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DCS liquid-based system is more effective than conventional smears to diagnosis of cervical lesions: Study in high-risk population with biopsy-based confirmation

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Abstract

Objective. To compare the performances of Papanicolaou test (PapTest) and of a new liquid-based cytology method, DNA-Citoliq® System (DCS), in a high-risk population, with histology confirmation.

Methods. Paired specimens of exfoliated cervical cells were collected under split-sample protocol. All patients were submitted to colposcopy and a biopsy taken when any atypical transformation zone was seen. Sensitivity, specificity, positive and negative predictive values, and overall accuracy of both conventional and DCS methods were computed in relation to histology.

Results. A total of 1095 patients were analyzed by two cytology methods and, in 425 (38.8%), histologically. There were significantly more adequate samples with DCS (98.63%) than with conventional (89.6%) smears ($P < 0.001$). ASCUS was diagnosed significantly more with DCS than with conventional Pap ($P < 0.001$). Conventional Pap misclassified as normal 55.4% (158/285) of cases with either LSIL or HSIL or cancer at histology, whereas DCS misclassified 31.2% (89/285) of cases ($P < 0.001$). DCS had a significantly higher sensitivity (70% and 91.3%) than the conventional Pap (49.8% and 72.8%) to detect both LSIL+ and HSIL+ at histology, respectively. On the other hand, specificity of conventional smear (88.2% and 85.2%) was significantly higher than DCS (75.4% and 70.9%) considering both LSIL+ and HSIL+ at histology, respectively.

Conclusions. This study confirms the superiority of the liquid-based cytology system DCS to detect cervical lesions. The rate of adequate DSC slides was significantly higher than with conventional cytology.
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Keywords: DCS system; Liquid-based cytology; Papanicolaou test; Cervical cancer; Cervical screening

Introduction

New innovations to optimize the screening of cervical cancer and the precursor lesions have been studied in recent years. A revolutionary methodology to improve cytological diagnosis was made possible with the development of a liquid medium that allows preservation of cell morphology for cytopathological evaluation and nucleic acids for molecular tests [1,2]. One such medium, the Universal Collection Medium (UCM) [DNA-Citoliq® System (DCS)], an alcohol-based fixative, has been recently developed and tested clinically [3,4]. The performance of this medium in preserving cell morphology was well documented in a recent study

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[5] such as its capacity to preserve samples for biomolecular investigation.

The apparent laboratorial simplicity of Pap smear actually involves different steps of a complex procedure. To produce a cervical sample with adequate squamous columnar cells representation requires skills in patient preparation, specimen collection, laboratory processing, and slide interpretation [6]. Despite adequate care in preparing the conventional smear, only about 20% of cells are effectively transferred to the slide [7]. In contrast, the liquid-based cytology provides clear background without cellular overlapping and the analyses can be more expeditious than the conventional smear [8]. In addition, in the liquid-based procedure, the cells ultimately transferred to the slide are likely to be more representative of the overall sample to be screened. All factors considered, liquid-based cytology has demonstrated higher sensitivity to detect cervical lesions than conventional smears [9–14].

Indeed, the clinical significance of an abnormal cytology can only be assessed by comparing cytology results with histology of biopsy specimens. It should be pointed out, however, that women with low grade lesions (LSIL) and ASCUS detected in liquid-based cytology are often not referred to colposcopy and therefore, do not undergo cervical biopsy [11]. Moreover, as most studies have generally included women from the general population, high grade lesions (HSIL) and cancers have usually been underrepresented [11,12]. There is a scarcity of data comparing the accuracy of liquid-based cytology with histological diagnosis in high-risk populations [13].

The goal of this investigation was to compare, in a split-sample protocol, the screening performance of conventional smears with the new liquid-based cytology method, DCS, in a high-risk population, using colposcopy followed by histology as "gold standard."

Materials and methods

After approval of the Institutional Review Board of both institutions involved in the project, 1095 consecutive women were prospectively enrolled in the study. All tests were performed in blind fashion protocol.

Our study focused on high-risk population defined as women presenting with any aceto-white positive lesion detected on speculum visual inspection, abnormal cytology, and/or previous history of cervical lesion. These women were referred to Pérola Biygnton Hospital, a public reference center for women genital diseases in São Paulo city, Brazil, where the study was carried out.

In all cases, cytological samples were collected in a split-sample protocol. Conventional samples, collected with Ayre's spatula and endocervical brush, were smeared into the slide and immediately fixed with polyethylene glycol. The same brush was again used for brushing the ectocervix and placed into tubes containing 1 ml of UCM®. The purpose was to prioritize for conventional smears the best conditions for the cytological investigation, and for liquid-based samples, the remaining material. Following collection of cervical specimens, all 1095 women have undergone colposcopy and guided biopsy, when applicable. Conization or hysterectomy was also taken into account, if available, in final histologic diagnosis. The samples for cytology and histology evaluation were processed at Pathology Division of Adolfo Lutz Institute, a reference laboratory in São Paulo.

Once at the laboratory, batches of 12 DCS samples were simultaneously prepared in 10 to 15 min. In DCS system, the specimen in the slide is contained in a 25-mm-diameter circle. Both conventional and DCS slides were stained according to the Papanicolaou method and classified according to Bethesda 2000 System [15]. Professionals trained in the evaluation of thin-layer slides manually screened all slides. The pair of DCS and conventional slides of each patient were examined by the same cytologist blinded to the result of its pair. All positive and suspicious cases were reviewed and forwarded to the senior cytopathologist for final diagnosis, also blinded to the result of the other slide preparation method and histological diagnosis. Histological specimens were initially evaluated according to WHO classification of squamous lesions in three classes (CIN 1, 2, and 3) [16], blinded to cytological results. However, for comparing with cytological diagnoses, two categories were adopted: LSIL and HSIL.

For the purpose of statistical analysis, squamous and glandular atypical findings of undetermined significance were grouped in one category; three different cutoffs of a positive cytology were used: ASCUS+, LSIL+, and HSIL+; two cutoffs of a positive histology were used: presence of any lesion and HSIL/cancer; histology was considered the gold standard. Women with no abnormality at the colposcopy were recorded as negative histology.

Sensitivity, specificity, positive and negative predictive values, and overall accuracy of both conventional and DCL methods were computed in relation to histology. Using Pearson's χ^2 test, differences in sensitivity and specificity of the two cytologic methods were compared. A difference was statistically significant if the P value was ≤ 0.05. Data were stored as excel files and analyzed using the SPSS statistical software, version 10.

Results

The mean age was 34.7 years (14 to 86). There were significantly more adequate samples with DCS (98.63%) than with conventional (89.6%) smears ($P < 0.001$). The main causes for conventional slide inadequacy were sample obscured by red blood cells followed by dense inflammatory infiltrate. For the DSC slides, the main cause was the presence of massive red blood cells. The screening prevalence of ASCUS and squamous intraepithelial lesions according to DCS and conventional Pap are listed in

Table 1
Cytology results (DCS) compared to colposcopy followed by histology findings ("gold standard")

Cytology results	Histologic results				Total
	Normal	LSIL	HSIL	Cancer	
Unsatisfactory	10	4	1	0	15
Normal	603	73	11	0	687
ASCUS/AGC	102	22	25	2	151
LSIL	61	42	29	0	132
HSIL	34	17	52	4	107
Cancer	0	0	2	1	3
Total	810	158	120	7	1095

Tables 1 and 2. ASCUS was diagnosed significantly more with DCS than with conventional Pap smear ($P < 0.001$). However, out of the 99 cases of ASCUS with DCS and normal with conventional Pap smear, there were 13 cases of HSIL and 17 cases of LSIL in histology. Overall, the conventional Pap smear misclassified as normal 55.4% (158/285) of cases with either LSIL or HSIL or cancer at histology, whereas DCS misclassified 31.2% (89/285) of cases ($P < 0.001$). Tables 3 and 4 summarize the diagnostic parameters of conventional and DCS preparations. DCS had a significantly higher sensitivity (70% and 91.3%) than the conventional Pap smear (49.8% and 72.8%) to detect both LSIL+ and HSIL+ at histology, respectively. On the other hand, specificity of conventional smear (88.2% and 85.2%) was significantly higher than DCS (75.4% and 70.9%) considering both LSIL+ and HSIL+ at histology, respectively.

Discussion

Although, ideally, a screening test for cervical lesions should have both perfect sensitivity and specificity, such test is not available. Therefore, the screening tool ought to prioritize sensitivity, so that no lesion would escape detection at the expense of a somewhat diminished specificity. Any positive result at screening, including ASCUS, simply means that further investigation is required to confirm the diagnosis. This study confirms the superiority of the liquid-based cytology system DCS as a screening test to detect cervical lesions. The rate of adequate DSC slides was significantly higher than with conventional cytology. Furthermore, DSC proved to be more sensitive than conventional smears to detect both histologically proved LSIL and HSIL in this high-risk population. Out of the 1095 patients enrolled in this study, 285 (26%) had LSIL, HSIL, or cancer at histology.

A screening test, as opposed to a diagnostic procedure, should have a low threshold to detect disease, i.e., should have high sensitivity. A case screened positive warrants further diagnostic investigation to confirm or rule out disease. Cervical cytology is no exception. Conventional cytology has long been known for its low sensitivity, attributed to inadequate sample collection and interpretation difficulties [17]. Higher sensitivity of liquid-based cytology has been well documented [18–21]. DSC, a novel liquid-based system, has similar cell morphology as ThinPrep and Autocyte [5]. As per this study protocol, DSC slides used residual cells. Despite favoring the conventional method, DSC proved to be a superior screening test, as demonstrated by its much higher sensitivity and positive predictive value to detect both LSIL and HSIL at histology. A direct-to-vial protocol could yield even better results, as reported by Vassilakos et al. [12]. DSC has been previously compared to the conventional method using a similar sample collection protocol, but with no histological confirmation [22].

Recently, Pan et al. [13], using ThinPrep methodology and colposcopy followed by biopsy when applicable, studied 1997 women in a high incidence area of cervical cancer in China. All 12 squamous cell carcinoma and 87% of the HSIL at histology were detected by that liquid-based cytology. Abufala et al. [14], also using ThinPrep, have showed a sensitivity of 76% with ThinPrep versus 68% with conven-

Table 2
Cytology results (conventional) compared to colposcopy followed by histology findings ("gold standard")

Cytology results	Histologic results				Total
	Normal	LSIL	HSIL	Cancer	
Unsatisfactory	80	21	12	1	114
Normal	644	95	30	1	770
ASCUS/AGUS	42	15	10	1	68
LSIL	29	22	25	0	76
HSIL	15	5	40	3	63
Cancer	0	0	3	1	4
Total	810	158	120	7	1095

Table 3
Sensitivity, specificity, positive (+PV) and negative (−PN) predictive values, and overall accuracy of DCS and conventional Pap for any histological alterations (LSIL+)

	DCS®	Conventional	*P* value
Sensitivity	70.00%	49.80%	<0.0001
Specificity	75.38%	88.22%	<0.0001
+ PV	49.87%	59.24%	0.037
− PV	87.77%	83.64%	0.032
Accuracy	73.98%	78.39%	0.023

Sensitivity: positive cytology results / total positive histologic results.
Specificity: negative cytology results / total negative histologic results.
PV = predictive value.

Table 4
Sensitivity, specificity, positive (+PV) and negative (−PN) predictive values, and overall accuracy of DCS and conventional Pap for HSIL+ at histology

	DCS®	Conventional	*P* value
Sensitivity	91.27%	72.81%	<0.0001
Specificity	70.86%	85.24%	<0.0001
+ PV	29.26%	39.34%	0.017
− PV	98.40%	95.97%	0.009
Accuracy	73.24%	83.79%	<0.0001

PV = Predictive value.

tional smears and specificity of 86% and 79%, respectively. However, Chacho et al. [23] did not find the ThinPrep system to be any more effective than conventional smears to detect invasive carcinomas or intraepithelial lesions.

Although DSC falls short from being an ideal screening test for cervical lesions, as it misses 31.2% of histologically confirmed lesions, it performs significantly better than conventional cytology which misses 55.4% of lesions. Considering only satisfactory samples, DSC missed only 4% of HSIL at histology, as compared to 10% with conventional cytology, the remaining being ASCUS or LSIL. Among the 687 cases with negative DSC results, 10.6% and 1.6% were LSIL and HSIL at histology, respectively. Similarly, among the 770 cases with negative results with the conventional cytology, 12.3% and 4% were LSIL and HSIL, respectively. In both situations, a false-negative colposcopy is the likely explanation for such discrepancies. Other studies have pointed out less than ideal diagnostic reproducibility with cytology and histology [24,25]. Possible reasons to explain this poor inter-observer agreement remain to be elucidated. However, quality assurance procedures in each step could minimize the errors, as advocated by Petry et al. [26], although such procedures are difficult to implement in routine care.

In conclusion, DCS, a screening method that can be easily implemented in clinical practice, is associated with fewer unsatisfactory samples and a significantly higher sensitivity when compared to conventional cytology. In addition, DCS has the advantage of collecting material for HPV-DNA Hybrid capture test, when deemed necessary.

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