

## Gynecologic Cytopathology

ACTA  
CYTOLOGICAActa Cytologica 2015;59:273–277  
DOI: 10.1159/000435801Received: April 23, 2015  
Accepted after revision: June 8, 2015  
Published online: August 13, 2015

# Critical Analyses of the Introduction of Liquid-Based Cytology in a Public Health Service of the State of São Paulo, Brazil

Adhemar Longatto-Filho<sup>a, e, h-j</sup> José Eduardo Levi<sup>d, e</sup> Toni Ricardo Martins<sup>d</sup>  
Diane Cohen<sup>f</sup> Lise Cury<sup>f</sup> Luisa Lina Villa<sup>c, e, g</sup> José Eluf-Neto<sup>b, f, g</sup>

<sup>a</sup>Laboratório de Investigação Médica (LIM) 14, <sup>b</sup>Department of Preventive Medicine and <sup>c</sup>Departamento de Radiologia e Oncologia, Faculdade de Medicina, and <sup>d</sup>Laboratório de Virologia, Instituto de Medicina Tropical, Universidade de São Paulo, <sup>e</sup>Instituto Nacional de Ciência e Tecnologia das Doenças do Papilomavírus Humano, <sup>f</sup>Fundação Oncocentro de São Paulo, and <sup>g</sup>Centro de Investigação Translacional em Oncologia, Instituto do Câncer do Estado de São Paulo, São Paulo, and <sup>h</sup>Molecular Oncology Research Center, Barretos Cancer Hospital, Pio XII Foundation, Barretos, Brazil; <sup>i</sup>Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, and <sup>j</sup>ICVS/3B's – PT Government Associate Laboratory, Braga, Portugal

## Key Words

Cervical cancer screening · Cytology in low-resource settings · Gynecologic cytology · Liquid-based cytology · Public health

## Abstract

**Objective:** The aim of this study was to compare the performance of the current conventional Pap smear with liquid-based cytology (LBC) preparations. **Study Design:** Women routinely undergoing their cytopathological and histopathological examinations at Fundação Oncocentro de São Paulo (FOSP) were recruited for LBC. Conventional smears were analyzed from women from other areas of the State of São Paulo with similar sociodemographic characteristics. **Results:** A total of 218,594 cases were analyzed, consisting of 206,999 conventional smears and 11,595 LBC. Among the conventional smears, 3.0% were of unsatisfactory preparation; conversely, unsatisfactory LBC preparations accounted for 0.3%. The ASC-H (atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion) frequency did not demonstrate any differences between the two

methods. In contrast, the incidence of ASC-US (atypical squamous cells of undetermined significance) was almost twice as frequent between LBC and conventional smears, at 2.9 versus 1.6%, respectively. An equal percentage of high-grade squamous intraepithelial lesions were observed for the two methods, but not for low-grade squamous intraepithelial lesions, which were more significantly observed in LBC preparations than in conventional smears (2.2 vs. 0.7%). The index of positivity was importantly enhanced from 3.0% (conventional smears) to 5.7% (LBC). **Conclusions:** LBC performed better than conventional smears, and we are truly confident that LBC can improve public health strategies aimed at reducing cervical lesions through prevention programs.

© 2015 S. Karger AG, Basel

## Introduction

The introduction of the Papanicolaou test (Pap test) proved to be effective in reducing mortality caused by cervical cancer in developed countries. Unfortunately, cervi-

cal cancer still represents a serious public health problem in poor and developing countries, including Brazil [1, 2], where this neoplasia remains very frequent, with about 16,000 new cases per year [3]. Worldwide, the Pap test is still used by the majority of cervical cancer screening programs as the primary laboratorial tool of prevention. The conventional Pap smear has a winning history on decreasing the incidence of and mortality from cervical cancer, with an estimated reduction of around 80%, but this is restricted to settings with high-quality cytology screening that is effectively and regularly executed, with a good population coverage [4]. The conventional Pap smear, however, is quite laborious, with various difficulties concerning collection, the preparation of glass slides and reading. On the other hand, Pap tests prepared with collection in liquid medium result in homogeneous preparations that facilitate reading and enable a better performance by cytotechnologists, significantly reducing the proportion of unsatisfactory smears, and allowing readings through an automated system [5]. Liquid-based cytology (LBC), besides representing the technical development of conventional cytology, also offers a single means of collecting samples that preserves nucleic acids and allows the transport and storage of samples at room temperature, and is quite suitable for screening algorithms that include human papillomavirus (HPV) testing [6, 7]. For over a decade, strong evidence indicating that Pap smears collected in liquid medium have a sensitivity and specificity similar to those of conventional cytology has been presented [8]. In addition, a number of recent publications have clearly demonstrated that LBC offers increased indexes of cytology positivity, and a consistent and remarkable reduction of unsatisfactory preparations [9–12]. Moreover, the best performance can be achieved with the use of computer-assisted screening programs, which is also feasible but not frequently employed globally with conventional Pap smears [13]. The São Paulo State Secretary of Health (SES) is responsible for the collection and analysis of approximately 2.5 million Pap tests per year, and the Fundação Oncocentro de São Paulo – FOSP (with the third-largest volume) performs about 250,000 of these tests. Under this scenario, molecular techniques could favor cervical cancer screening because of its very high negative predictive value (>99%) and high sensitivity [14]. Data from the Hospital Cancer Registry of the FOSP show that the proportion of women under 30 years of age with invasive cervical cancer is small. Between January 2000 and April 2012, 13,760 invasive cervical cancer cases were accounted for in the State of São Paulo, of which 436 (3.2%) occurred in women between

the age of 25 and 29 years, and 122 (0.9%) cases were in women aged between 20 and 24 years. The comparison of cervical cancer survival rates indicates a huge difference among developed and underserved countries, attributable to differences in access to early diagnosis, quality of screening and the existence or lack of programs of screening, as well as the provision of adequate treatment [15–17]. This study aimed to evaluate the preliminary results of the introduction of LBC to the daily routine of Pap tests analyzed in the FOSP laboratory, and to compare results with conventional Pap smears performed over the same period.

## Material and Methods

The samples for this study were collected from women participating in the Projeto Região Oeste of the Health Care Facilities opportunistic program: Basic Health Units (supported by Faculdade de medicina da Universidade São Paulo – FMUSP) and the Interlagos Hospital and Maternity Center, from December 2013 to December 2014. The target population was women of any age that spontaneously visited the Public Health Ambulatories associated to the FOSP. The conventional Pap smears were collected from several ambulatories distributed in São Paulo State with similar sociodemographic characteristics to those where LBC was introduced. The comparison of the performance of both methodologies was made by evaluating the overall results of each during the period of study. The Ethics Committee of the Faculty of Medicine, University of São Paulo, approved this study (No. 075/13).

For the liquid-based Pap test cells were collected from the endocervical junction and placed in the recipient containing the liquid fixer. The whole brush head was placed in the recipient containing BD SurePath™ (BD Diagnostics – TriPath, Burlington, N.C., USA) liquid and sent to the FOSP laboratory for the preparation of glass slides according to manufacturer's instructions using the semiautomated BD PrepMate™ and BD PrepStain™ (BD Diagnostics – TriPath) tools. Before starting the study, the FOSP team received training in the preanalytical phase of cytological and molecular examinations, as well as in the LBC reading.

## Results

A total of 218,594 cases were analyzed: 206,999 conventional smears and 11,595 LBC preparations. Table 1 shows the number of women analyzed by each test according to age. Among the conventional smears, 3.0% were unsatisfactory preparations, which corresponds to 6,239 cases; conversely, unsatisfactory LBC preparations accounted for 0.3%, or 29 cases, predominantly represented by samples collected from atrophic cervixes (table 2). Table 3 shows the distribution of cytological results between the preparations performed with liquid medium

**Table 1.** Number of women analyzed by each test according to age

| Age distribution, years | Cytology preparation |                     |
|-------------------------|----------------------|---------------------|
|                         | LBC, n (%)           | conventional, n (%) |
| 11–24                   | 1,523 (13.1)         | 30,957 (15.0)       |
| 25–44                   | 5,040 (43.5)         | 92,285 (44.6)       |
| 45–64                   | 4,075 (35.1)         | 71,609 (34.6)       |
| 65+                     | 957 (8.3)            | 12,148 (5.9)        |
| Total                   | 11,595 (100.0)       | 206,999 (100.0)     |

**Table 2.** Number of unsatisfactory and satisfactory preparations performed with liquid medium and conventional methodologies

| Sample adequacy | Cytology preparation |                     |
|-----------------|----------------------|---------------------|
|                 | LBC, n (%)           | conventional, n (%) |
| Unsatisfactory  | 29 (0.3)             | 6,239 (3.0)         |
| Satisfactory    | 11,566 (99.7)        | 200,760 (97.0)      |
| Total           | 11,595 (100.0)       | 206,999 (100.0)     |

**Table 3.** Distribution of cytological results between the preparations performed with liquid medium and conventional methodologies

| Cytological result | Cytology preparation |                     | Total, n (%)    |
|--------------------|----------------------|---------------------|-----------------|
|                    | LBC, n (%)           | conventional, n (%) |                 |
| ASC-H              | 43 (0.4)             | 1,007 (0.5)         | 1,050 (0.5)     |
| ASC-US             | 337 (2.9)            | 3,253 (1.6)         | 3,590 (1.6)     |
| Invasive carcinoma | 1 (0.0)              | 19 (0.0)            | 20 (0.0)        |
| HSIL               | 18 (0.2)             | 419 (0.2)           | 437 (0.2)       |
| LSIL               | 256 (2.2)            | 1,427 (0.7)         | 1,683 (0.8)     |
| Negative           | 10,940 (94.4)        | 200,874 (97.0)      | 211,814 (96.9)  |
| Total              | 11,595 (100.0)       | 206,999 (100.0)     | 218,594 (100.0) |

**Table 4.** Distribution of endocervical representation by LBC and conventional methodologies

| Endocervical cells detection | Cytology preparation |                     | Total, n (%)    |
|------------------------------|----------------------|---------------------|-----------------|
|                              | LBC, n (%)           | conventional, n (%) |                 |
| Yes                          | 6,575 (56.7)         | 120,162 (58.0)      | 126,737 (58.0)  |
| No                           | 5,023 (43.3)         | 86,913 (42.0)       | 91,936 (42.0)   |
| Total                        | 11,598 (100.0)       | 207,075 (100.0)     | 218,673 (100.0) |

and conventional methodologies. Of note, there was no difference in ASC-H (atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion) between the two methods. In contrast, ASC-US (atypical squamous cells of undetermined significance) was recorded in almost twice as many LBC cases as in conventional smears – 2.9 versus 1.6%, respectively. An equal percentage of high-grade squamous intraepithelial lesion (HSIL) was observed for the two methods, but not for low-grade squamous intraepithelial lesion (LSIL), which was more frequently observed in LBC preparations than in conventional smears (2.2 vs. 0.7%). Invasive carcinoma was detected in 1 case prepared with LBC technology and in 20 conventional smear cases, which proportionally was very similar, with no perceived differences between the two methods. The index of positivity was considerably

enhanced ( $p < 0.0001$ ) from 3.0% (conventional smears) to 5.7% (LBC). Finally, table 4 shows the distribution of endocervical representation in both preparations. No differences were observed between either methodology, which implies, in part, a similar performance of sample collection.

## Discussion

The results achieved in this study are very encouraging because the index of positive results for the conventional smears compared to LBC preparations rose from 3.0 to 5.7%, almost doubling the number of cases, representing a significant impact for public health cervical cancer strategies. São Paulo State has more than 40 million in-

habitants, and FOSP represents an important institution for cancer registration, training of public health professionals, and quality control of cytopathology procedures. The frequencies of squamous columnar junction in both methodologies were similar. The accuracy of sample collection probably influenced the results we observed. It is important to note, for example, that the frequencies of positive HSIL were similar by both methodologies, and no important gain was detected with the introduction of LBC, as has been anticipated in other studies [9, 18]. The same proportion of invasive cancers was obtained with both methodologies. However, for settings with an elevated prevalence of high-grade lesions, LBC is proven to be more effective for detecting HSIL than conventional smears [10–12]. We hypothesize that these promising results would be even more relevant if LBC was applied in a large casuistic population. Importantly, the remarkable increase of LSIL exceeded our expectations. LSIL alterations were significantly more frequent in LBC samples than conventional preparations, which endorsed the observations reported in many previous studies [9, 19, 20]. LSIL is recognized as a reversible/transitory lesion; however, the impact of the augmented frequency of LSIL might signify an increase of HSIL after colposcopic examination if LSIL persists [21]. This fact is particularly evident if high-risk HPV tests are judiciously applied in LSIL cases; the LSIL result combined with a positive high-risk HPV test is strongly associated to a high-grade lesion on biopsy [21]. This evidence encouraged us to continue our research and initiate a project that involves the combination of LBC, slide reading preparation and automation, and HPV testing, which will be implemented in the near future. Moreover, LBC plus a HPV DNA test detects more high-grade lesions and avoids many abnormal cytology results without a clinically significant increase in the number of unnecessary colposcopies [21].

One of the most remarkable results we achieved relates to the adequacy of cytological samples. The FOSP laboratory performs more than 200,000 Pap tests annually. The proportion of unsatisfactory cases was very high in conventional smears, totaling more than 6,000 cases. If one considers that the Brazilian Government funds more than 10 million PAP tests each year with the aim of prevention, 3% represents approximately 300,000 women to be recalled. However, the indexes of unsatisfactory cases in Brazil prompt far less optimism than we found in the FOSP laboratory; not surprisingly, robust evidence indicates that in most settings reflex HPV triage is likely to be the optimal strategy for managing women presenting with ASC-US results [22]. The higher number of positive

cases in the LBC arm was also associated with the augmented number of ASC-US. This is not surprising, and the higher frequency of ASC-US in LBC is related to the 'learning curve' period that can vary among cytotechnologists, dependent on their particular skills. The LBC preparation-associated ASC-US frequency tends to decrease over time and establish a slight superior baseline as compared with conventional smears [12]. Moreover, significant improvements are expected in accomplishing the correct diagnosis over time for intraepithelial lesions [23].

Finally, LBC offers an opportunity to introduce a high level of internal quality assurance in cytological diagnoses with a reproducible use of computer-guided image screening [24] that potentially reduces the margin of false-negative results and enhances the sensitivity of the Pap test evaluation [25], besides permitting the implementation of combined analyses of cytology and HPV molecular tests [26]. It is important to mention that between 2009 and 2011 the National Institute of Science and Technology of the Diseases Associated to the Papillomavirus (INCT-HPV) performed an assessment study of molecular techniques and proposed new algorithms for cervical cancer screening, which showed a very high negative predictive value (>99%) and high sensitivity [14]. Currently, the INCT-HPV is engaged with FOSP to initiate projects towards screening improvements. Our standards of positivity are comparable to the best-practice laboratories in cytopathology. In the near future, we are planning to improve our efforts in cervical cancer prevention with the combination of HPV testing and cytology automation.

### Acknowledgments

The authors are indebted to BD Brazil, which partially supported the study by providing the SurePath™ collection kits and equipment. The authors independently performed the study design, results evaluation and writing of the manuscript.

### References

- 1 Ferlang J, Shin HR, Forman D, Forman D, Mathers C, Parkin DM: Estimates of the worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;127:2893–2917.
- 2 Arbyn M, Castellsague M, de Sanjose S, Bruni L, Saraiya M, Bray F, Ferlay J: Worldwide burden of cervical cancer in 2008. *Ann Oncol* 2011;22:2675–2686.
- 3 INCA: ESTIMATIVA 2014: incidência de câncer no Brasil. <http://www.inca.gov.br/estimativa/2014/>.



- 4 Lyng E, Antilla A, Arbyn M, Segnan N, Ronco G: What's next? Perspectives and future needs of cervical screening in Europe in the era of molecular testing and vaccination. *Eur J Cancer* 2009;45:2714–2721.
- 5 Longatto-Filho A, Schmitt FC: Gynecological cytology: too old to be a pop star but too young to die. *Diagn Cytopathol* 2007;35:672–673.
- 6 Longatto-Filho A, Schmitt FC: Cytology education in the 21st century: living in the past or crossing the Rubicon? *Acta Cytol* 2010;54:654–656.
- 7 Schmitt FC, Longatto-Filho A, Valent A, Vielh P: Molecular techniques in cytopathology practice. *J Clin Pathol* 2008;61:258–267.
- 8 Isidean SD, Franco EL: Embracing a new era in cervical cancer screening. *Lancet* 2014;383:493–494.
- 9 Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J: Liquid compared with conventional cervical cytology: a systematic review and meta-analysis. *Obstet Gynecol* 2008;111:167–177.
- 10 Zheng B, Austin RM, Liang X, Li Z, Chen C, Yan S, Zhao C: Bethesda System reporting rates for conventional Papanicolaou tests and liquid-based cytology in a large Chinese college of American pathologists-certified independent medical laboratory: analysis of 1,394,389 Papanicolaou test reports. *Arch Pathol Lab Med* 2015;139:373–377.
- 11 Scapulatempo C, Fregnani JH, Campacci N, Possati-Resende JC, Longatto-Filho A, RODEO Study Team: The significance of augmented high-grade squamous intraepithelial lesion detection on Pap test examination: partial results from the RODEO study team. *Acta Cytol* 2013;57:489–494.
- 12 Fregnani JH, Scapulatempo C, Haikel RL Jr, Sacchetto T, Campacci N, Mauad EC, Longatto-Filho A, RODEO Study Team: Could alarmingly high rates of negative diagnoses in remote rural areas be minimized with liquid-based cytology? Preliminary results from the RODEO Study Team. *Acta Cytol* 2013;57:69–74.
- 13 Saieg MA, Motta TH, Fodra ME, Scapulatempo C, Longatto-Filho A, Stiepcich MM: Automated screening of conventional gynecological cytology smears: feasible and reliable. *Acta Cytol* 2014;58:378–382.
- 14 Navarro C, Fonseca AJ, Sibajev A, Souza CI, Araújo DS, Teles DA, Carvalho SG, Cavalcante KW, Rabelo WL: Cervical cancer screening coverage in a high-incidence region. *Rev Saude Publica* 2015;49:1–8.
- 15 Allemanni C, Weir HK, Carreira H, et al: Global surveillance of cancer survival 1995–2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* 2015;385:977–1010.
- 16 The NHS Information Centre, Screening and Immunisations Team: Cervical screening programme, England, 2011. <http://www.cancerscreening.nhs.uk/cervical/cervical-statistics-bulletin-2010-11.pdf>.
- 17 Nygård JF, Skare GB, Thoresen SØ: The cervical cancer screening programme in Norway, 1992–2000: changes in Pap smear coverage and incidence of cervical cancer. *J Med Screen* 2002;9:86–91.
- 18 Renshaw AA, Young NA, Birdsong GG, Styer PE, Davey DD, Mody DR, Colgan TJ: Comparison of performance of conventional and ThinPrep gynecologic preparations in the College of American Pathologists Gynecologic Cytology Program. *Arch Pathol Lab Med* 2004;128:17–22.
- 19 Ronco G, Cuzick J, Pierotti P, Cariaggi MP, Dalla Palma P, Naldoni C, Ghiringhello B, Giorgi-Rossi P, Minucci D, Parisio F, Pojer A, Schiboni ML, Sintoni C, Zorzi M, Segnan N, Confortini M: Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial. *BMJ* 2007;335:28.
- 20 Longatto-Filho A, Naud P, Derchain SF, Roteli-Martins C, Tatti S, Hammes LS, Sarian LO, Eržen M, Branca M, de Matos JC, Gontijo R, Maeda MY, Lima T, Costa S, Syrjänen S, Syrjänen K: Performance characteristics of Pap test, VIA, VILI, HR-HPV testing, cervicography, and colposcopy in diagnosis of significant cervical pathology. *Virchows Arch* 2012;460:577–585.
- 21 Siebers AG, Arbyn M, Melchers WJ, van Kemenade FJ, Vedder JE, van der Linden H, van Ballegooijen M, Bekkers RL, Bulten J: Effectiveness of two strategies to follow-up ASC-US and LSIL screening results in The Netherlands using repeat cytology with or without additional hrHPV testing: a retrospective cohort study. *Cancer Causes Control* 2014;25:1141–1149.
- 22 Vanni T, Legood R, Franco EL, Villa LL, Luz PM, Schwartzmann G: Economic evaluation of strategies for managing women with equivocal cytological results in Brazil. *Int J Cancer* 2011;129:671–679.
- 23 Cummings MC, Marquart L, Pelecanos AM, Perkins G, Papadimos D, O'Rourke P, Ross JA: Which are more correctly diagnosed: conventional Papanicolaou smears or Thinprep samples? A comparative study of 9 years of external quality-assurance testing. *Cancer Cytopathol* 2015;123:108–116.
- 24 Stein MD, Fregnani JH, Scapulatempo C, Mafra A, Campacci N, Longatto-Filho A, RODEO Study Team from Barretos Cancer Hospital: Performance and reproducibility of gynecologic cytology interpretation using the FocalPoint system: results of the RODEO Study Team. *Am J Clin Pathol* 2013;140:567–571.
- 25 Wilbur DC, Black-Schaffer WS, Luff RD, Abraham KP, Kemper C, Molina JT, Tench WD: The Becton Dickinson FocalPoint GS Imaging System: clinical trials demonstrate significantly improved sensitivity for the detection of important cervical lesions. *Am J Clin Pathol* 2009;132:767–775.
- 26 Monsonego J, Hudgens MG, Zerat L, Zerat JC, Syrjänen K, Smith JS: Risk assessment and clinical impact of liquid-based cytology, oncogenic human papillomavirus (HPV) DNA and mRNA testing in primary cervical cancer screening (The FASE Study). *Gynecol Oncol* 2012;125:175–180.