

Could Alarming High Rates of Negative Diagnoses in Remote Rural Areas Be Minimized with Liquid-Based Cytology? Preliminary Results from the RODEO Study Team¹

José Humberto T.G. Fregnani^a Cristovam Scapulatempo^a Raphael L. Haikel Jr^a
Teóclito Saccheto^a Natália Campacci^a Edmundo C. Mauad^a
Adhemar Longatto-Filho^{a–d}



^aBarretos Cancer Hospital, Pio XII Foundation, Barretos, and ^bLaboratory of Medical Investigation 14, Faculty of Medicine, University of São Paulo, São Paulo, Brazil; ^cLife and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, and ^dICVS/3B's, PT Government Associate Laboratory, Guimarães, Portugal

Key Words

Cytology · Papanicolaou test · SurePath · Cytological screening · Liquid-based cytology

Abstract

Objective: It was the aim of this study to compare diagnostic performances of the BD SurePath™ liquid-based Papanicolaou test (LBC) and the conventional Papanicolaou test (CPT) in cervical samples of women from remote rural areas of Brazil. **Study Design:** Specimens were collected by mobile units provided by Barretos Cancer Hospital. This report evaluates the manual screening arm of the RODEO study. Of 12,048 women seen between May and December 2010, 6,001 were examined using LBC and 6,047 using CPT. **Results:** Comparative (LBC vs. CPT) outcomes were: all abnormal tests, 2.1 versus 1.0%; ASC-US (atypical squamous cells

of unknown significance), 0.7 versus 0.1%; ASC-H (atypical squamous cells with possible high-grade squamous intraepithelial lesions) and AGC (atypical glandular cells), 0.4 versus 0.3%; LSIL (low-grade squamous intraepithelial lesions), 0.7 versus 0.3%; HSIL (high-grade squamous intraepithelial lesions), 0.4 versus 0.2%, and unsatisfactory, 0.03 versus 0.08%. The LBC arm detected significantly more lesions (ASC-US+) than CPT ($p < 0.001$); however, when we divided the diagnoses into two groups, ASC-H– (negative/ASC-US/LSIL) and ASC-H+ (ASC-H/AGC/HSIL), the difference was not statistically important ($p = 0.213$). **Conclusions:** With inherent difficulties in patient recruitment and patient compliance with cancer screening, best test performance including human papillomavirus test capability are vitally necessary in Brazil's struggle to reduce cervical cancer.

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¹ Adriana Cruvinel Carloni^a, Naiara Correa Nogueira-de-Souza^a, Maíra Stein^a, Fábio de Paula Mateus^a, Eduardo Tadeu da Silva^a, Michele M. Castro Alves^a, Fábio Cardoso de Lima^a, Erlaine Martins Suriano^a.

J.H.T.G.F. and C.S. contributed equally to this work.

Introduction

Cervical cancer prevention can be likened to a three-legged stool, with the seat representing cancer reduction and the legs comprising patient access [1], accurate and reliable testing [2], and a responsive medical infrastructure [1, 3]. Brazil's geography and the socioeconomic depression of rural Brazil challenge public health authorities charged with reducing cervical cancer [2].

Barretos Cancer Hospital remains among Brazil's most active cancer hospitals, and in answering geographic challenges, the hospital has, for decades, used mobile units (MUs) to enable the collection and preparation of patient samples, colposcopy examinations and minor surgical procedures. Under the auspices of the hospital and with the cooperation of local government authorities, physicians, nurses and ancillary service professionals work with community agents to recruit and educate target populations for cancer prevention programs [3].

Due in part to its historical role in reducing cervical cancer prevalence in the late 1950s and 1960s, the conventional Papanicolaou (Pap) test (CPT) is Brazil's benchmark cervical cancer screening test. Unfortunately, incidence and mortality of cervical cancer remain high among Brazil's rural poor, and failure to remedy this situation lies in part with collecting, preparing and fixing cervical cytology samples, whose simplicity has been overstated and whose complexity has been underappreciated [4, 5]. The BD SurePath™ liquid-based Pap test (LBC) standardizes fixation, slide preparation and slide staining. It is a technical advancement that also opens the door for computer-assisted slide analysis, human papillomavirus detection and other molecular testing [6]. The RODEO study will evaluate the impact of MU-based LBC on cervical disease detection, and this publication offers preliminary results of the implementation of LBC.

Material and Methods

Study Design

RODEO is a prospective multi-center study evaluating 30,000 women from Barretos Cancer Hospital (Barretos, Brazil) as well as from Leonor Mendes de Barros Hospital and Hospital das Clínicas of São Paulo State University (São Paulo, Brazil). It is intended to offer best practices for the control and prevention of cervical cancer among Brazilian women, especially among those from impoverished, generally rural, high-risk areas including regions of the Amazon River basin. The current report offers preliminary data of conventional and direct-to-vial collections obtained by the MUs of Barretos Cancer Hospital (fig. 1).

Eligibility Criteria and Consent

Women were considered eligible for the study if their clinical assessment allowed for cervical cytological examination. Participants gave informed consent to participate in the study protocol that was approved by the ethics committee of Barretos Cancer Hospital (No. 244/2009).

Group Allocation

The first examination of the day was random sampling; thereafter, sampling alternated in accordance with the time of day (e.g. morning or afternoon). When the MUs remained in an area for several days, LBC and CPT samplings were performed on alternate days.

Sample Collection and Preparation

The CPT sample was collected with a wooden Ayre spatula and a Rovers® Cervex-Brush® Combi, smeared on a glass slide and spray fixed. The LBC sample was collected with a Rovers Cervex-Brush Combi, transferred into an LBC fixative and sent to the laboratory, where slides were prepared according to the manufacturer's instructions.

Cytological Interpretation

The cytology classification system of Brazil reflects the 2001 Bethesda System [7].

Statistical Analysis

Data were exported to SPSS for Windows® version 17.0 (SPSS Inc., Chicago, Ill., USA). The t test was used to compare women's ages. Differences between the two sampling systems were compared using the χ^2 and Fisher's exact test, with significance levels set at 5%. Confidence intervals for proportions were calculated.

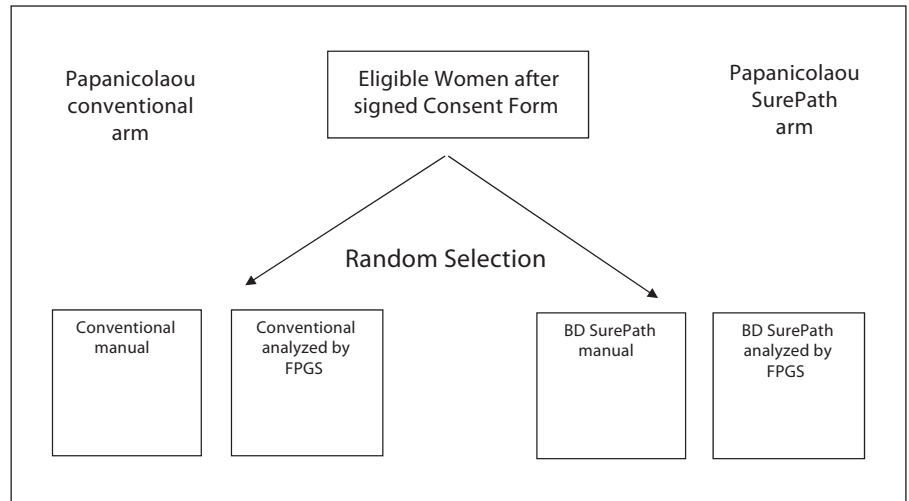
Results

The study set comprised 12,048 consecutively examined women. The study interval was from May to December 2010. 6,001 (49%) individuals, with a mean age of 46.5 ± 12.5 years, were examined using LBC, and 6,047 (51%) individuals, with a mean age of 46.1 ± 13.3 years, were examined using CPT.

The geographic distribution of source material from seven Brazilian states was as follows: Acre (0.24%), Goiás (1.68%), Minas Gerais (2.71%), Mato Grosso do Sul (3.11%), Mato Grosso (27.9%), Rondônia (8.31%) and São Paulo (55.97%) (fig. 2).

Comparative (LBC vs. CPT) outcomes were: all normals, 97.9 versus 99.0%; all abnormal, 2.1 versus 1.0%; unsatisfactory tests, 0.03 versus 0.08%. Data are further detailed in table 1. Table 2 presents the frequency of negative + ASC-US (atypical squamous cells of unknown significance) + LSIL (low-grade squamous intraepithelial lesions) and ASC-H (atypical squamous cells with possible

Fig. 1. Study design flow charts representing the comparison of CPT versus LBC. In this present study, only preliminary results from the manual arm were analyzed. The computer-assisted analyses of Focal Point Guided Station (FPGS) were not reported.



high-grade squamous intraepithelial lesions) + AGC (atypical glandular cells) + HSIL (high-grade squamous intraepithelial lesions), according to the type of cytology preparation: 99.2 and 0.8% for LBC and 99.4 and 0.6% for CPT, respectively. Table 3 shows the distribution of LBC cytology results according to the study period. There was a clear reduction in ASC-US as the study progressed. A similar pattern was observed for ASC-H.

Discussion

In Brazil, the cervical cancer screening and control program has been implemented at the national level more than 30 years ago. Various authors have observed that for the country as a whole, mortality from cervical cancer has remained virtually stable. Cervical cancer is ranked second among the leading causes of death from cancer in Brazil's female population, with a downward trend for the country as a whole, a decline in the State capitals, but a stable trend among its interior municipalities [8].

Methodological advances have proven essential to outcome improvement in gynecological cytology and cancer prevention. The CPT performance falls short of that of LBC. The main reasons for the improved performance of LBC are the control of fixation, cell transfer (with the randomized distribution of abnormal cells) and presentation, affording uniform and consistently stained monolayers [4].

In the hands of seasoned health care providers, as exemplified by the members of the MU teams, LBC appeared to outperform CPT. Larger sample sizes (compris-



Fig. 2. The route of MUs in seven Brazilian States representing more than 6,000 km of traveling.

ing around 27,500–34,500 patients in each arm) would be needed to demonstrate statistically improved detection of abnormal tests (ASC-H and higher) and a meaningful reduction in unsatisfactory samples. Likewise, although HSIL appears twice as often in the LBC group, a statistically valid separation requires larger sample sizes.

Table 1. Overall distribution of cytological examination according to the type of cytology preparation and the 2001 Bethesda System classification

Pap test result	Type of cytology preparation					
	LBC			CPT		
	n	%	95% CI	n	%	95% CI
Negative	5,872	97.9	95.4–100	5,981	99.0	96.5–100
ASC-US	39	0.7	0.5–0.9	6	0.1	0.04–0.2
ASC-H	24	0.4	0.3–0.6	21	0.3	0.2–0.5
LSIL	41	0.7	0.5–0.9	19	0.3	0.2–0.5
HSIL	22	0.4	0.2–0.6	14	0.2	0.1–0.4
AGC	1	0.02	0.004–0.09	1	0.02	0.004–0.09
Total	5,999	100.0	–	6,042	100.0	–

Unsatisfactory cases were excluded. 95% CI = 95% Confidence interval for proportions. χ^2 test, $p < 0.001$.

Table 2. Distribution of Pap test results according to the type of cytology preparation

Pap test result	Type of cytology	
	LBC	CPT
ASC-H–(negative/ASC-US/LSIL)	5,952 (99.2)	6,006 (99.4)
ASC-H+(ASC-H/AGC/HSIL)	47 (0.8)	36 (0.6)
Total	5,999 (100.0)	6,042 (100.0)

Figures in parentheses are percentages. Prevalence rate = 1.3, $p = 0.213$ (χ^2 test).

Table 3. Distribution of cytology results according to the study period

Cytology result (LBC)	Number of cases in respective study periods						Total
	≤1,000	1,001–2,000	2,001–3,000	3,001–4,000	4,001–5,000	>5,000	
Negative	969 (16.5)	983 (16.7)	980 (16.7)	971 (16.5)	978 (16.7)	990 (16.9)	5,871 (100.0)
ASC-US	15 (38.5)	8 (20.5)	4 (10.3)	5 (12.8)	4 (10.3)	3 (7.7)	39 (100.0)
ASC-H	6 (25.0)	3 (12.5)	3 (12.5)	8 (33.3)	3 (12.5)	1 (4.2)	24 (100.0)
LSIL	6 (14.6)	3 (7.3)	9 (22.0)	8 (19.5)	11 (26.8)	4 (9.8)	41 (100.0)
HSIL	4 (18.2)	1 (4.5)	4 (18.2)	8 (36.4)	3 (13.6)	2 (9.1)	22 (100.0)

Figures in parentheses are percentages.

ASC-US was reported more frequently in the LBC arm of the study (0.7 vs. 0.1%); however, with increasing experience, there was a clear decrease in ASC-US reporting, and this was interpreted as a learning curve effect. If ASC-US and ASC-H are not taken into consideration, the LSIL + HSIL proportions for LBC remain twice that of CPT (1.1 vs.

0.5%). Among high-risk Brazilian women, LBC has been shown to outperform CPT [9]; however, its superiority has not been borne out among low-risk women [9–12]. Nonetheless, in the setting of a community whose population numbers in the millions, small proportional differences equate to significant differences among actual patients.

Unfortunately, this study also exposes a dark side to cytology screening in Brazil. For the sake of comparison, mortality from cervical cancer in Victoria (Australia) declined gradually over time, and since 2002, it has been around 1.0 per 100,000 women (1.4 per 100,000 women for Australia as a whole; 2008 International Cancer Screening Network data of the US National Cancer Institute), which is among the lowest cervical cancer death rates in the world. Over the same interval, Brazil's mortality rate was about 10.9 per 100,000 women – more than 10-fold greater than that of Victoria. However, one must take into consideration that there are differences in incidence and mortality depending on how the data are collected. There is urgent need to improve the quality of population-based cancer registries in low-income countries [13].

One of the most disturbing aspects of this preliminary report, and one that should affect Brazil's approach to cervical cancer screening, is that preliminary data show an alarmingly high 'negative' rate, regardless of the mode of cytological screening (97.9% for LBC and 99.0% for CPT). Of unequivocal abnormal diagnoses, the reported data show a HSIL + LSIL rate of between 1.1 and 0.5% (based on both LBC and CPT diagnoses). These outcomes, compared to the 2010 results of the Victorian Cervical Cytology Registry, showed that the proportion of Pap tests with identical squamous cell abnormalities was 6.4% – a rate between 5- and 12-fold higher for Australian as compared to Brazilian women at a time when cervical cancer was 10-fold lower for Australian versus Brazilian women [14]. The reasons for the low rates of cytological alterations detected at screening are certainly ample and complex, but we can hypothesize that differences in cervical lesion prevalence (very low) in rural areas could influence the Pap performance. Furthermore, the low sensitivity of the Pap test is well recognized even in optimal scenarios. On the other hand, the training of all professionals involved in screening programs based on cytology could be revised, the theoretical issues amplified and periodical re-training programs considered to increase the quality of sample collection and preparation, as well as the slide analyses. Moreover, formal examination of theory and practice could be implemented with rigid proficiency exams. The workload should be officially stipulate to 40–50 slides per day. Currently, there are only recommendations for best practices, and the cytotechnicians are free to read more than that for 8 h. The skills and salaries of cytotechnicians could also be raised. However, all these options will certainly increase the very low prices of Pap tests in Brazil. We are now aiming for a new

concept of cytological screening in Barretos Cancer Hospital, and the introduction of LBC is a first step to improve quality. Investments in the education of cytotechnicians are also being implemented by integrating these professionals in graduation courses. The augmented frequency of HSIL in the LBC arm certainly endorses the next steps that comprise automation for reading slides (Focalpoint BD system is under evaluation) and improvements in the internal quality control system for cytological diagnoses that is currently based on the revision of 10% of negative cases and the revision of all ASC-US+ cases.

Given the current astronomical discrepancies on cytological alteration frequencies, one wonders whether Brazil should 'read the writing on the proverbial wall' and altogether abandon gynecological cytology screening in favor of high-risk human papillomavirus testing, which has shown great promise among Latin American and Eastern European populations as a stand-alone detection method for cervical cancer and its immediate precursors, cervical intraepithelial neoplasia 2 and 3 [15].

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Disclosure Statement

The authors declare that there are no individual competing interests.

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Erratum

In the article by Fregnani et al., entitled '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 Cytologica 2013;57:69–74, DOI: 10.1159/000343046], the RODEO Study Team needs to be included in the list of authors so that it reads:

José Humberto T.G. Fregnani^a Cristovam Scapulatempo^a Raphael L. Haikel Jr^a
Teóclito Sacchetto^a Natália Campacci^a Edmundo C. Mauad^a
Adhemar Longatto-Filho^{a-d} Rodeo Study Team¹

^aBarretos Cancer Hospital, Pio XII Foundation, Barretos, and ^bLaboratory of Medical Investigation 14, Faculty of Medicine, University of São Paulo, São Paulo, Brazil; ^cLife and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, and ^dICVS/3B's, PT Government Associate Laboratory, Guimarães, Portugal

¹ Adriana Cruvinel Carloni, Naiara Correa Nogueira-de-Souza, Maíra Stein, Fábio de Paula Mateus, Eduardo Tadeu da Silva, Michele M. Castro Alves, Fábio Cardoso de Lima, Erlaine Martins Suriano (all with affiliation ^a).