

Squamous lesions with glandular involvement: conventional cytology *versus* liquid-based cytology

Oliveira T* ¹†, Azevedo V¹ †, Dominguez R²

¹School of Health – Polytechnic Institute of Porto (ESS-P.PORTO), Portugal

²Anatomical Pathology Service, Pedro Hispano Hospital; Local Health Unit of Matosinhos – EPE, Portugal

† These authors contributed equally to this project

Received: March 2017/ Published: November 2017

<https://doi.org/10.26537/citotech.v0i3.2284>

***Corresponding Author:**

Telma Oliveira

telmah_18@hotmail.com

ABSTRACT

Portugal is the Western European country with the highest incidence rate of cervical cancer. Its main cause is the human papillomavirus (HPV) infection, and the most common method for its prevention and detection is the conventional and/or liquid-based cervicovaginal cytology. Conventional cytological smears have more disadvantages than the liquid-based ones; nevertheless, when it comes to glandular lesions, both the conventional and the liquid-based cytology present some limitations. This paper is about a 31 year-old woman whose results of the cytological screening tests differed from those of the histological ones. With the publication of the present work, we aim to highlight the limitations of the conventional smear when compared to the liquid-based cytology for the diagnosis of glandular lesions.

Key-words: conventional cytology, liquid-based cytology, high-grade squamous intraepithelial lesion, glandular involvement, HPV

INTRODUCTION

Nearly one thousand new cases of cervical cancer are registered every year in Portugal, making this the most common tumour of the female genital tract (almost 6% of all cancers affecting women)¹.

Cervical cancer is the fourth most common cancer in Europe², and Portugal is the Western European country with the highest incidence rate³.

The precursor lesions of cervical cancer (cervical intraepithelial neoplasia - CIN) and squamous intraepithelial lesions (SIL) are divided into two groups: low-grade lesions (genital warts, CINI/LSIL – low-grade squamous intraepithelial lesion) and high-grade lesions (CINII-III/HSIL – high-grade squamous intraepithelial lesion). The histological classification is based on cytomorphological and architectural criteria which include the amount of epithelial thickness surrounded by immature cells, the level until which it is possible to observe mitotic figures, as well as the presence of atypical mitotic figures, nuclear stacking, pleomorphism and loss of cell polarity⁴.

Squamous lesions may involve endocervical glands, which occurs mostly with high-grade squamous intraepithelial lesions. The literature says that endocervical glands are involved in nearly 40% of all high-grade lesions, which is nearly four times more than in low-grade lesions⁴.

The cytological result is often vague, with both the conventional cytology and the liquid-based cytology (LBC) presenting some limitations. When it comes to the diagnosis of glandular lesions, the conventional cytology is more sensitive, whereas the liquid-based cytology is more specific⁵. Liquid-based cytologies lead to a significant reduction in the number of “undetermined significance” results, as they offer a clean background with cells distributed in a uniform way; they also take less

time to perform the screening and ensure a better treatment of samples with blood and inflammatory cells⁷.

CASE PRESENTATION

The starting point for this case is the conventional cytology of a 31 year-old woman, performed in the context of a screening at a health centre of the Local Health Unit of Matosinhos.

The smear presented a background obscured by blood and inflammation (**Fig.1A**) with some bare nuclei and clusters of atypical cells, hindering, however, a proper assessment of the cytoplasmic and nuclear features (**Fig.1B** and **Fig.1C**). The cytological result was “Atypical Squamous Cells, cannot exclude HSIL” (ASC-H).

The patient was then referred to the consultation at the same Hospital, where she underwent an LBC and a cervical biopsy.

As illustrated by **Fig. 2**, the liquid-based cytology had a clean background where it was possible to observe some bare nuclei and clusters of atypical cells, as well as eccentric and slightly hyperchromatic nuclei with an increased nuclear-cytoplasmic ratio. However, it was not possible to assess the chromatin features or to precisely perceive if the cell clusters were squamous or glandular (**Fig.2A** and **Fig.2B**). The result of the liquid-based cytology was ASC-H and atypical endocervical glandular cells (AGC).

The histology of the biopsy showed a progressive atypia with dysplastic nuclei in nearly two thirds of the epithelial thickness (**Fig.3**), matching a diagnosis of CIN II.

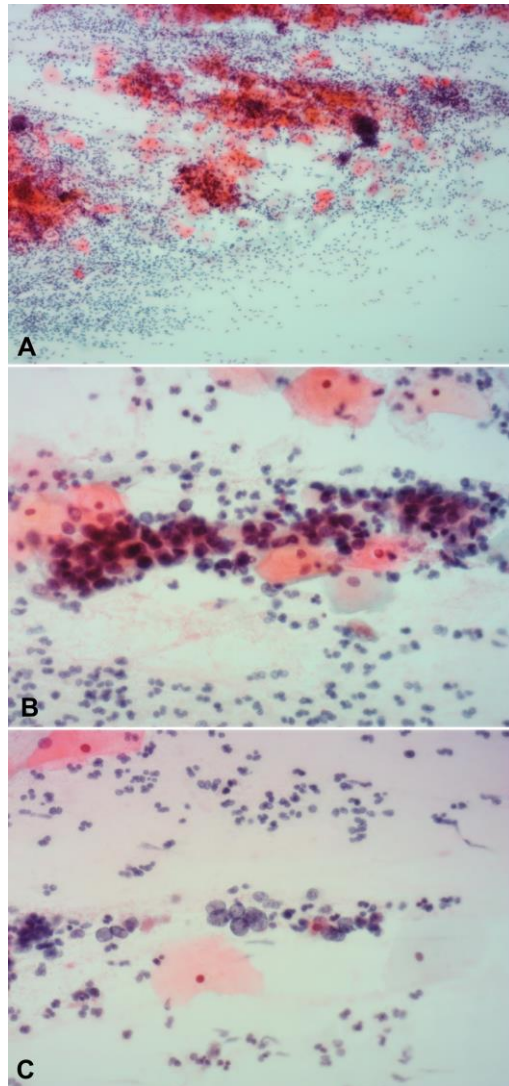


Fig. 1 – Conventional smear; sample limited by excess of inflammation: (A) background obscured by blood and inflammation (100x); (B) group of bare nuclei present in the smear (400x); (C) group of bare nuclei present in the smear (400x). Papanicolaou stain.

The LBC sample was used to perform the HPV detection and typification through the Abbott® test. The result was positive for HPV type 16.

The biopsy specimen was used to carry out an immunohistochemical dual-staining test with p16 and ki67, which resulted positive for both markers, with cellular proliferation in nearly two thirds of the epithelial thickness (Fig. 4A) and in the endocervical glands (Fig. 4B).

In the course of the follow-up, the patient was submitted to a cone biopsy; the histological exam indicated the presence of dysplastic cells all over the epithelial thickness and in the endocervical glands (Fig.5), resulting in a diagnosis of “carcinoma in situ with endocervical glands involvement”. Chronic cervicitis was also observed.

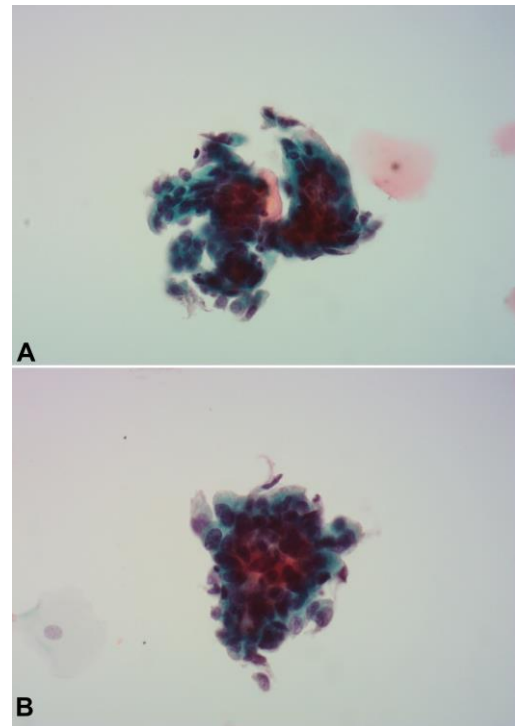


Fig. 2 – In A and B it is possible to observe clusters of atypical cells and hyperchromasia; however, it was not possible to conclude about the squamous and/or glandular origin. Liquid-based cytology - ThinPrep®, Papanicolaou stain, 400x.

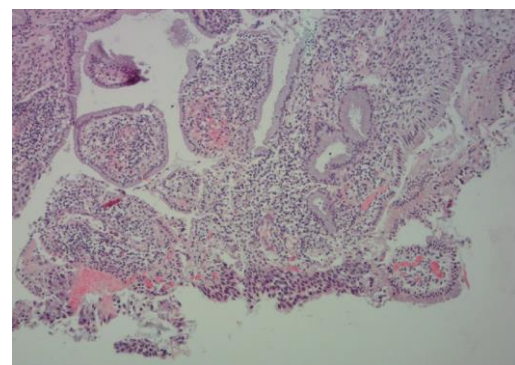


Fig. 3 – Representation of the histological features of the cervical biopsy. It is possible to observe the presence of dysplastic cells in nearly two thirds of the epithelial thickness. Haematoxylin-eosin stain, 100x.

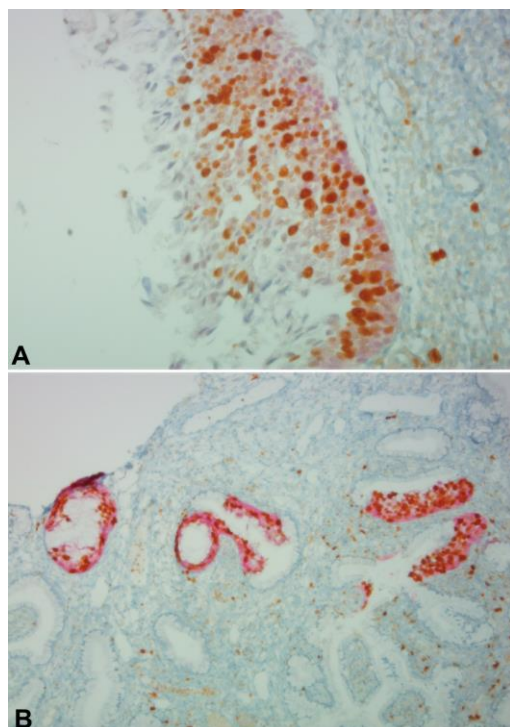


Fig. 4 – Immunohistochemical staining with markers ki67 and p16: **(A)** positive result for ki67/p16 in two thirds of the squamous epithelium (400x); **(B)** positive result for ki67/p16 in the endocervical glands (200x).

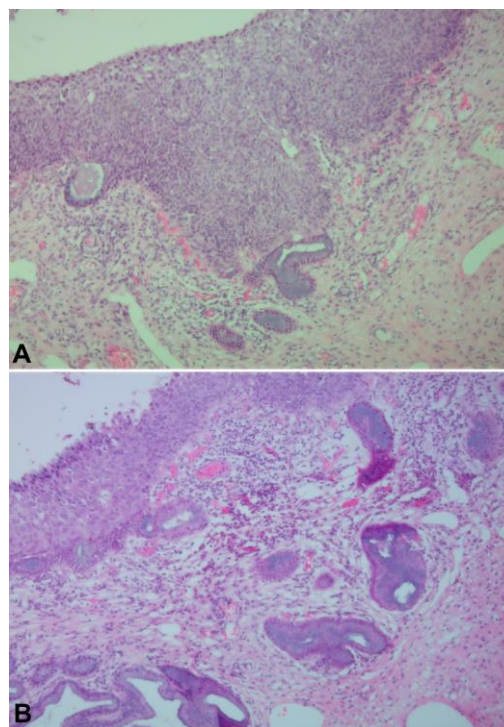


Fig. 5 – Histological features of the cone biopsy specimen, in which it is possible to observe the invasion of the entire epithelial thickness by dysplastic cells **(A)**, endocervical glands involved by masses of dysplastic cells **(B)**. Haematoxylin-eosin stain, 100x.

ANALYSIS AND DISCUSSION

This study allowed us to consider the advantages of the LBC when compared to the conventional cytology.

The conventional smear of this case showed scarce groups of atypical cells and bare nuclei; nevertheless, there could be other atypical cells relevant to the diagnosis but unable to be observed, due to the excess of inflammation and blood. The liquid-based cytology also showed similar clusters of atypical cells, but their nuclei and cytoplasm were more distinctive, with the advantage that the sample background was not obscured by blood or inflammation. This suggests that the limitations associated with the quality of the conventional smear might affect the sensitivity and specificity of the cytological diagnosis and, consequently, the follow-up of the lesion.

The cytological study of the collected samples did not support the attribution of a specific result of “high-grade squamous intraepithelial lesion with endocervical cells involvement”, due both to the limitations of the conventional smear, and to the difficulty in interpreting the scarce, atypical cells present in the LBC.

The excessive amount of blood may have obscured the atypical cells present in the sample, which could have resulted in a wrong diagnosis of “negative for intraepithelial lesion or malignancy” (NILM).

The cytology’s sensitivity and specificity were improved by developing the LBC, thus reducing the hematic and inflammatory background, distributing cells in a more homogeneous way and allowing a proper fixation and staining⁷.

According to the Bethesda system, this type of squamous lesion with glandular involvement exhibited some cytological characteristics which are similar or even identical to those of the glandular lesions⁷.

The cell clusters of lesions with glandular involvement can be wrongly thought of as having glandular origin. Lesions of squamous origin are characterized by disarrayed clusters of cells displaying loss of cohesion and flattening of the cells in the cluster's periphery. The cytoplasm is finely vacuolated, which might suggest a glandular process; however, on a closer look, it is possible to observe that those clusters do not exhibit features of glandular differentiation, such as basal nuclei, columnar and plane formations and pseudostratification. An important thing to consider when determining the squamous or glandular origin is the observation of squamous and dysplastic cells close to the atypical clusters⁷.

Besides the cellular architecture, nuclear characteristics can also help to identify the lesion's origin. The chromatin pattern of squamous lesions is not as coarsely granular as that of adenocarcinoma *in situ* (AIS)⁶; some nucleoli can also be observed in an HSIL with glandular involvement, despite being less evident in the AIS⁷.

It should be emphasised, however, that a squamous lesion can coexist with a glandular lesion⁷.

The difficulty in diagnosing squamous lesions with glandular involvement can be reduced by a combined interpretation of the cytology and the histology, as well as other complementary exams, such as HPV testing and an immunohistochemical study with p16 and ki67.

Most of the glandular tumours are associated with the HPV infection, with types 16 and 18 being the most frequent⁸. On the other hand, and according to previous studies, the majority of the high-grade intraepithelial lesions, CIN II and CIN III, exhibit strong,

diffuse positivity for p16, as well as high ki67 scores⁹. In this case, the presence of high-risk HPV and the positive result for the p16/ki67 dual staining allowed us to conclude that the DNA of the virus was already integrated in the host's cells⁶.

This case allowed us to correlate a cytological result of ASC-H and AGC with a histological diagnosis of CIN II in the biopsy and a "carcinoma *in situ* with endocervical glandular involvement" in the cone biopsy specimen.

CONCLUSION

By presenting this case, we intended to highlight the degree of limitation of conventional cytology when compared to the LBC, namely in cases of squamous lesions with glandular involvement. These cases require a more careful observation of the cell morphology, architecture and nuclear characteristics, due to its implications to the follow-up and treatment of the patient.

The cytological result of this case was ASC-H and AGC. The cone biopsy, HPV testing and immunohistochemical study allowed to reach a specific diagnosis, leading to an appropriate follow-up and treatment. However, it is important to note that only the LBC sample allowed the observation of the cytomorphological features of both squamous and glandular lesions.

ACKNOWLEDGEMENTS

We would like to thank the Anatomical Pathology Service of the Pedro Hispano Hospital, EPE, namely Dr. Mrinalini Honavar, who gave us permission to use this case study, and Dr. Fátima Magalhães, for her support in the image selection. A special thanks to the technician Amaro Frutuoso, for his availability and collaboration in the laboratory issues and in the image acquisition.

REFERENCES

1. InfoCancro - Tudo sobre cancro [Internet]. InfoCancro - www.infocancro.com. 2017 [cited 21 December 2016]. Available from: <http://www.roche.pt/sites-tematicos/infocancro/index.cfm/tipos/cancro-do-utero/>
2. Fact Sheets by Population [Internet]. Globocan.iarc.fr. 2017 [cited 20 December 2016]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
3. Oncologia I. Cancro do colo do útero - O que é? | Instituto CUF de Oncologia [Internet]. Saudecuf.pt. 2017 [cited 21 December 2016]. Available from: <https://www.saudecuf.pt/oncologia/o-cancro/cancro-do-colo-do-utero>
4. Nagi CSchlosshauer P. Endocervical glandular involvement is associated with high-grade SIL. *Gynecologic Oncology*. 2006;102(2):240-243.
5. Moreira M, Longatto Filho A, Castelo A, de Barros M, Silva A, Thomann P et al. How accurate is cytological diagnosis of cervical glandular lesions?. *Diagnostic Cytopathology*. 2008;36(4):270-274.
6. Gupta, N., Nijhawan, R., Srinivasan, R., Suri, V., Rajwanshi, A. and Singh, V. (2015). Liquid-based cytology versus conventional cytology for evaluation of cervical Pap smears: Experience from the first 1000 split samples. *Indian Journal of Pathology and Microbiology*, 58(1), p.17.
7. Nayar RWilbur D. *The Bethesda system for reporting cervical cytology*. 3rd ed. Springer; 2015
8. McCluggage W. New developments in endocervical glandular lesions. *Histopathology*. 2012;62(1):138-160.
9. von Knebel Doeberitz M, Reuschenbach M, Schmidt D, Bergeron C. Biomarkers for cervical cancer screening: the role of p16INK4to highlight transforming HPV infections. *Expert Review of Proteomics*. 2012;9(2):149-163.