

## Chapter 6

# The Development of a Novel Oral Cytologic Grading System

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Cervical cytology gained popularity with the publications of Papanicolaou and Traut, who demonstrated the diagnostic value of exfoliative cytology in the detection of carcinoma of the uterine cervix [1]. A variety of classification systems for reporting cervical cytology have been adopted since. In the “Papanicolaou” (Pap) classification, a specific “class” provided a level of concern about the presence of cancer cells. For example, class I smears contained benign cells and class V smears contained cells definitively diagnostic of malignancy.

The Pap classification system gradually became outdated as it had many variations and failed to keep abreast with the recent scientific advances in cervical carcinogenesis and precursor lesions [2]. Reagan encouraged the use of the term dysplasia for precancerous lesions, and dysplastic lesions were then subdivided by degree of abnormality and cell type, severe keratinizing dysplasia being an example [3, 4]. Richart introduced the term “Cervical Intraepithelial Neoplasia” (CIN) in 1967 to promote the idea of a continuum of precursor lesions [5].

The use of multiple classification systems for reporting results of cervical cytology soon caused widespread confusion among many laboratories and clinicians culminating in the development of a standard grading method. In 1988, the Bethesda system was developed to provide a uniform scheme for reporting cervical cytology, through a workshop convened by the National Cancer Institute (NCI) [2]. Subsequently, two additional workshops were held in 1999 and 2001 to address the inherent deficiencies of the new system and the role of evolving technologies and scientific advances in reporting [6, 7]. The 2001 Bethesda System received many

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inputs from cytologists and cytotechnologists and was attended by more than 400 participants, preceded by internet discussion groups [7].

While the Bethesda classification method represents one of the great success stories in cervical cytology, there has been little appetite for the adoption of a universal grading for oral cytology. This could be explained by the general lack of interest in oral cytology, which is due to a high percentage of false negative diagnoses [8, 9], attributed to great variation in technical quality and cellularity of oral smears as well as the use of inadequate sampling procedures. The lack of a standardized method for reporting oral cytology adversely affects proper management of patients with oral lesions.

Many investigators have used or continue to use a three-tiered oral cytologic grading system on adequate samples [10–12], whilst others have failed to provide one [13–17]. In a study performed in 1983 to evaluate the role of fine needle aspiration (FNA) in squamous cell carcinomas of the head and neck, Feldman et al. reported 229 FNAs, 42 of which were from the oral region [10]. The FNA results were reported in one of four categories: unsatisfactory, negative, suspicious, or positive for malignancy. Similar criteria were used by Scher et al. in evaluating FNAs from the oral cavity, oropharynx and nasopharynx [11]. In this study, suspicious FNA specimens were proven to be malignant in 100% of the cases that underwent subsequent biopsy.

The brush biopsy (CDx Laboratories, Suffern, NY, USA) was introduced in 1999 as a potential oral cancer case-finding device. In a prospective multicenter study to determine the sensitivity and specificity of oral brush biopsy (OralCDx®) for detection of pre-cancerous and cancerous lesions of the oral mucosa, Sciubba et al. reported results as positive, atypical or negative [12].

Currently, most available oral diagnostic tissue tests are expensive, time-consuming, invasive and not within easy reach of the vast majority of the world population which needs these investigations the most. These diagnostic procedures also require training and present logistical issues, e.g. infection control, transport of samples, turnaround times, communication of results and patient travel for recall. Though these issues may not present too many constraints to patients and clinicians with access to well-equipped and easily accessible healthcare systems, there is a pressing requirement to develop simpler, inexpensive and minimally invasive devices/methodologies which enable the diagnosis of clinically significant oral lesions with a high degree of accuracy. This need is all the more evident in rural and economically disadvantaged, medically underserved areas. The presently existing oral cytology techniques are largely experimental and are undergoing extensive testing.

For now, the conventional Pap smear for cervical smears provides better diagnostic information through its more elaborate Bethesda grading system than the OralCDx® for oral smears. The emergence of Liquid-Based Cytology (LBC) in recent years with dramatic improvements in technical quality and cellularity of the cytology specimens [18] has provoked a new interest in oral cytology. This inspired the authors to conduct a recent study [19] using an economical liquid-based preparatory method (Shandon PapSpin™) and propose an oral cytologic grading method analogous to the Bethesda System for reporting cervical cytology (Table 6.1).

**Table 6.1** Oral/oropharyngeal cytologic grading system

Grading system
<i>Specimen adequacy</i>
Adequate for evaluation (note the presence of basal/parabasal cells)
Inadequate for evaluation (specify reason, e.g. obscuring elements, unlabelled or broken slides)
<i>General categorization</i>
A: Normal
B: Reactive <sup>a</sup>
C: Atypical-probably reactive/low grade including low grade squamous intraepithelial lesion (LSIL)
D: Atypical-Probably high grade
E: High grade squamous intraepithelial lesion
F: Invasive squamous cell carcinoma
G: Other neoplasms: Specify

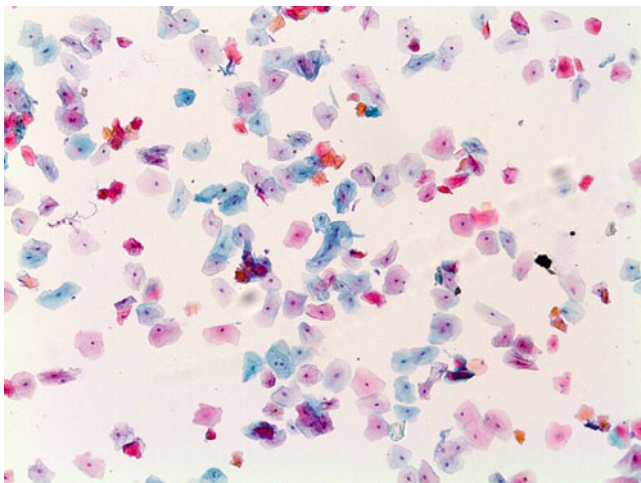
<sup>a</sup>The reactive category includes hyperkeratosis, inflammatory, infective, repair & chemo/radiation changes

The preservation of the traditional cytomorphologic criteria rendered by the PapSpin™ method allows standardization through both conventional and liquid-based cytologic techniques. Using this classification, the terminology for reporting results obtained by oral cytology examination is discussed.

## Adequacy Criteria

The 2001 Bethesda guidelines for reporting cervical cytology [7] consider a conventional smear as adequately cellular, if the smear has an estimated minimum of at least 8,000–12,000 well preserved (not obscured by blood or inflammation) squamous cells. Conventional smears with more than 75% of the squamous cells obscured by blood or inflammation are designated as unsatisfactory for evaluation. Since liquid-based preparations (LBPs) have more random sampling of cell constituents, a lower minimum cellularity of 5,000 well preserved squamous cells is required.

Reference images are provided for the estimation of conventional smear cellularity; laboratories are not to count individual cells [20]. Cell numbers on LBPs are reproducibly evaluated by estimates of representative fields. Kujan et al. in 2006 reinforced the method to draw a line across the center of each preparation and to evaluate ten discontinuous fields across the middle diameter of each preparation using the 40× objective lens. Only well-visualized squamous cells are counted. Large groups containing more than five squamous cells are counted on only one plane of focus. The average cell count from the ten fields (40×) is measured. An average of at least 7 cells/field is required in order to achieve the minimum of 5,000 cells/preparation [14].

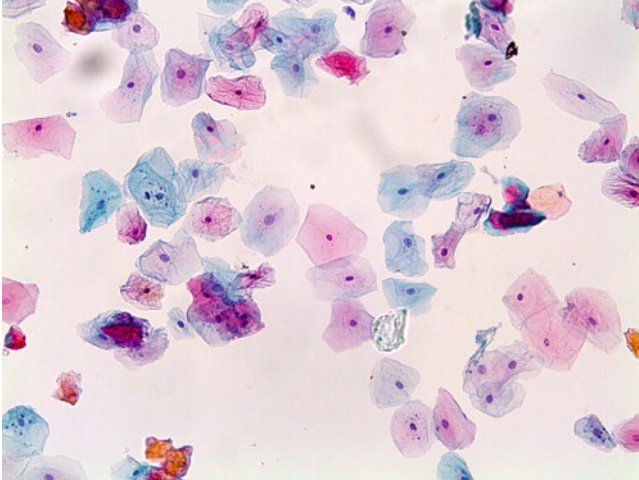


**Fig. 6.1** LBC (PapSpin). An adequate oral sample. Approximately 60% of the diameter of the preparation (5 mm circle) is covered by cellular material (Pap stain,  $\times 100$ )

While the Bethesda System provides a set of numeric criteria for minimal squamous cellularity of adequate cervical cytology specimens, such criteria are not well defined for oral cytology specimens. Conventional oral smears are generally more hypocellular than cervical smears and for this reason some investigators consider smears with at least 30 well preserved intermediate or parabasal cells (not obscured by blood, exudate or necrosis) as adequately cellular for evaluation [15], while others have failed to describe the criteria used [13, 16, 17] or have simply adhered to the adequacy criteria proposed by the Bethesda System [14]. In a study of normal oral mucosa using the SurePath™ LBC method and applying the Bethesda adequacy criteria, only two of the 150 (1.3%) specimens evaluated were considered inadequate, however, only 6 of the 150 slides studied contained basal cells.

In general, a suitable oral cytologic specimen should contain a representative sample of superficial, intermediate and parabasal/basal cells; and it is therefore important to assess the presence of the latter cells within specimens of adequate squamous cellularity (Table 6.1). The proper application of the Transepithelial Brush Biopsy Technique (TBBT) [12, 19, 21, 22] results in a significantly improved harvest of basal and parabasal cells. With TBBT, the brush is firmly applied to the lesion and rotated a number of times until pin point (punctuate) bleeding is provoked. This ensures a full thickness epithelial sampling via a minimally invasive procedure and provides the oral pathologist with a more representative cytologic specimen of the brushed mucosal epithelium.

In our recent study using the PapSpin™ LBC technique [19], a specimen was considered inadequate if less than 30% of the diameter of the circle of cells (5 mm) was covered by cellular material (Fig. 6.1). Although we deem this a simple and time saving adequacy criterion for LBPs, it may prove difficult for other non-calibrated examiners to reproducibly apply this principle. It is therefore prudent



**Fig. 6.2** LBC (PapSpin). Even distribution of oral superficial and intermediate cells (Pap stain,  $\times 400$ )

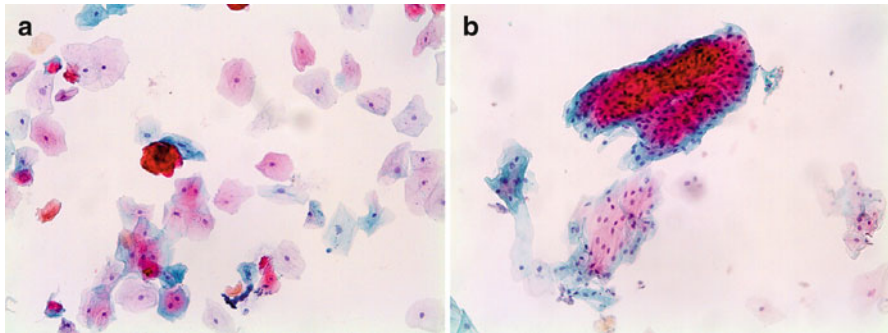
to state that future prospective studies will have to confirm this proposed standard or suggest alternative assessment criteria to determine the minimum squamous cellularity of oral cytologic specimens. Until such time, it is recommended to use the adequacy criteria proposed by the 2001 Bethesda system.

## General Diagnostic Categorization

### *A: Normal*

The oral stratified squamous epithelium consists of several layers. The basal layer appears as a single, well organized row of darkly staining cells that rest on a basement membrane. These cells together with the immediately superficial parabasal cells (Fig. 6.4a) are responsible for the continuous renewal of the oral epithelium. As the cells progressively migrate toward the surface, they acquire increased cytoplasm, the nature of which is eosinophilic due to increased cytokeratin protein deposition. Thus, the most mature cells in the upper layers of the epithelium have extremely small pyknotic nuclei and abundant eosinophilic cytoplasm and are known as superficial cells (Fig. 6.2).

The intermediate cells are characterized by pale grooved nuclei, the open chromatin of which may occasionally reveal a small chromocenter (Fig. 6.2). The cytoplasm of intermediate cells may contain abundant glycogen particles and appear clear in Pap-stained preparations.



**Fig. 6.3** (a) LBC (PapSpin). Hyperkeratosis. An orangeophilic aggregate of anucleated superficial cells (Pap stain,  $\times 200$ ). (b) Parakeratotic plaque (Pap stain,  $\times 200$ )

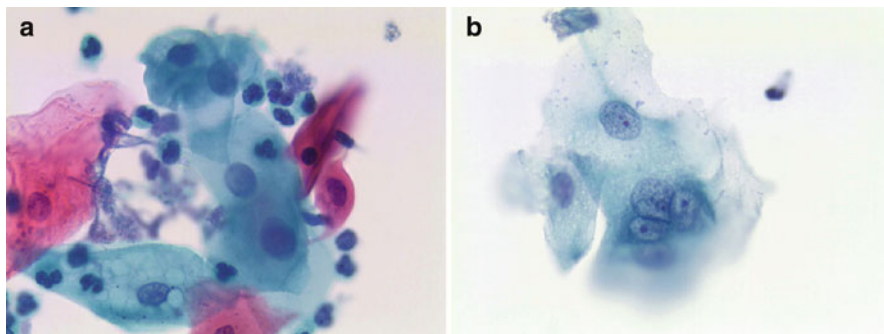
It is not surprising to see basal and parabasal cells in smears/preparations from atrophic oral epithelia. The basal/parabasal cells have scanty basophilic (protein poor) cytoplasm and nuclei that are larger than those of intermediate cells. This results in a high nuclear/cytoplasmic ratio. However, their nuclear membrane is smooth and regular and the chromatin is pale and delicate (Fig. 6.4a). In keratinized masticatory mucosa (hard plate and gingiva), anucleated keratotic superficial cells may be seen (orthokeratosis) or parakeratotic cells/plaques (parakeratosis— Fig. 6.3a, b) and these should be regarded as normal. However, the finding of hyperkeratotic cells derived from a white mucosal lesion in the presence of normal basal cells should be included in the reactive category. The parakeratotic cells generally have orangeophilic cytoplasm and small pyknotic nuclei, in which no residual chromatin structure is identified. Abnormally configured keratotic cells should be viewed with caution as they may herald an underlying dysplastic/malignant process.

## ***B: Reactive***

Oral squamous cells undergo reactive cytological alterations in the presence of a number of conditions. For this reason the reactive category is further subcategorized into infectious, inflammatory, repair and chemo-/radiotherapy induced changes.

### **(1) Inflammation/Infective**

In the presence of an inflammatory/infective process, the smear/preparation often contains an infiltrate of acute inflammatory cells with predominance of neutrophils (except

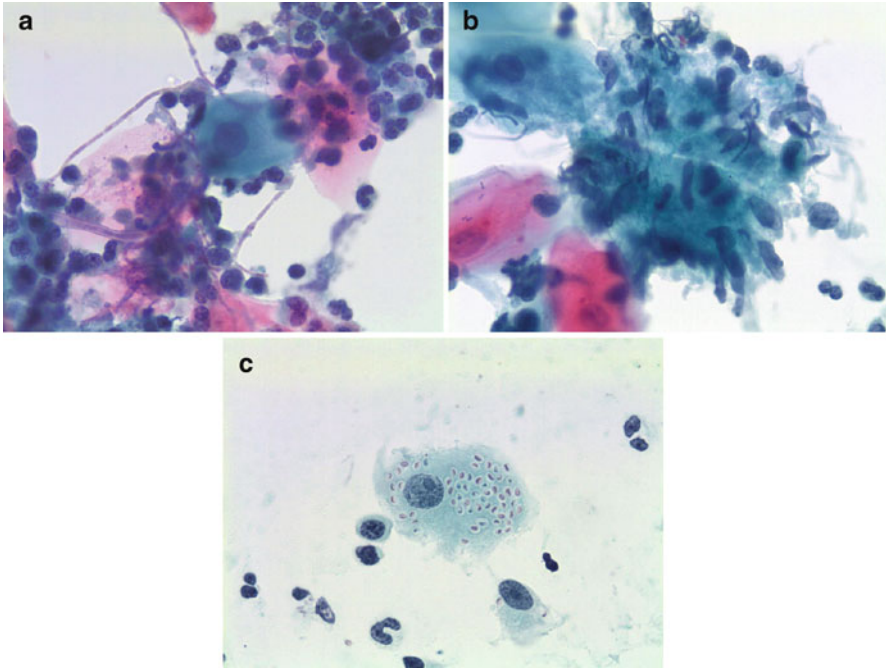


**Fig. 6.4** (a) LBC (PapSpin). Reactive (inflammatory)-an aphthous ulcer. In addition to the normal intermediate cells (*left*), this image shows cells (*center*) with a generous body of mature cytoplasm and mild nuclear enlargement with an attendant slight increase in N:C ratio. Hyperchromasia is not evident and the nuclear outlines are smooth. A basal/parabasal cell is seen on the right. The cell shows a marked increase in N:C ratio, resembling a high grade squamous intraepithelial lesion, but hyperchromasia is minimal and the nuclear outline is smooth (Pap stain,  $\times 1,000$ ). (b) Reactive. Multinucleated cell with abundant cytoplasm. The nuclei are normochromatic and the nuclear outlines are smooth. The chromatin is finely granular and evenly distributed. Small nucleoli are noted, suggesting a reactive process (Pap stain,  $\times 1,000$ )

in automated liquid-based preparations). Eosinophils are rarely seen in fixed oral smears/preparations, since the distinctive red granules are lost. A dense inflammatory infiltrate may partially or fully obscure oral squamous epithelial cells in conventional oral smears. This together with the hypocellular nature of the oral smears in general may result in inadequate specimens.

The inflammatory infiltrate is markedly reduced in liquid based preparations based on cytocentrifugation (e.g. PapSpin<sup>™</sup>) and is almost non-existent in automated liquid-based preparations, e.g. Thin Prep<sup>™</sup> (Cytec Corporation, Boxborough, MA). Nevertheless, given the large number of inflammatory/infective conditions encountered in the oral region, the presence of these cells is desirable, allowing a precise cytologic diagnosis to be made.

The cells in these subcategories often have a generous body of cytoplasm and demonstrate mild nuclear enlargement with an attendant slight increase in nuclear to cytoplasmic (N:C) ratio (Fig. 6.4a). Cells with hyperchromatic nuclei are not seen, however, small perinuclear halos, cytoplasmic vacuolation (Fig. 6.4a), and bi-/multinucleated cells (Fig. 6.4b) may be seen as part of the reactive changes. *Candida* spp. cause the most common fungal infection of oral mucosa and are detected in oral smears/preparations as budding yeasts and pseudohyphae (Fig. 6.5a). The finding of granulomas in oral cytologic smears/preparations underlines the importance of oral cytology as a rapid and reliable test for diagnosing oral granulomatous conditions, e.g. tuberculosis and histoplasmosis (Fig. 6.5b, c).



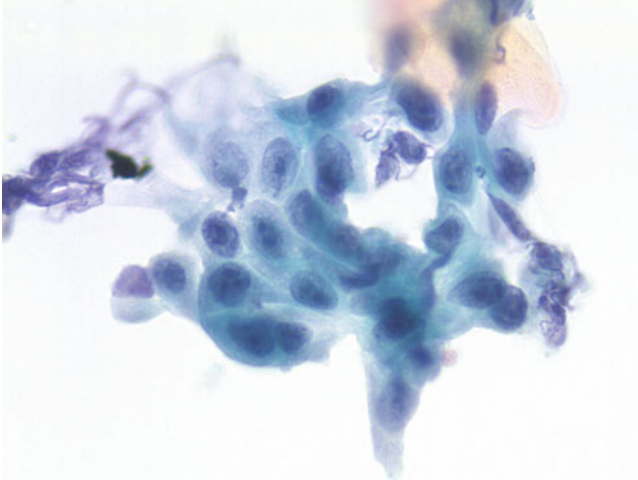
**Fig. 6.5** (a) LBC (PapSpin). Reactive (infective)—this image shows pseudohyphae of *Candida* spp. with a marked acute inflammatory cell response. The cell in the center displays reactive cellular changes, mild nuclear enlargement with an attendant increase in N:C ratio. However, nuclear hyperchromasia is not present and the nuclear outline is smooth. (b) Granuloma from an irregular ulcer with rolled/heaped up margins on the hard palate, clinically thought to be a squamous cell carcinoma. The image shows a cluster of epithelioid cells arranged in a syncytial fashion. The cells have oval to slightly bent nuclei and delicate cytoplasm (Pap stain,  $\times 1,000$ ). (c) Histoplasmosis. In addition to granulomas, a macrophage is seen containing numerous small intracellular round to oval bodies, 1–5  $\mu\text{m}$  yeast cells, surrounded by a small light halo (Pap stain,  $\times 1,000$ )

## (2) Repair

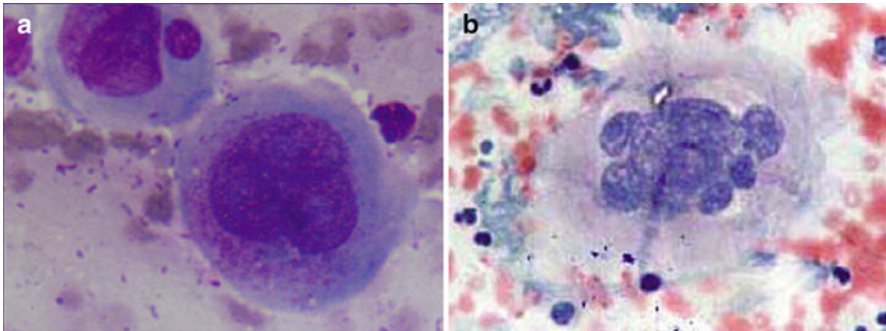
The oral cavity is a common site where a number of benign ulcerative conditions occur. These include aphthous ulcers, vesiculo-bullous diseases and traumatic ulcers, all of which are characterized by episodes of ulceration and regeneration (repair). Most cytologic examples of repair are characterized by cells arranged in cohesive flat streaming sheets that are often associated with inflammatory cells.

The cells demonstrate enlarged but pale nuclei, with even nuclear contours and prominent nucleoli (Fig. 6.6). This cytomorphology may resemble that of a squamous cell carcinoma, however, no single cells with similar cytomorphology are identified in repair which is a clue to the correct cytologic diagnosis.





**Fig. 6.6** LBC (PapSpin). Healing erosive lesion on the palate in a patient with oral mucous membrane pemphigoid. The cells are arranged in a flat streaming sheet. The nuclei are enlarged and show smooth to slightly irregular nuclear outlines and single prominent nucleoli. Mild hyperchromasia is seen although chromatin structure and distribution remains finely granular. No single cells with similar cytomorphology were identified, a key feature to correct diagnosis (Pap stain,  $\times 1,000$ )



**Fig. 6.7** (a) Radiation changes. Multinucleation and micronucleation (Giemsa,  $\times 1,000$ ). (b) Nuclear budding (H & E  $\times 1,000$ ). (Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology in diagnosing premalignant or malignant oral lesions. *Mol Cancer* 2006; 5: 1)

### (3) Radiation and Chemotherapy Induced Oral Cytological Changes

The cytology of radiation change is similar to that described in the Pap test. There is enlargement of both the cytoplasm and the nucleus, leading to large cells termed macrocytes (Fig. 6.7a). However, the N:C ratio remains unchanged [23]. Nuclear alterations such as nuclear budding, micronucleation and multinucleation may be seen (Fig. 6.7b) [24, 25]. Radiation also results in reactive cellular alterations, e.g. repair as discussed previously.

In a study comparing post-radiation changes in normal and malignant oral cells, it was found that various morphological abnormalities demonstrated a consistent significant increase with radiation dose [26]. Similar changes are expected to be induced by chemotherapeutic agents [27].

## **Atypical Changes**

Atypical squamous cell alterations belong to a spectrum of cellular morphological changes which fall between normal limits or reactive on the one end, and frankly dysplastic process (indicative of a squamous intraepithelial lesion—SIL) on the other end. Reasons for the atypical diagnosis include lack of specific cytologic features or insufficient number of cells with characteristic cytologic features.

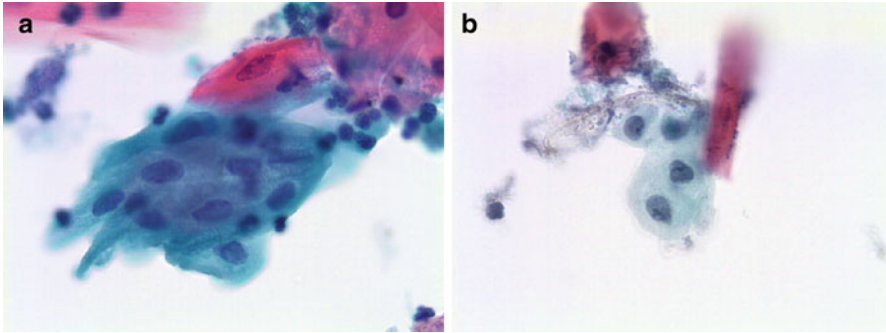
Since any meaningful classification should bear a close correlation to the biological behavior of its respective lesions and since the current guidelines for the treatment of oral dysplastic lesions advocate active treatment of high grade dysplasias [28], which are more likely to be aneuploid [29] with high risk of progression to squamous cell carcinomas [30], the term atypical is further subcategorized to illustrate the probabilities of low grade/reactive and high grade lesions.

### ***C: Atypical Probably Reactive/Low Grade (Atypical-RL)***

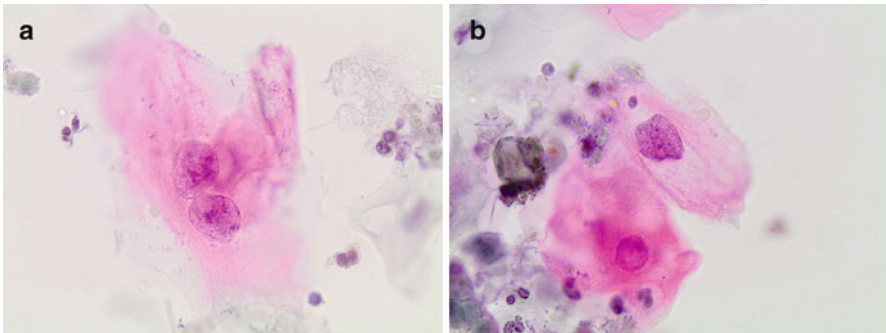
The atypical-RL cells resemble superficial cells, intermediate cells, reactive cells and the cells observed in low grade squamous intraepithelial lesions (LSILs), in terms of size, cytoplasmic volume and staining characteristics. The distinction between these cell types is somewhat subjective and is primarily based on nuclear morphology. It may therefore be wise to include LSIL as a subcategory in this category, the inclusion of which has no clinical/therapeutic implications. The nuclei of atypical-RL cells are enlarged (although still smaller than the cells seen in LSIL so that the N:C ratio is less than 3:1) and possess slightly irregular or smooth nuclear membranes. The nuclei are mildly hyperchromatic or hypochromatic and do not demonstrate the coarse granularity often seen in LSIL. The presence of acute inflammatory cells may favor a reactive change rather than a dysplastic process (Fig. 6.8a, b).

### ***Low-Grade Squamous Intraepithelial Lesion***

The cells of low-grade squamous intraepithelial lesion (LGSIL) are large with fairly abundant “mature” well-defined cytoplasm. As mentioned, the cytoplasmic volume and staining characteristics are similar to those of intermediate and superficial cells.

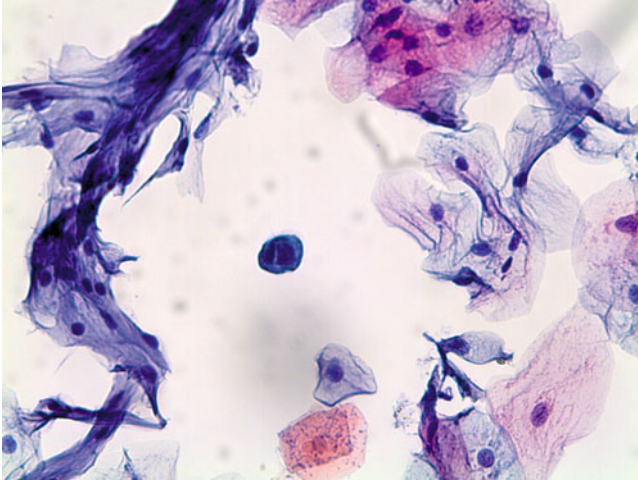


**Fig. 6.8** (a) LBC (PapSpin). Atypical-reactive/low-grade. The cells in this image show nuclear enlargement with an attendant increase in N:C ratio, compared with the normal intermediate cell nucleus seen top right. The cells demonstrate irregularities of nuclear contours. The cytoplasm looks slightly immature (denser). Some neutrophil polymorphs are noted. The cell with the bright orangeophilic cytoplasm exhibits a degenerate nucleus. The presence of inflammatory cells favors a reactive process but a low grade squamous intraepithelial lesion cannot be completely excluded (Pap stain,  $\times 1,000$ ). (b) Atypical-reactive/low-grade. The nuclei are mildly enlarged with slight nuclear membrane irregularities; however, the nuclei are normochromatic (Pap stain,  $\times 1,000$ )



**Fig. 6.9** (a) LBC (PapSpin). LGSIL. A large binucleated cell is seen in this image with markedly enlarged nuclei and abundant cytoplasm with a slight increase in N:C ratio. Slight irregularity of the nuclear membrane is seen. The chromatin is coarsely granular and evenly distributed. No nucleoli are identified (Pap stain,  $\times 1,000$ ). (b) A large cell is seen in the center with cytomorphological features of LSIL ( $\times 1,000$ )

These cells often stand out at screening magnification. Large hyperchromatic nuclei are seen, three times the size of the intermediate cell nuclei, which results in a slightly increased N:C ratio. Slight irregularities of the nuclear membranes are visible. The chromatin is coarsely granular and uniformly spread. Nucleoli are not a feature of LGSIL (Fig. 6.9a, b).



**Fig. 6.10** LBC (SurePath). Atypical/high-grade. A single cell is seen in the center with enlarged and intensely hyperchromatic nucleus. The scanty cytoplasm results in a high N:C ratio. The nuclear membrane outline is strikingly irregular. The cytomorphology is that of a HGSIL, however, because this is the only cell observed, the diagnosis was limited to Atypical/high-grade (Pap stain,  $\times 400$ )

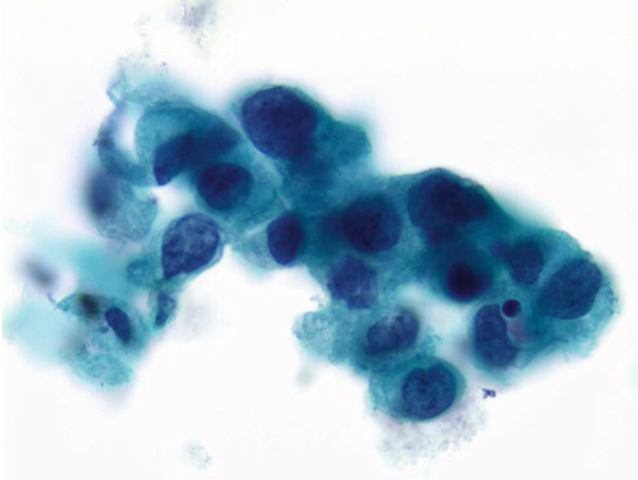
### ***D: Atypical Probably High Grade (Atypical-H)***

This subcategory is used if the cytologic features fall qualitatively short of high-grade squamous intraepithelial lesion (HGSIL), or if an insufficient number of cells indicative of a HGSIL are present (Fig. 6.10). Atypical-H cells are usually scarce. The nuclei of atypical-H cells are markedly enlarged and are three times the area of a normal intermediate squamous cell nucleus, with marked reduction in cytoplasmic volume and an attendant increase in N:C ratio. The nuclei may be hyperchromatic or show variable hyperchromasia; the nuclear membranes are slightly irregular and nucleoli are absent. More prominent nuclear membrane irregularities are features of a HGSIL.

### ***E: High-Grade Squamous Intraepithelial Lesion***

The cells of high-grade squamous intraepithelial lesion (HGSIL) may be arranged as single cells, streaming sheets or syncytial clusters. A syncytium is a three dimensional group of closely packed and haphazardly arranged hyperchromatic nuclei with molding. No cell borders can be perceived. Mitotic figures and apoptotic bodies can be seen in large syncytial clusters.

The cells of HGSIL possess markedly enlarged and hyperchromatic nuclei (which may be the same size or less than that of a LSIL) with scanty dense cytoplasm and



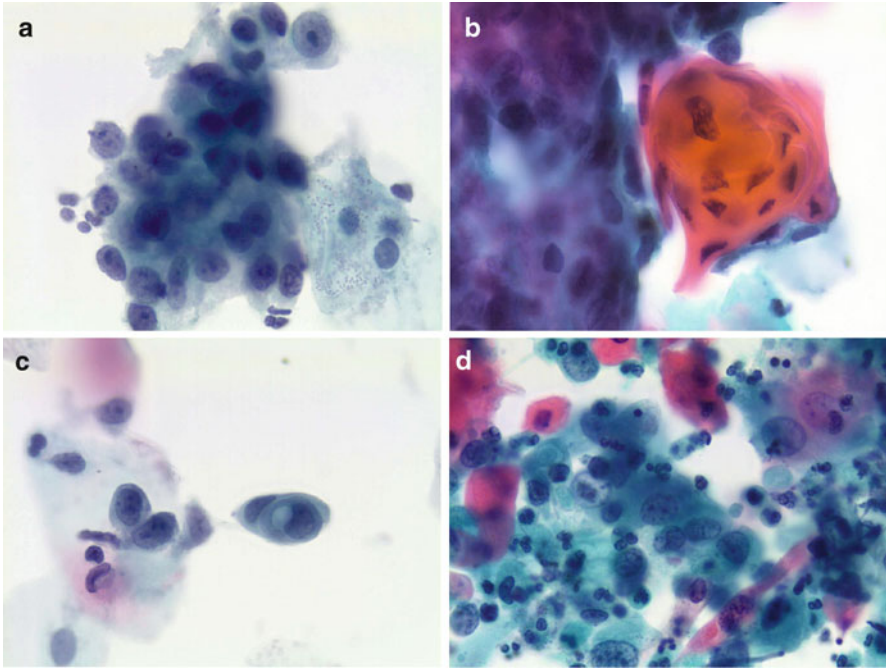
**Fig. 6.11** LBC (PapSpin). HGSIL—In this high grade lesion, the cells show high N:C ratios and nuclear hyperchromasia with coarsely granular chromatin. The nuclear membranes are irregular, and the cytoplasm has a hard (dense) appearance (Pap stain,  $\times 1,000$ )

thus a very high N:C ratio. Irregularities of nuclear contour are striking, especially in liquid-based preparations. The chromatin pattern is finely or coarsely granular but is uniformly distributed. Nucleoli are not seen (Fig. 6.11).

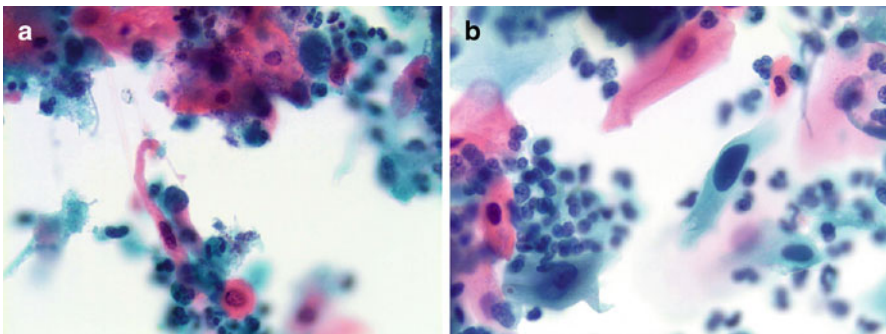
### ***F: Invasive Squamous Cell Carcinoma***

In invasive squamous cell carcinoma (SCC) the cells are found as syncytial groups. Pronounced nuclear alterations are features of invasive SCC and include marked variation in nuclear shape and size, irregularities of nuclear membrane, prominent irregular nucleoli, variably sized and irregularly distributed chromatin clumps separated by areas of clearing (Fig. 6.12a). There is often shedding of isolated single cells with malignant nuclear features.

In keratinizing squamous cell carcinomas, there is usually evidence of atypical keratin pearls. (Fig. 6.12b) Occasionally some malignant cells may display a cannibalistic behavior (“Cell in Cell”—Fig. 6.12c) and abnormally configured keratotic cells with bizarre shapes (spindling, tadpole shapes or long cytoplasmic projections—(Figs. 6.12d and 6.13a, b). The abnormal keratotic cells are evidence of abnormal maturation and possess dense orange/blue or pink cytoplasm and intensely hyperchromatic nuclei. Although they are often associated with an invasive SCC, they may be encountered in non-invasive lesions, and the correct



**Fig. 6.12** (a) LBC (PapSpin). Invasive squamous cell carcinoma-haphazard arrangement of variably sized cells in syncytial arrangement typical of carcinoma. This contrasts with the streaming arrangement seen in repair. The nuclei demonstrate chromatin clearing, prominent irregular nucleoli and irregular nuclear outlines (Pap stain,  $\times 1,000$ ). (b) Invasive squamous cell carcinoma—an atypical keratin pearl, pathognomonic of keratinizing squamous cell carcinoma (Pap stain,  $\times 1,000$ ). (c) Invasive squamous cell carcinoma—“cell in cell” (cannibalism) (Pap stain,  $\times 1,000$ ). (d) Invasive squamous cell carcinoma—In addition to the carcinoma cells this image shows an abnormally configured keratotic cell with long cytoplasmic projections, bright pink cytoplasm, and intensely hyperchromatic irregular nucleus (*bottom right*). A parakeratotic cell and an abnormal cytoplasmic fragment are seen on the left (Pap stain,  $\times 1,000$ )



**Fig. 6.13** (a, b) LBC (PapSpin). Abnormally configured spindled keratotic cells and small parakeratotic cells (Pap stain,  $\times 1,000$ )

interpretation of carcinoma is based on the presence of nuclear abnormalities described, identified by careful study of numerous cells and cell groups.

The background may show necrosis, which may occasionally collect at the periphery of the cell groups (clinging necrosis) and a scattering of (abnormal) cytoplasmic fragments. The syncytial arrangement of carcinoma cells, together with their prominent nucleoli and nuclear pallor (clearing) may simulate repair, however, the finding of flat, streaming cohesive sheets of cells in repair contrasts the haphazard arrangement of cells in invasive SCC. In addition, no single cells with malignant nuclear features are identified in repair and the chromatin remains pale, uniform and finely granular.

## Verrucous Carcinoma

The diagnosis of verrucous carcinoma can only be made on histologic sections that are deep enough to demonstrate the downward pushing epithelium in relation to the underlying submucosa. Generally there is no evidence of cellular atypia in the absence of malignant transformation/invasion and hence the cytological features of verrucous carcinoma are non-specific; they usually demonstrate abundant parakeratosis and unremarkable epithelial cells.

## Future Perspectives

While still at its infancy, this grading system provides a standardized and uniform method of reporting for the practising oral pathologist. To further validate the newly proposed classification scheme and discover the best cut-off value for distinguishing reactive/low grade lesions from high grade/squamous cell carcinoma, the authors propose a simple and easy scoring method based on nine cytologic characteristics [19] which may well increase the specificity of the oral cytology test in a manner similar to that of the Pap test (Table 6.2). It was found that a cytologic score of <3 indicated a reactive/low grade lesion and a cytologic score of 3 or more indicated a high grade lesion or invasive squamous cell carcinoma, with high sensitivity (95%) and specificity (96%) (Fig. 6.14).

While the newly proposed oral cytologic scoring system shows promise to be simple, reliable and reproducible, future large scale studies including an acceptable large number of clinical Class I lesions (clinically worrisome that would typically raise suspicion for premalignancy or invasive cancer) and Class II lesions (primarily not strongly suspicious for a high-grade dysplasia or an invasive carcinoma and would otherwise not trigger an invasive diagnostic procedure) will have to confirm its applicability and usefulness and determine the optimal score for each cytologic diagnostic category. This is ideally achieved through prospective multicenter

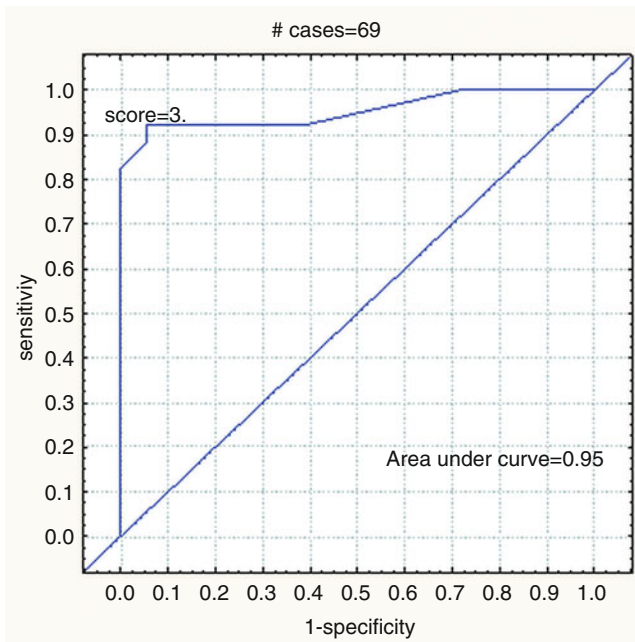
**Table 6.2** Oral/oropharyngeal cytologic scoring system

Scoring system	
Irregular nuclear membrane	Yes (1)/No (0)
Irregular chromatin distribution/hyperchromasia	Yes (1)/No (0)
Prominent nucleoli	Yes (1)/No (0)
Abnormal cell shapes <sup>a</sup>	Yes (1)/No (0)
Parakeratotic cells <sup>b</sup>	Yes (1)/No (0)
Necrosis	Yes (1)/No (0)
Syncytial groups (>10 cells) <sup>c</sup>	Yes (1)/No (0)
Irregular nucleoli	Yes (1)/No (0)
Abnormal cytoplasmic fragments	Yes (1)/No (0)

<sup>a</sup>Abnormal cell shapes: Abnormally configured keratotic cells with spindling, tadpole shapes or long cytoplasmic projections

<sup>b</sup>Parakeratotic Cells: Cells with dense orangeophilic cytoplasm and small hyperchromatic degenerate nuclei. The nuclear-to-cytoplasmic ratio is low

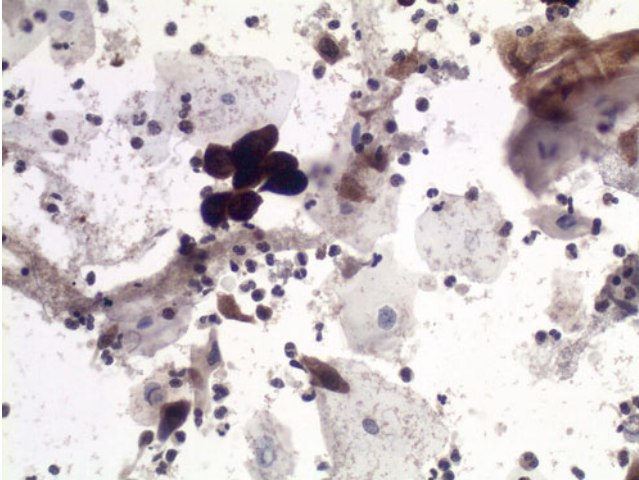
<sup>c</sup>Syncytial groups: Pleomorphic cells seen in three-dimensional clusters



**Fig. 6.14** A score of 3 was found to be the optimal cut-off value to discriminate between reactive/low-grade and high-grade/invasive SCC

collaborative studies in oral cancer screening with possible application of molecular diagnostics and immunocytochemistry for detection of predictive cellular antigens (e.g. D2-40/Podoplanin—Fig. 6.15).





**Fig. 6.15** D2-40 (Podoplanin) immunocytochemistry. This image shows strong staining of tumor cells, weak to moderate staining of dysplastic cells and lack of staining of reactive and normal intermediate, and superficial cells (x400)

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