Accuracy of Diagnosis of Atypical Glandular Cells—Conventional and ThinPrep

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The incidence of glandular cervical malignancy is steadily increasing. Glandular abnormalities are more frequently diagnosed on cervical smears.

In this study, we attempt to evaluate our experience with glandular cytology and to assess the sensitivity and specificity of the ThinPrep (TP) over conventional Papanicolaou (Pap) smears.

Glandular abnormalities during a 3-yr period from October 2000 to October 2003 were retrieved from our cytology database. The study group comprised smears from 369 patients, 272 conventional Pap smears (CPSs) and 97 TP from a total of 400,184 smears.

The types of glandular abnormalities were tabulated following a modified Bethesda classification. Correlation with histology and follow-up cytology was achieved in all but six patients.

Significant lesions were identified in 116/272 (PPV 42.6%) of CPSs and 58/97 (PPV 58.9%) TPs. Pure glandular abnormalities numbered 125 conventional and 51 TP; significant lesions identified in this group were 36/125 (PPV 28.8%) CPS and 26/51 (PPV 50.9%) TP.

Statistical analysis showed significant differences for positive predictive values of TP and CPS and a suggestion of increased sensitivity. The main limiting factor was small numbers of glandular abnormalities and a desirable longer study time. Diagn. Cytopathol. 2006;34:614–619.

Key Words: cervical; glandular; ThinPrep; PPV

Papanicolaou (Pap) smears have a reported sensitivity of 80% and a specificity of 99%.[1] With conventional Pap smears (CPSs), false-negative rates varying from 1.5 to 55% have been documented,[2] and these are due largely to sampling and preparation errors, the presence of blood/mucus acting as obscuring agents, as well as screening and interpretation errors.

ThinPrep (TP; CYTYC Corp., Boxborough, MA) is a new technique of collection and preparation of cervical material, overcoming some of the limiting problems posed with the Pap smear. The collected sample is suspended in a liquid medium containing a preservative and mucolytic. The preparation involves 'controlled membrane collection and transfer of cells', with production of a monolayered cytology slide.

Numerous studies have compared the performance of TP and CPS. In a comprehensive quantitative survey, Abulafia et al. concluded that TP tends to be more sensitive and specific than conventional smears in detecting cervical dysplasia.[3] Most of these studies focus on squamous cell lesions of the cervix. Only a few studies have examined atypical glandular cells (AGC)—diagnosis and outcome in liquid-based and conventional preparations.[4–8] The reports are conflicting, varying from no difference to increased or decreased detection of glandular lesions on TP versus conventional. The current overall impression is an increased sensitivity and specificity in the detection of pre-neoplastic and neoplastic endocervical glandular lesions with liquid-based preparations.[4]

Diagnostic Medlab is the largest community laboratory in New Zealand, processing ~120,000 smears annually. Most smears stem from screening a multicultural group, including both high and low risk populations. In 1998, the TP Pap test was introduced as an optional alternative to the CPS. Since there is a fee for TP, patient choice determines the specimen type. The percentage of smears received in liquid base has steadily increased. We have noticed an improvement in diagnosis of both low-grade and high-grade squamous lesions and an overall drop in the unsatisfactory rate.

This study evaluates the accuracy of diagnosis of glandular lesions (positive predictive values (PPVs)), and attempts to assess the performance of TP versus CPS.

Materials and Methods

Material for the study was sourced in two ways: For the period October 2000 to October 2003, the computer records of smears with a glandular abnormality were retrieved. Glandular abnormality is defined according to a modified

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Table I. Total Number AGUS With a Breakdown of Diagnostic Categories

	C	*T*
AGUS-NOS	121	51
AGUS-FR	86	17
AGUS-FN (sugg. AIS)	19	9
AIS	11	6
Adenocarcinoma	10	5
AGUS, endometrial	11	8
Endometrial adenocarcinoma	14	1
	272	97
Total no. of smears	*293,935*	*106,249*

Table II. Conventional AGUS-NOS

Cytology	*Histo/cyto follow-up*
AGUS/HGSIL	51 (42.1)[a]
HGSIL only	39 (76.4)
AIS only	2 (4)
HGSIL + HPV	3 (5.8)
AIS + SIL	4 (7.8)
No follow-up	3
AGUS/ASCUS	31 (25.6)
HGSIL only	4 (13)
LGSIL	3 (9.7)
AIS + SIL	2 (6.5)
Invasion SCC	1 (3.2)
NAD	21 (67.7)
AGUS-NOS	39 (32.3)
HGSIL	4 (10.2)
AIS alone	1 (2.6)
AIS + HGSIL	2 (5.1)
Adenocarcinoma	1 (2.6)
NAD	31 (79.4)
Total	*121 (44.5)*

[a]Values in parentheses indicate percentages.

Table III. ThinPrep AGUS-NOS

Cytology	*Histo/cyto follow-up*
AGUS/HGSIL	22 (43.1)[a]
HGSIL	13 (59)
AIS	4 (18.1)
LGSIL	1 (4.5)
NAD	4
AGUS/ASCUS	8 (15.7)
HGSIL	6 (75)
Adenocarcinoma endoCx	1 (12.5)
NAD	1
AGUS-NOS	21 (41.2)
HGSIL	3 (14.3)
AIS	3 (14.3)
AIS/HGSIL	4 (19)
HPV	2 (9.5)
NAD	9
Total	*51 (52.6)*

[a]Values in parentheses indicate percentages.

Bethesda classification, and includes abnormal glandular cells of undetermined significance-NOS (AGUS-NOS), abnormal glandular cells-favour reactive (AGUS-FR), abnormal glandular cells of uncertain significance-favour neoplastic (AGUS-FN), (suggestive of AIS), adenocarcinoma in situ (AIS) and adenocarcinoma (endocervical or endometrial).

Table IV. Conventional AGUS-FR

Cytology	*Histo/cyto follow-up*
AGUS/HGSIL	2 (2.3)[a]
HGSIL	1 (50)
LGSIL	1 (50)
AGUS/ASCUS	24 (28)
HGSIL	2 (8)
LGSIL	2 (8)
NAD	20 (84)
AGUS-FR	60 (69.7)
HGSIL	4 (6.6)
AIS	1 (1.6)
LGSIL	5 (8.3)
NAD	50 (83.5)
Total	*86 (31.6)*

[a]Values in parentheses indicate percentages.

Table V. ThinPrep AGUS-FR

Cytology	*Histo/cyto follow-up*
AGUS/ASCUS	4 (23.5)[a]
HGSIL	2 (50)
NAD	2 (50)
AGUS	13 (76.5)
LGSIL	1 (7.6)
NAD	10 (76)
No follow-up	2
Total	*17 (17.61)*

[a]Values in parentheses indicate percentages.

Table VI. Conventional AGUS-FN

Cytology	*Histo/cyto follow-up*
AIS/HGSIL	4 (21)[a]
HGSIL alone	1 (25)
AIS/HGSIL	1 (25)
NAD	1 (25)
No follow-up	1
AIS/ASCUS	6 (31.6)
AIS/HGSIL	4 (66.8)
LGSIL	1 (16.6)
NAD	1 (16.6)
AIS	9 (47.4)
AIS/HGSIL	2 (22.2)
AIS alone	5 (55.5)
Metastatic adenocarcinoma	1 (11.1)
EndoCx polyp	1
Total	*19 (7)*

[a]Values in parentheses indicate percentages.

Table VII. ThinPrep AGUS-FN

Cytology	*Histo/cyto follow-up*
AIS/ASCUS	2 (22.2)[a]
AIS	2 (100)
AIS	7 (77.8)
AIS	5 (71.4)
AIS/SIL	1 (14.3)
LGSIL	1 (14.3)
Total	*9 (9.3)*

[a]Values in parentheses indicate percentages.

Table VIII. Conventional AIS and Conventional Adenocarcinoma

Cytology	*Histo/cyto follow-up*
Conventional AIS	11 (4)[a]
AIS/HGSIL	2 (18.2)
AIS/HGSIL	2 (100)
AIS/ASCUS	2 (18.2)
AIS/HGSIL	2 (100)
AIS	7 (63.6)
HGSIL	1 (14.3)
AIS	3 (42.8)
AIS/HGSIL	2 (28.0)
NAD	1
Conventional adenocarcinoma	10 (3.7)
EndoCx adenocarcinoma	2
Endometrial adenocarcinoma	2
Metastatic	4
AIS/HGSIL	1
NAD-inflammatory	1

[a]Values in parentheses indicate percentages.

Table IX. ThinPrep AIS and ThinPrep Adenocarcinoma, NOS

Cytology	*Histo/cyto follow-up*
AIS	6 (6.2)[a]
AIS (ASCUS)	1 (16.7)
HGSIL	1
AIS	5 (83.3)
AIS	3
Adenocarcinoma	2
Adenocarcinoma	5 (5.2)
Adenocarcinoma	3
SCC	1
AIS	1

[a]Values in parentheses indicate percentages.

Smears from patients with a histological diagnosis of AIS, endocervical adenocarcinoma or endometrial adenocarcinoma in the same timeframe, were retrieved. There were six patients identified, four of whom had positive smears and were included in the aforementioned retrieval; the remaining two had negative smears, reviewed and confirmed (true negatives) and were excluded from the total numbers.

Smears were collected by practice nurses, general physicians or specialist gynaecologists. For CPS, material was collected with cytobrush/wooden spatula or cervibroom, transferred onto glass slides, fixed with an alcohol-based preservative and submitted to the laboratory. For liquid base, the material was collected with a plastic spatula and brush and transferred into a vial containing PreservCyt. The gold standards were histology, which included biopsies, endometrial curettings, pipelle and Lletz excision biopsies, and follow-up cytology, depending on the recommended guidelines as set down by the New Zealand National Cervical Screening Registry.

These reports retrieved from computer database of the laboratory formed the study material. As part of the routine cyto/histo correlation, all cytology slides are reviewed when there is discordance with histology. We have not changed the original cytological diagnosis, as we are attempting to assess our screening performance.

Statistical analysis included calculation of the PPV, which is defined as the proportion of positive test results that are true positives; an attempt at assessing sensitivity, which is the proportion of patients with the disease who have a positive test result, and specificity, which is the proportion of patients without the disease who have a negative test result.

Table X. Conventional AGUS/Endometrial Carcinoma

Cytology	*Histo/cyto follow-up*
Endometrial (NOS)	11
Endometrial adenocarcinoma	1
NAD	10
Endometrial adenocarcinoma	14
Endometrial adenocarcinoma	9
Metastatic	2
NAD	3
Total	*25 (9.2)*

Table XI. ThinPrep AGUS/Endometrial Carcinoma

Cytology	*Histo/cyto follow-up*
Endometrial (NOS)	8
Endometrial adenocarcinoma	1
HGSIL	1
NAD	6
Adenocarcinoma	1
Complex hyperplasia with atypia	1
Total	*9 (9.3)*

Results

The number of smears processed in the 3-yr period from October 2000 to October 2003 totalled 400,184 (293,935 CPS and 106,249 TP). A total of 369 (0.09%) patients comprised the study group, 272 CPS and 97 TP smears (Table I).

The diagnostic category with the largest number was AGUS-NOS, comprising 121 CPS (Table II) and 51 TP (Table III) abnormalities. Concomitant squamous abnormalities were identified in 82 CPS (51 high-grade SIL and 31 ASCUS), and 30 in the TP group (22 high-grade SIL and 8 ASCUS). Histological correlation supports a concordance rate of 61% in CPS group (50/82) (61%). Only a small number (2/82) had a pure glandular abnormality (2.4%) and an additional few (6/82) had dual abnormalities (7.32%). In the TP group, concordance with squamous abnormality numbered 20/30 (a concordance rate of 66%) and pure glandular 5/30 (16.1%). In smears with abnormal glandular cells only (AGUS-NOS), significant abnormality was detected on histology in 8/39 CPS (PPV = 20.5%) and 10/21 TP (PPV = 47.6%) (P value = 0.032).

AGUS-FR comprised 86 CPS and 17 TP (Tables IV and V). Approximately 30% of CPS (26/86) and 23.5% of TP (4/17) had associated squamous cytological abnormalities, 6/26 (23%) CPS and 2/4 (20%) TP were confirmed with a

squamous abnormality on histology. The remaining 20/26 (76.9%) CPS and 12/17 (70.5%) TP revealed normal or inflammatory histology. Of the 60 CPS with pure AGUS-FR, 5 had high-grade and 5 had low-grade lesions (PPV = 66%). The remaining (50/60) CPS and (10/13) TP, making up the major proportion, were normal or inflammatory.

In the AGUS-FN category (Tables VI and VII), a good concordance of both glandular and squamous lesions was achieved. Of 19 within the CPS group, 13 (PPV = 68.4%) and 8/9 (PPV = 88.8%) TP had significant abnormality on histology. There was almost 100% concordance with pure glandular neoplastic diagnosis, histological lesions confirmed in all but one CPS smear.

The diagnosis of AIS numbered 17 (11 CPS and 6 TP), (Tables VIII and IX). Histology was available in all patients, confirming 9/11 CPS with AIS, 1/11 HGSIL and only 1 with no abnormality (95% concordance). The 6 TP had significant pathology on histology—3 AIS, 2 invasive adenocarcinoma and 1 HGSIL (100% concordance).

Diagnosis of invasive endocervical adenocarcinoma had similar concordance—9/10 CPS and 5/5 TP with significant lesions on histology.

Identification of AGUS (probably endometrial) resulted in 25 CPS and 9 TP. Significant pathology was confirmed histologically in 12/25 CPS and 3/9 TP (Table X and XI).

Of a total of 26 neoplastic pure glandular conventional cytology, 17 had histological confirmation of a glandular lesion and 6 had a squamous lesion. Within the TP group, 14 of 17 were confirmed with a glandular neoplasm and 3 with significant squamous lesion.

Discussion

There are numerous studies comparing the sensitivity and specificity of liquid-based preparations to CPS smears. These studies vary greatly in patient population, screening versus diagnostic, low risk versus high risk, gold standard as cytology versus histology, etc. Statistical comparison of studies is therefore limited and difficult. Abulafia et al.[3] in 2003 published a quantitative survey of studies of accuracy of TP versus CPS. A total of 27 studies (17 comparing cytology and 10 using histology as gold standard) were examined and a conclusion of 89% concordance between cytology was calculated. There was greater sensitivity and, to a lesser extent, specificity with TP over CPS. These studies focused on squamous lesions predominantly.

The identification of glandular lesions has played a lesser role in smear diagnosis. This is due to a number of factors—Pap smear was originally designed to identify squamous abnormalities, the number of glandular abnormalities is fewer, there is great inter-observer variation in deciding the origin of glandular cells (endocervical endometrial or metastatic) and there are no defined precursor lesions as for squamous malignancy.

In recent years, there has been a shift of attention to glandular lesions because of an absolute increase in glandular abnormalities,[9] more expertise in recognising and confirming glandular abnormalities and more treatment options. Simsur A et al.[10] showed no consensus among observers in both the origin of cells (endometrial versus endocervical) and in diagnosis (κ score < 0.4). At the same time, there are studies showing that the presence of AGCs in smears is associated with a higher incidence of significant pathology,[11–13] indicating that closer surveillance or more aggressive management is warranted. Earlier studies have shown a higher pick up of significant lesions in AGUS-FN over AGUS-FR, but this has not been substantiated in more recent studies[13] (Figs. C-1a and b). This, together with the higher incidence of significant abnormalities identified on follow-up, prompted the change in Bethesda to AGC in 2001. AGCs are diagnosed more accurately when the slides are evaluated by experienced cytologists and cytopathologists.[14]

There are only a few published studies on the comparison of diagnosis of glandular abnormalities with TP and CPS smears. The bigger studies are hospital-based and serve high-risk populations.[4–6] In all of these studies, as in the present one, the absolute numbers are small. From the literature, the overall sensitivity is increased with TP and there appears to be a greater specificity. Wang et al.,[15] also in a hospital-based study, is the only one to show no difference in specificity between TP and CPS, but highlighted a higher pick-up rate of glandular pathology in smears diagnosed as AGUS-FN.

Several studies examine the accuracy of diagnosing endometrial adenocarcinoma with both methods,[8,16] concluding that the TP contributed to an increase in the detection of endometrial adenocarcinoma.

Abnormal glandular lesions formed a small percentage of the total number of smears in our population (0.09%). There was similar overall incidence of glandular abnormalities detected with CPS (0.09%) and TP (0.09%) in the study period. Significant lesions were histologically confirmed in 116/272 CPS (PPV = 42.6%) and 58/97 TP (PPV = 59.8%) ($P = 0.003$ proportions are significantly different). Pure glandular abnormalities numbered 125 CPS and 51 TP and significant histological lesions were confirmed in 36/125 CPS (PPV = 28.8%) and 26/51 TP (PPV = 50.9%) ($P = 0.006$ proportions are significantly different), supporting an increase in sensitivity. This was due largely to a decrease in the numbers within the AGUS-FR group (60/272 CPS, 22% versus 13/97 TP, 13%). Our results also support the literature which favours a higher incidence of significant pathology in the category of AGUS-FN over AGUS-FR. In the AGUS-FN group 14/19 CPS and 8/9 TP had significant lesions confirmed on biopsy, whereas only 8/86 CPS and 2/17 TP had confirmed significant lesions in the AGUS-FR group. A breakdown of the significant lesions, focussing on glandular

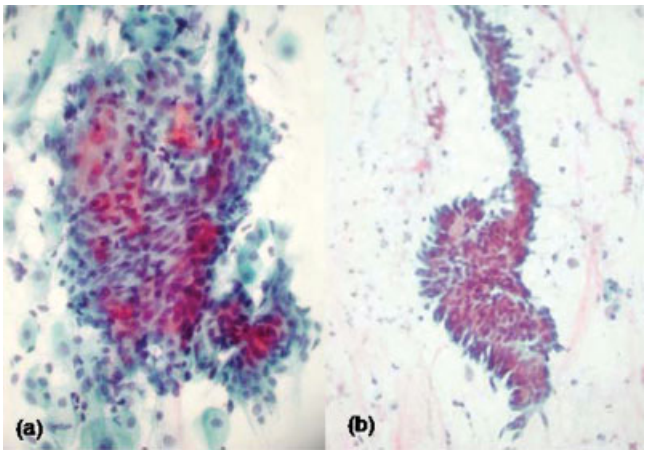

Fig. C-1. Photomicrographs of reactive endocervical cells (a) and AIS (b) on TP.

abnormalities alone, confirms a higher specificity—65% for CPS (17/26) and 82% for TP (14/17).

Endometrial cells are frequently identified in smears and the criteria within CPS are well documented. However, if there are clinical indications in post-menopausal women (>50 yr), follow-up is recommended. With the liquid-based preparations there is more optimal fixation, and endometrial cells appear larger with open nuclei and nucleoli (Figs. C-2a and b). There needed to be a shift in the criteria for atypia within endometrial cells on TP. A learning curve emerged in our laboratory as evidenced by the following finding. AGUS, probably endometrials, numbered 8 in the 2000–2003 period, 2 of which showed significant histology. In the 2003–2005 period, 4 AGUS, probably endometrials, were diagnosed, two of which then showed significant histology.

Screening times for TP are similar to CPS with well-trained cytologists. There is a longer learning curve with glandular lesions, mainly due to small numbers and less experience. A good clinical history, age of patient and a high level of awareness is helpful in accurate diagnosis.

Detailed statistical analysis was limited for several reasons, and the numbers of glandular lesions in proportion to the total number of smears is small and the number of liquid-based samples constitutes an even smaller number. The comparison stats utilised the Fisher exact tests and, with the individual groups, the limitation of small numbers have to be considered.

There was a statistically significant difference in the overall concordance (116/272 CPS and 58/97 TP), P value = 0.003. This could partly be explained on the squamous component of the smears and histology concordance. However, selecting out the pure glandular lesions on cytology also produced statistically significant results (36/125 CPS and 26/51 TP), P value = 0.006, suggesting that the histo/cyto correlation of significant lesions is better with TP than

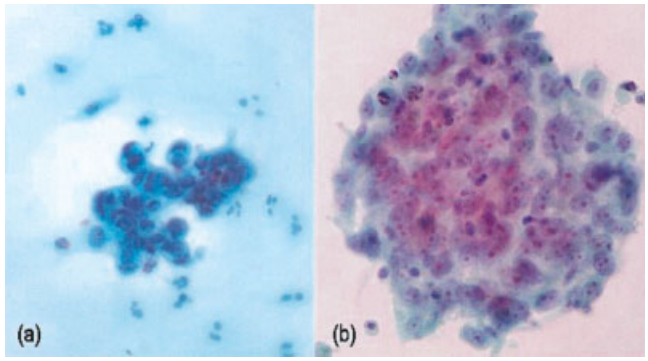

Fig. C-2. Photomicrographs of normal endometrial cells (a) and endometrial carcinoma (b) on TP.

with CPS. Since there is a choice bias of TP over CPS at the initial level, we make an assumption that P (disease given the women chose TP) is the same as P (disease given the women chose CPS) then we can compare sensitivity. The stats can suggest a more sensitive test on the calculated ratio even if we cannot quote the exact level of sensitivity. Calculation shows a ratio of 0.14 (<1), suggesting that TP is more sensitive than CPS.

Conclusion

Even with several limitations, which include patient bias, small numbers of liquid-based samples and small numbers of glandular lesions, we have shown a higher PPV with TP and an increased specificity and sensitivity. Interpretation of glandular lesions on liquid-based samples is a learning curve, improving with experience and teaching.

A longer study period to retrieve larger numbers of glandular abnormalities is desirable.

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