath LBC preparations, all images used in this review are taken from slides prepared using this methodology.

Cell types and identification criteria

The cervix is covered by stratified squamous epithelium on the ectocervix and columnar epithelium lining the endocervical canal. The area at which the two types meet is termed the squamocolumnar (SC) junction. The exact location of this junction changes throughout a woman's life. Before puberty and after the menopause it lies within the endocervical canal; however, during reproductive years hormonal influences cause eversion of the columnar epithelium onto the ectocervix and so the SC junction appears at varying distances from the external os. The acidic pH of the vagina stimulates the process of metaplasia with eventual replacement of the columnar epithelium by squamous cells. This area is known as the transformation zone and has a characteristic naked-eye appearance visible to the smear taker.

The multilayered squamous epithelium appears opaque in comparison to the more reddened columnar epithelium, the single cell layer of which allows underlying blood vessels to be seen. The transformation zone may contain visible patches of metaplasia within the columnar epithelium and these may be the source of benign metaplastic HCCG observed in LBC samples. However, the transformation zone is also the area where most cervical abnormalities arise, and thus cervical sampling targets this area.

Several cell types may present as HCCG, and may be normal or abnormal. During screening, each individual group encountered must be assessed. Hyperchromatic crowded cell groups are easily detected with a x10 microscope objective. Low power can be very useful in revealing the arrangement of cells within the group. Disorganised or chaotic architecture, indicative of abnormal cells, is sometimes more apparent at low power than at higher magnification, as is the overall hyperchromasia of these cell groups.[11] It is also vital to examine them at higher

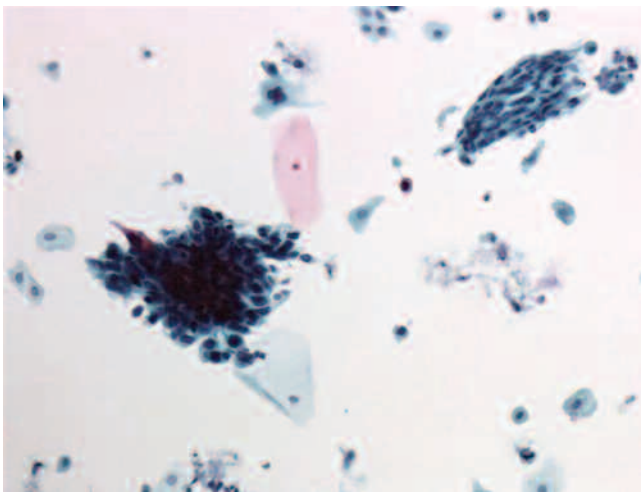

Fig. 10. High-grade squamous dyskaryosis in atrophy. The group on the right is a normal atrophic cluster; the group on the left is abnormal (original magnification x200; image provided by Dr. D. N. Rana).

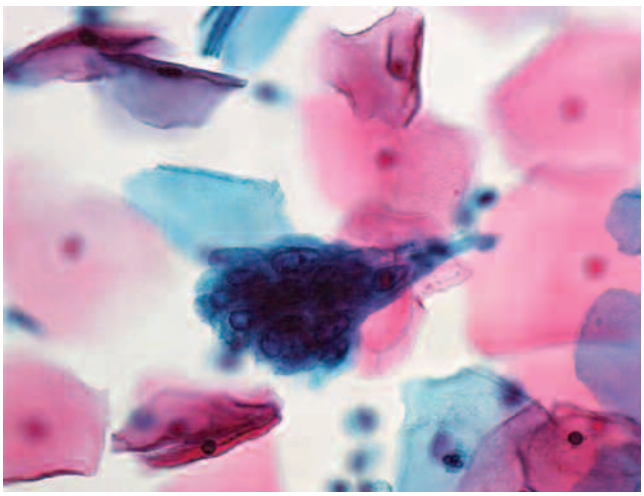

Fig. 11. Squamous cell carcinoma. Nuclei show prominent nucleoli with chromatin clearing (original magnification x400; image provided by Dr. D. N. Rana).

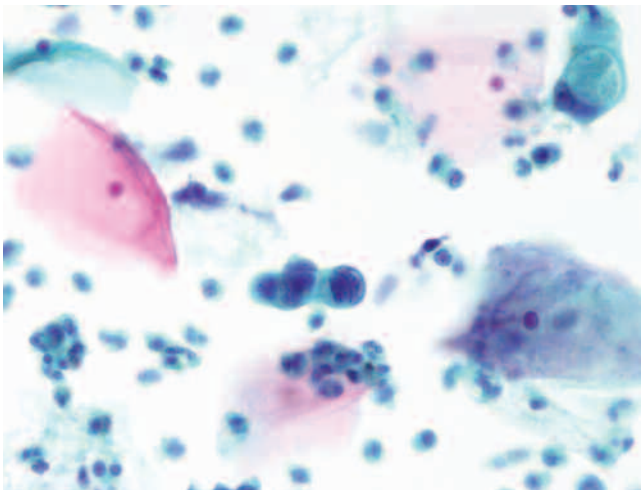

Fig. 12. 'Cell in cell' feature of squamous cell carcinoma (original magnification x400).

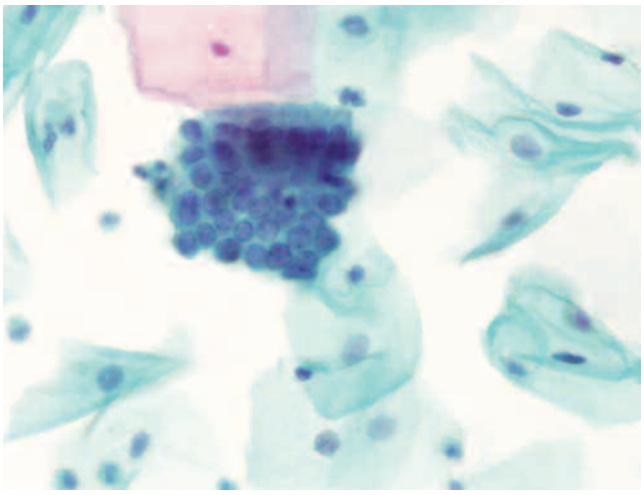

Fig. 13. Normal endocervical cells. The top edge of the group shows the palisade formation of elongated cells and basal nuclei (original magnification x400).

magnification to reveal finer details of both the group and the individual cells.

As HCCG are three-dimensional, it is important to look at each plane of focus. Cells at the edge of the group are often easier to examine and provide the best cellular detail and clues to cell type. Criteria used for identification in both SurePath and ThinPrep LBC preparations are largely identical to those for conventional smears. This includes size and shape of cells, amount and appearance of cytoplasm, size and shape of nuclei and position within the cell, nuclear/cytoplasmic (N/C) ratio, chromatin pattern, nuclear outline, presence of nucleoli and mitotic figures, and arrangement of cells within the group.

Further assistance may often be found by comparison with cells present elsewhere on the slide. However, it must be remembered that individual cells within groups are often smaller than single cells of the same type and that HCCG may be the only abnormality present.[11] Cells and cell groups in LBC preparations tend to present a more 'rounded' appearance than seen in conventional smears, with more visible cytoplasm in single cells from high-grade dyskaryosis. This can lead to problems in classifying cells. One study found that severe squamous dyskaryosis presenting as HCCG is more likely to be interpreted as a glandular lesion on LBC slides.[1]

The initial decision to be made on examining any HCCG is whether the group comprises non-epithelial elements, squamous or glandular cells. Only when this is clear can the screener evaluate whether the group is normal or abnormal.

Non-epithelial elements include clusters of bacteria (Fig. 2), histiocytes and neutrophils seen in inflammation (Fig. 3), and aggregates of lymphocytes observed in follicular cervicitis. Such groups do not normally present interpretative problems on screening.

Squamous cells

Normal squamous cells tend to have a centrally placed nucleus, regardless of the degree of maturation. The amount of cytoplasm increases and N/C ratio decreases as cells mature. The chromatin pattern is finely dispersed and

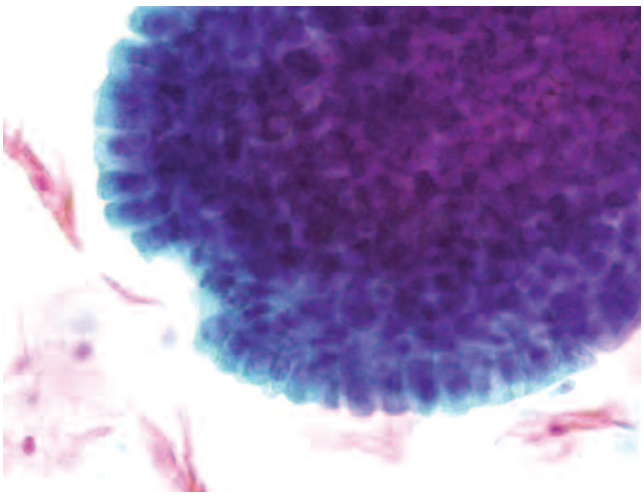

Fig. 14. Microbiopsy of normal endocervical cells showing palisade formation at the edge of the group (original magnification x400).

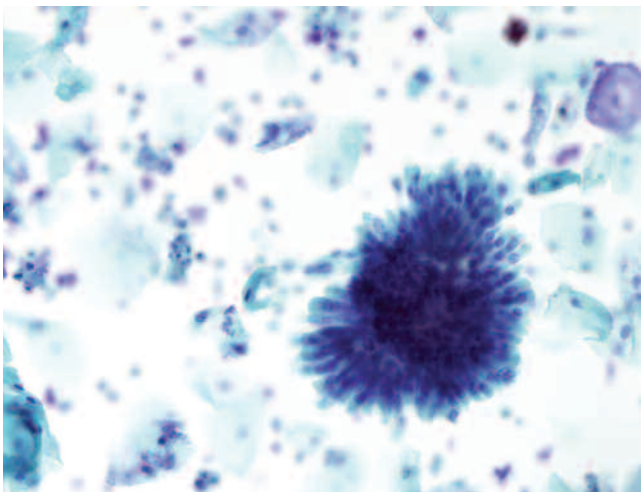

Fig. 15. Starburst presentation of normal endocervical cells (original magnification x200).

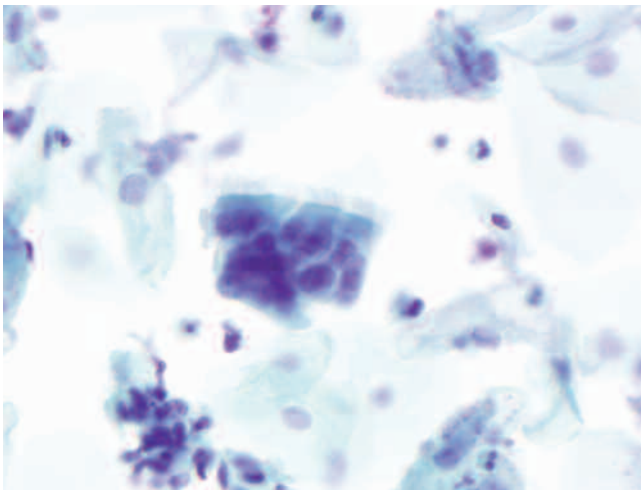

Fig. 16. Multinucleated endocervical cells with prominent nucleoli. The cells have terminal bars and cilia which are features of benign cells (original magnification x400).

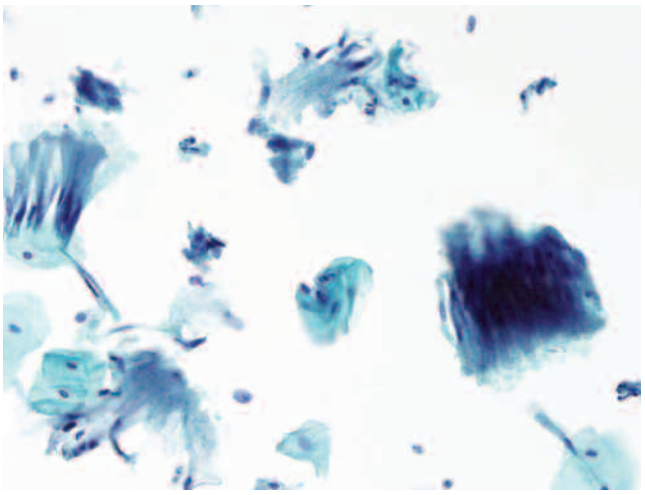

Fig. 17. Endocervical cells with a distorted and elongated appearance characteristic of acetic acid artefact (original magnification x200).

nuclear outline is smooth. Normal squamous cells presenting as HCCG are smaller parabasal cells, such as those in atrophic samples, and immature metaplastic cells from the transformation zone where individual cell size is also small. Normality can be assessed by group architecture, which will be regular and organised, with cells evenly spaced in any plane of focus. In immature metaplastic cells, cytoplasm is dense and cyanophilic, with rounded cell borders and centrally placed nuclei (Fig. 4). Reactive cells, such as those from areas of repair (Fig. 5), may contain nucleoli, and the chromatin may become slightly more granular. The group architecture shows a 'streaming' appearance of cells orientated in the same direction, but cells remain evenly spaced.

In normal clusters, all nuclei within the HCCG should have a similar appearance. However, atrophic samples can contain hyperchromatic clusters of pleomorphic cells with high N/C ratios, which can be difficult to confirm as benign groups. Clues are the absence of mitotic figures in normal atrophic groups, and degenerate and indistinct chromatin.[11] Cell borders may be indistinct and groups may appear syncytial. Clusters of bare nuclei are another feature of atrophy and may appear similar to endometrial cells. Diagnostic clues are the absence of cytoplasm and their resemblance to the nuclei found in syncytial sheets (Fig. 6). The differential diagnosis is important, as endometrial cells are an abnormal finding in post-menopausal women, with clinical significance.

Dyskaryotic HCCG always represent a high-grade lesion.[11,13] Hyperchromatic crowded cell groups from high-grade squamous dyskaryosis (Fig. 7) are identified by studying the architecture and nuclear features of the group. Abnormal cells present a crowded and chaotic architecture, increased N/C ratio, sometimes with little obvious cytoplasm, pleomorphic nuclei and coarse, granular chromatin. However, the nuclear outline is often smooth and lacks the bulbous protrusions and deep notches often associated with dyskaryosis. Mitotic figures may be present (Fig. 8) and nucleoli, although usually absent, may be present when dyskaryosis involves endocervical glands.

The presence of micronucleoli is characteristic of gland

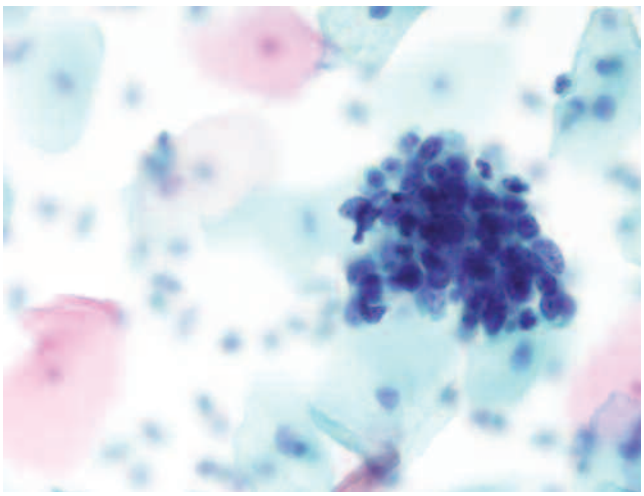

Fig. 18. Hyperchromatic crowded cell group from cervical glandular intraepithelial neoplasia (CGIN) resembling severe squamous dyskaryosis (original magnification x400).

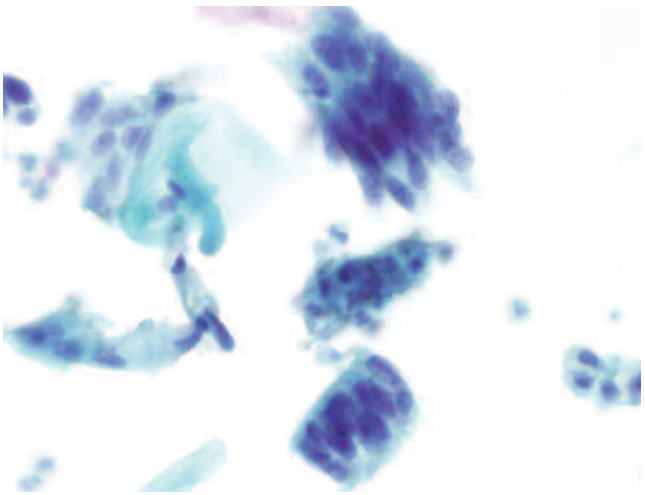

Fig. 19. Cell groups from CGIN. The lower group shows pseudostratification; the upper group shows feathering of nuclei at the lower edge (original magnification x400).

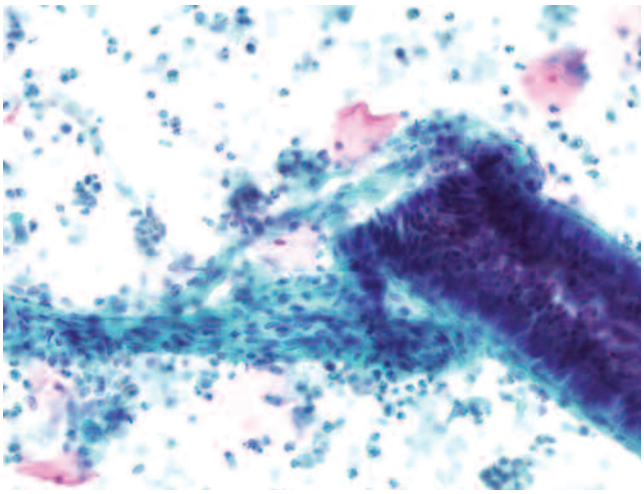

Fig. 20. Lower uterine segment (LUS) sample. To the right of the picture is an endometrial tubular structure, and the associated stromal fragments are to the left (original magnification x200).

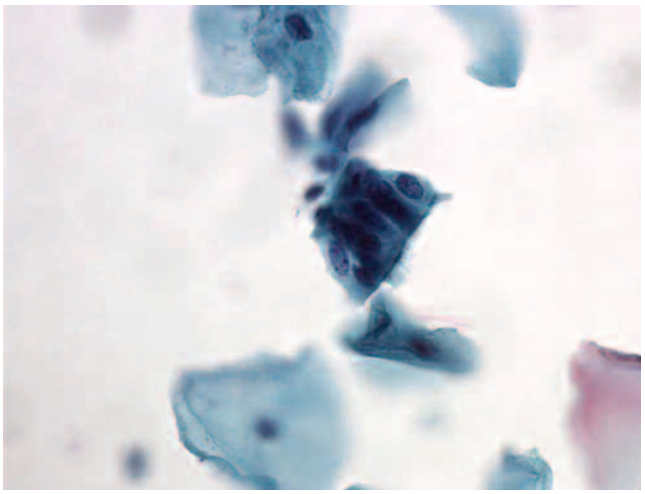

Fig. 21. Tubo-endometrioid metaplasia (TEM). The group shows pseudo-stratification but the presence of terminal bars and cilia mean it is benign (original magnification x400; image provided by Dr. D. N. Rana).

involvement (Fig 9).[18] It is important in this instance not to associate the presence of nucleoli with benign reactive changes, where cells would be evenly spaced within the group. Single dyskaryotic cells are often present in the rest of the sample, which is a further guide to the abnormal nature of the groups.

Dyskaryotic HCCG in atrophy can be difficult to diagnose, as normal atrophic cells can show variation in nuclear size and shape. However, polarity is maintained in benign groups whereas abnormal HCCG will show a more chaotic architecture. Chromatin in abnormal cells will often show dyskaryotic features, and will be crisper and more distinct than in normal atrophic cells. The presence of mitoses is another clue to the neoplastic nature of an atrophic group, although absence of mitotic figures does not mean the group is normal. Cells at the edge of benign HCCG may show a degree of maturation, whereas abnormal clusters do not.[11] The comparison between normal and abnormal atrophic groups is illustrated in Figure 10.

Squamous cell carcinoma (SCC) may also present as HCCG. Nuclei may show marked pleomorphism and prominent, sometimes multiple nucleoli. Chromatin distribution can be very irregular, sometimes with clear areas or 'windows' within the nucleus (Fig. 11).

Sometimes there will be features in the rest of the sample to assist with diagnosis. These include the 'cell in cell' pattern (Fig. 12) and the bizarre keratinised tadpole or fibre cells seen in keratinising SCC. However, it must be remembered that these features may not be present. The characteristic background diathesis of necrotic debris or fibrin is not as apparent on LBC slides as in conventional smears, but traces may still be present. Samples from SCC will often also contain severely dyskaryotic cells.

Glandular cells

Most HCCG of glandular origin are composed of benign cells of either endocervical or endometrial origin. Normal endocervical sheets show a honeycomb pattern of evenly distributed cells, with round to oval central nuclei and small nucleoli. Seen in this presentation, each nucleus is

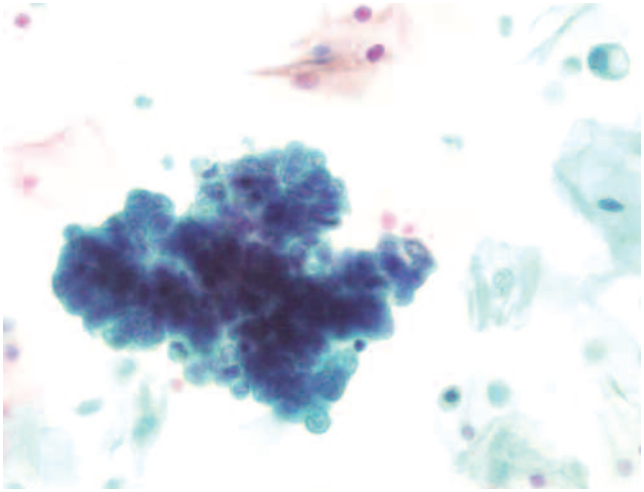

Fig. 22. Hyperchromatic crowded cell group of normal endometrial cells (original magnification x400).

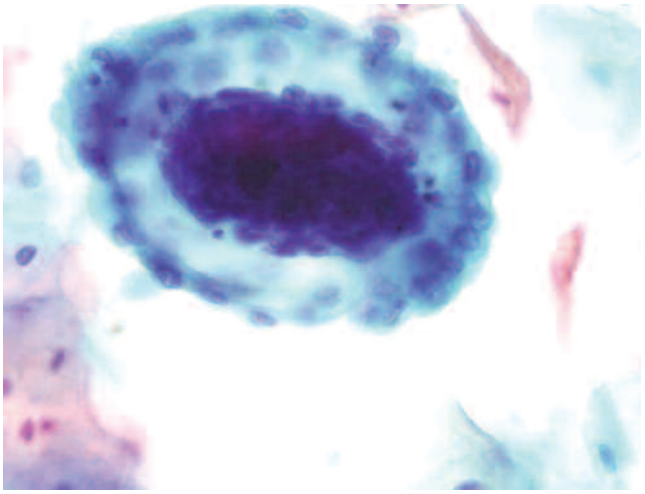

Fig. 23. Biphasic endometrial cell group with dense stromal core and paler epithelial cell rim, giving the characteristic 'top hat' appearance (original magnification x400).

surrounded by a thin rim of cytoplasm, with considerable depth of focus to the cell. Terminal bars and cilia may be present, especially in cells from the upper endocervical canal. When endocervical cells are observed in palisade formation, nuclei are situated at the base of the elongated cells and this feature may be visible at the edge of sheets (Fig. 13) and larger microbiopsies (Fig. 14). Vacuolated cytoplasm is a feature of normal secretory endocervicals. 'Starburst' groups (Fig. 15) are a particular presentation in SurePath LBC samples.

Reactive endocervical cells can show multinucleation, with more prominent nucleoli, smooth nuclear membranes and a degree of pleomorphism and hyperchromasia. The N/C ratio remains normal, with the presence of cilia indicating benign cells (Fig. 16).

Figure 17 illustrates the distorted endocervical groups caused by cervical sampling after the application of acetic acid to the cervix during colposcopic examination. Cells appear shrunken and elongated and groups may be difficult to interpret. There is the potential for false-positive reports, as these patients have known or suspected cervical abnormalities, hence their attendance at the colposcopy clinic.

Diagnostic criteria for glandular lesions in LBC slides are similar to those for conventional smears.[19] One study found better preservation of nuclear detail and more subtle presentation of architectural features in LBC.[20] Hyperchromatic crowded cell groups comprising abnormal endocervicals from cervical glandular intraepithelial neoplasia (CGIN) show a rounded, disorganised structure with crowded, overlapping nuclei. Chromatin distribution is coarse and uneven. Rosette formation within groups, with nuclei towards the outer edge, is characteristic of CGIN but is not always present in LBC preparations. Cells at the edge of the group may retain the elongated endocervical shape and show subtle feathering, but differentiation between severe squamous dyskaryosis involving endocervical crypts and CGIN may be difficult (Fig. 18).

One recent paper[21] suggests that the centre of these clusters is the key to correct interpretation. Neoplastic glandular HCCG have a central area only three or four cells thick, which it is possible to see through, while those from high-grade CIN show a much denser and crowded centre. In addition, glandular nuclei may be more elongated than the more rounded ones seen in squamous cells. Pseudostratified strips of abnormal endocervicals (Fig. 19) are a diagnostic feature of CGIN, although these may not always be present.

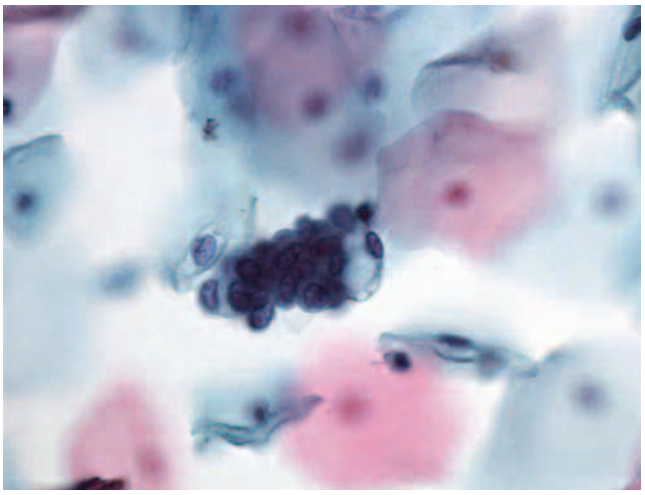

Fig. 24. Intrauterine contraceptive device (IUCD) changes in endometrial cells. The group shows vacuolation of the cytoplasm – a so-called 'bubblegum' cluster (original magnification x400; image provided by Dr. D. N. Rana).

As mentioned previously, the presence of HCCG in patients who have had previous surgery for cervical abnormality can be a particular diagnostic problem and the potential for false-positive reporting is significant. Sampling of the lower uterine segment (LUS) is commonplace, producing numerous endometrial tubular structures (Fig. 20), with a stromal core and palisade edges. Separate stromal fragments may also be seen and their presence helps to differentiate between LUS and CGIN, where stromal fragments are absent.[13,22]

Tubo-endometrioid metaplasia (TEM) is another benign finding in LBC samples following cervical surgery. It produces HCCG of small endocervical-type cells, which may show a degree of crowding, feathering and inverted rosettes

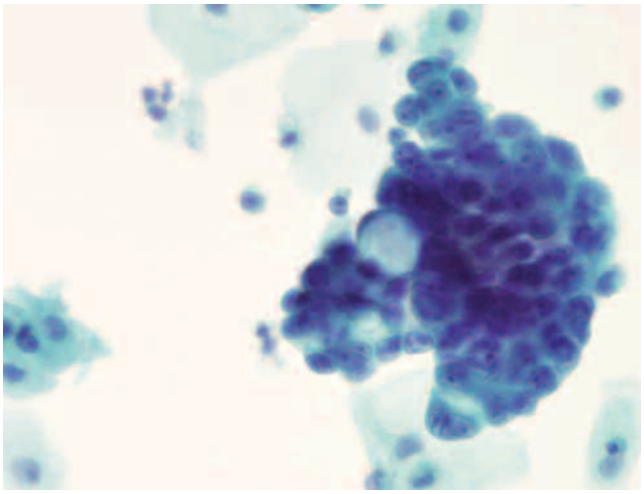

Fig. 25. Abnormal endometrial cells showing enlarged nuclei and prominent nucleoli (original magnification x400).

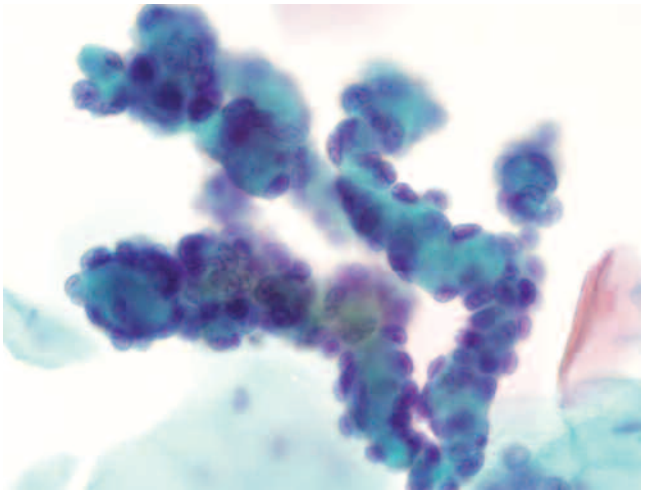

Fig. 26. Papillary cell cluster suspicious for ovarian carcinoma (original magnification x400).

with the nuclei towards the centre. Strips may be present that show some pseudostratification, and these features (Fig. 21) make it the most important diagnostic pitfall for CGIN. However, the presence of terminal bars and cilia are characteristics of TEM and are absent in CGIN. In addition, the edges of cell strips will be parallel rather than the sometimes tapered bird-tail appearance seen with pseudostratified strips from CGIN.

Normally exfoliated endometrial cells (Fig. 22) appear as small HCCG sometimes containing apoptotic bodies, debris and neutrophils. Cytoplasm is poorly defined and nuclei vary in shape and are situated eccentrically. Figure 23 shows a biphasic group forming the characteristic 'top hat', with a dark stromal core and rim of paler epithelial cells.

Age and last menstrual period (LMP) is essential information for interpreting endometrial HCCG. Endometrial cells should not be present after day 12 (mid-cycle) and their presence beyond this may have clinical significance in women over 40 years old, suggesting endometrial pathology. The presence of an IUCD should be noted, as it may lead to endometrial cells shedding throughout the cycle. Such groups often have vacuolated cytoplasm, producing characteristic 'bubblegum' clusters (Fig. 24).

Directly sampled endometrial cells appear better preserved and may contain mitotic figures depending on the menstrual phase at the time of sampling. This again highlights the need for accurate clinical information. The overall appearance of groups will be uniform, however, and stromal fragments may be present.

Abnormal endometrial cells from hyperplasia or adenocarcinoma may also present as HCCG. Enlarged nuclei and nucleoli (Fig. 25) are not found in normal endometrials and so indicate malignancy. Vacuoles containing ingested debris and neutrophils may also be present.

Very occasionally, HCCG may represent malignancy from other parts of the gynaecological tract (e.g., ovary, Fallopian tube or peritoneum). Cells may be degenerate but show characteristic features of adenocarcinoma. Papillary fragments (Fig. 26) and the presence of psammoma bodies (Fig. 27) may indicate ovarian origin. Clinical and radiological correlation is important in such cases.

Other rare presentations of HCCG include small-cell neuroendocrine carcinoma (Fig. 28) and metastatic cancer, the presence of which may be indicated by clinical information. Once again, groups usually show obvious malignant features and look out of place and 'stand out' from the normal background population.[2]

Future developments

Introduction of LBC has facilitated adjunctive tests such as molecular techniques for high-risk HPV, as excess cellular material remains in the fixative vial following preparation of the screening slide. The development of various antibodies to help identify malignant cells has led to studies on their potential use in cervical cytology, particularly with cells difficult to identify on morphology alone, including HCCG. One recent study used this technique to distinguish CGIN from TEM in a cell-block made from residual LBC sample.[23] There have been other promising results leading to the development of a commercially available antibody (ProEx C; TriPath Imaging, Burlington, NC) to detect dysplasia specifically for use with LBC samples. However, detailed discussion of immunocytochemistry and molecular techniques is outside the scope of this review.

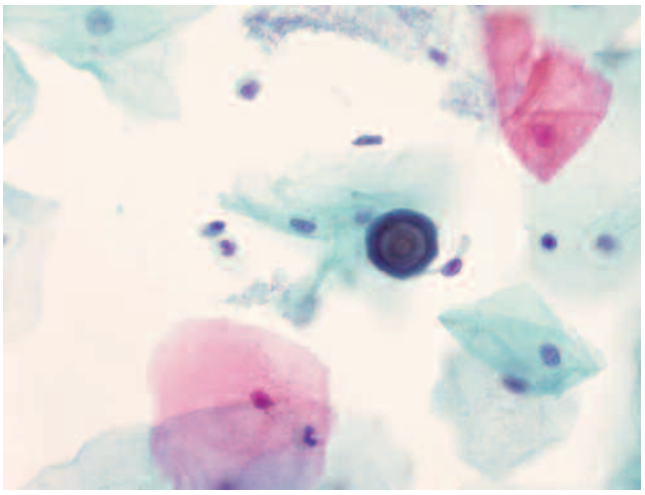

Fig. 27. Psammoma body showing the characteristic concentric ring structure (original magnification x400).

The introduction of LBC has also facilitated the use of semi-automated screening systems, originally developed using conventional smears. Previous studies using cytomorphological assessment and digital analysis of HCCG concluded that there were significant differences in nuclear area between malignant and benign cells.[24] Current systems use computerised image analysis of cell morphology and algorithms to analyse and interpret slides. Two main products are currently operating: FocalPoint slide profiler (TriPath Imaging) for SurePath LBC samples, and ThinPrep Imaging System (Hologic) for use with ThinPrep LBC slides. Both systems can be linked to microscopes, enabling location-guided manual review of the most abnormal areas identified by the computer. This manual review of selected fields is an integral part of both systems. Hyperchromatic crowded cell groups, both normal and abnormal, may be identified in one or more of the fields of interest and so screener interpretation of these groups remains.

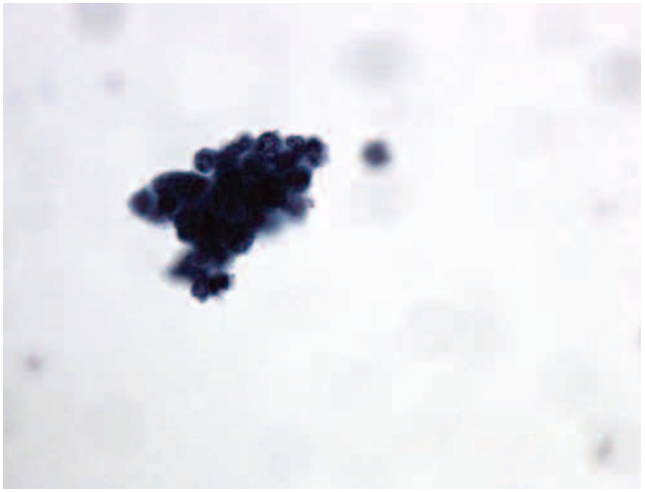

Fig. 28. Hyperchromatic crowded cell group from small-cell neuroendocrine carcinoma (original magnification x600; image provided by Dr. D. N. Rana).

Conclusions

Interpretation of cells in cytology is largely a subjective procedure. It presents the screener with continuing challenges, the production of an accurate diagnosis being its ultimate aim. The numerous cell types and their different presentations, often with subtle differences between normal and abnormal forms, is no better illustrated than with HCCG. While computer analysis and biomarkers would seem to be the main focus of current research, as yet there is no definitive answer to the problem of morphological HCCG interpretation. No replacement has yet been found for the human element in screening. Awareness of the potential significance of HCCG and continuing cytology education for screeners, including morphological interpretation of these groups, is essential. This, combined with advances in immunocytochemistry and image analysis, hopefully will lead to a more sensitive screening test and further reduction in the incidence of cervical cancer. □

References

1 Renshaw A, Mody D, Wang E, Haja J, Colgan T. Hyperchromatic crowded cell groups in cervical cytology – differing appearances and interpretations in conventional and ThinPrep preparations. *Arch Pathol Lab Med* 2006; **130** (3): 332–6.

2 DeMay RM. *The art and science of cytopathology*. Chicago: ASCP Press, 1996.

3 Karrer HE. Cell interconnections in normal human cervical epithelium. *J Biophys Biochem Cytol* 1960; **7** (1): 181–5.

4 Gumbiner BM. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* 1996; **84**: 345–7.

5 De Boer CJ, Van Dorst E, Van Krieken H *et al*. Changing roles of cadherins and catenins during progression of squamous intraepithelial lesions in the uterine cervix. *J Pathol* 1999; **155** (2): 505–15.

6 Feratovic R, Lewin SN, Sonoda Y *et al*. Cytological findings after fertility-sparing radical trachelectomy. *Cancer Cytopathol* 2008; **114** (1): 1–6.

7 Faraker CA, Boxer ME. Rapid review (partial rescreening) of cervical cytology. Four years experience and quality assurance implications. *J Clin Pathol* 1996; **49**: 587–91.

8 Robertson JH, Woodend B. Negative cytology preceding cervical cancer: causes and prevention. *J Clin Pathol* 1993; **46** (8): 700–2.

9 Lee KR. Adenocarcinoma *in situ* with a small cell (endometrioid) pattern in cervical smears. *Cancer* 1999; **87** (5): 254–8.

10 Ruba S, Schoolland M, Allpress S, Sterrett G. Adenocarcinoma *in situ* of the uterine cervix. Screening and diagnostic errors in Papanicolaou smears. *Cancer* 2004; **102** (5): 280–7.

11 DeMay RM. Hyperchromatic crowded cell groups: pitfalls in Pap smear diagnosis. *Am J Clin Pathol* 2000 ; **114** (Suppl 1): S36–43.

12 Frabie WJ. Error reduction and risk management in cytopathology. *Semin Diagn Pathol* 2007; **24** (2): 77–8.

13 Chivukula M, Austin RM, Shidham VB. Evaluation and significance of hyperchromatic crowded cell groups (HCCG) in liquid based paps. *Cytojournal* 2007; **4**: 2.

14 Pajtler M, Audy-Jurković S, Skopljanac-Mačina L, Antulov J, Barišić A, Miličić-Juhas V. Rapid cervicovaginal smear screening: method of quality control and assessing individual cytotechnologist performance. *Cytopathology* 2006; **17** (3): 121–6.

15 NHS Cervical Screening Programme (NHSCSP). *Audit of invasive cervical cancers*. Publication No 28, 2006 (www.cancerscreening.nhs.uk/cervical/publications/nhscsp28.html).

16 NHS Cervical Screening Programme (NHSCSP). *Achievable standards, benchmarks for reporting and criteria for evaluating cervical cytopathology.* Publication No 1, 2000 (www.cancerscreening.nhs.uk/cervical/publications/cc-02.html).

17 Schooland M, Sterrett GF, Knowles SA, Mitchell KM, Kurinczuk JJ. The inconclusive-possible high-grade epithelial abnormality category in Papanicolaou smear reporting. *Cancer* 1998; **84** (4): 208–17.

18 Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep cytology. *Diagn Cytopathol* 2002; **26** (3): 181–5.

19 Biscotti CV, Gero MA, Toddy SM, Fischler DF, Easley KA. Endocervical adenocarcinoma *in situ*: an analysis of cellular features. *Diagn Cytopathol* 1997; **17** (5): 326–32.

20 Ozkan F, Ramzy I, Mody DR. Glandular lesions of the cervix on thin-layer Pap tests. Validity of cytologic criteria used in identifying significant lesions. *Acta Cytol* 2004; **48** (3): 372–9.

21 Thiryayi SA, Marshall J, Rana DN. Differentiating between endocervical glandular neoplasia and high-grade squamous intraepithelial lesions in endocervical crypts: cytological features in ThinPrep and SurePath cervical cytology samples. *Diagn Cytopathol* 2009; **37** (5): 315–9.

22 Lee KR, Genest DR, Minter LJ, Granter SR, Cibas ES. Adenocarcinoma *in situ* in cervical smears with a small cell (endometrioid) pattern: distinction from cells directly sampled from the upper endocervical canal or lower segment of the endometrium. *Am J Clin Pathol* 1998; **109** (6): 738–42.

23 Narine N, Rana DN, McVey RJ, Fitzmaurice R. Ancillary testing in liquid-based cytology to distinguish cervical glandular neoplasia from tuboendometrial metaplasia in a young woman. *Diagn Cytopathol* 2010 Feb 24 (Epub ahead of print; www3.interscience.wiley.com/journal/121450078/issue).

24 Harris MV, Cason Z, Benghuzzi H, Tucci M. Cytomorphological assessment of benign and malignant dense hyperchromatic groups in cervicovaginal smears. *Biomed Sci Instrum* 2000; **36**: 349–54.