ORIGINAL ARTICLE

Characteristics of false-negative ThinPrep cervical smears in women with high-grade squamous intraepithelial lesions

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Objective To examine the cellular characteristics and other possible factors affecting the correct prediction of ThinPrep cervical smears from patients with high-grade squamous intraepithelial lesions.

Design Retrospective study.

Setting Private anatomical pathology service, Hong Kong.

Patients Patients (n=98) with biopsy-confirmed high-grade squamous intraepithelial lesion of the uterine cervix encountered between 2004 and 2006.

Main outcome measures Correct prediction of the cervical ThinPrep Papanicolaou tests.

Results There were no significant differences in age, hormonal status, degree of inflammation, and the presence or absence of endocervical/metaplastic cells between the true-positive and false-negative groups. There was a significant difference in the number of abnormal cells present and the screening time between the two groups. Approximately 79% (34/43) false-negative smears had 100 or less abnormal cells, while only 11% (5/45) true-positive smears had 100 or less abnormal cells (P<0.001). The true-positive smears were also more likely to contain koilocytic cells and abnormal cells with hyperchromatic nuclei.

Conclusions The number of abnormal cells present, the presence or absence of koilocytic cells, and the presence or absence of abnormal cells with nuclear hyperchromasia appeared to be independent factors affecting the correct prediction of smears from patients with high-grade squamous intraepithelial lesions.

Introduction

The Papanicolaou (Pap) test has proved to be the most effective cancer-screening test ever developed. However, there is a limit to the sensitivity of detecting abnormal cells present in a smear. Studies have shown that some high-grade squamous intraepithelial lesion (HSIL) smears were more likely to be reported incorrectly as negative than others.[1-4] These false-negative (FN) Pap smears usually had fewer abnormal cells, had lesional cells with fine nuclear chromatin (pale dyskaryosis), were small, and sometimes constituted fewer than 25% of the cells on the smear. The use of liquid-based Pap tests, such as the ThinPrep Pap test, resulted in statistically significant improvements in both diagnostic yield and specimen adequacy,[5] but more recent meta-analyses indicated that there may be no difference between the two tests.[6-8] Additionally, the use of a computerised imager with ThinPrep Pap tests appeared to yield more HSIL than manual screening.[7,8] It is therefore important to study the characteristics of FN liquid-based HSIL smears and determine whether the factors affecting the accuracy of conventional smears in this regard also apply.

Our laboratory reports on over 80 000, mostly liquid-based Pap smears per year. The patients were referred to us from general practitioners and gynaecologists in private practice in Hong Kong. We used the Bethesda system (2001) for the interpretation of cervical smears. In 2006, we encountered a low-grade squamous intraepithelial lesion (LSIL) rate of 0.0167, an ASCUS (atypical squamous cell of undetermined significance) rate of 0.0168, an HSIL rate of 0.0046, and an ASC-H (atypical squamous cells cannot rule out high-grade lesion) rate of 0.00148. Of all the cases with follow-up biopsy results available

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for correlation, 82% of LSIL smears were confirmed by biopsy, as were 77% of HSIL smears.

Methods

As a standard internal quality control protocol, all biopsy-proven HSIL cases that had yielded negative smears within 30 months prior to the biopsy were reviewed.[9] Between 2004 and 2006, 166 such cases reported as 'negative for intraepithelial lesion or malignancy' were identified. Specimens that were reported as ASCUS, ASC-H, and LSIL were not included. Upon review, 43 of them revealed the presence of dysplastic cells that were either missed or misinterpreted during initial screening, and constituted the FN group. The control true-positive (TP) smears reported by us had been accessed in our laboratory in 2006, and were from consecutive patients with biopsy-proven HSIL lesions. The age of the patients, the degree of inflammation, the presence or absence of endocervical/metaplastic cells, and the screening time for each case were retrieved from computer records. The degree of inflammation was graded semi-quantitatively into absent, mild, moderate, and severe. The number of abnormal cells and the number of abnormal cell clusters in each smear were counted manually. Abnormal cell cluster was defined as aggregates of 15 or more abnormal cells.[10] We also examined the nuclear size and nuclear staining property of the lesional cells. Increased nuclear size was defined as two times or more the size of normal intermediate cell nuclei in the same smear. Nuclear hyperchromasia was defined as visibly darker staining of the lesional cell nuclei, as compared to intermediate cell nuclei in the same smear. Koilocytic cells were squamous cells with enlarged, irregular, and hyperchromatic nuclei, surrounded by a peri-nuclear halo.

Results

Between the years 2004 and 2006, there were a total of 166 biopsy-proven HSIL lesions with negative Pap smears obtained 30 months or less prior to the biopsy. Upon review of these FN smears, 123 did not show any dysplastic cells even with hindsight and diligent searching and were believed to be due to sampling errors. Forty-three (26%) smears, however, showed the presence of dysplastic cells that were either missed (in 39 patients, accounting for 91%), or misinterpreted during the initial screening process (in four patients, or 9%). There were no significant differences in age, degree of inflammation, nuclear size of lesional cells, and the presence or absence of endocervical/metaplastic cells between the TP and FN groups (Table). The mean screening time was significantly longer in the TP than in the FN group. Koilocytes were more frequently present in the former. Smears that were TP were also more likely to contain lesional cells with hyperchromatic nuclei. There was a significant difference in the total number of abnormal cells and number of abnormal cell clusters between the two groups. Specifically, 86% (37/43) having FN smears contained two or less

出現高度鱗狀上皮內病變的女性接受液基薄層細胞學製片作宮頸塗片檢測結果呈假陰性的特點

目的 對出現高度鱗狀上皮內病變的病人所接受的液基薄層細胞學製片宮頸塗片檢測，檢視影響能否得出正確預測結果的細胞特點及其他可能影響結果的因素。

設計 回顧研究。

安排 香港私營的病理解剖科服務。

患者 2004至2006年期間，98位經細胞切片檢查確定在宮頸出現高度鱗狀上皮內病變的病人。

主要結果測量 液基薄層宮頸塗片（帕帕尼科尼拉烏試驗）的正確預測。

結果 在結果為真陽性和結果為假陰性兩組之間，在年齡、激素水平、炎症程度，以及有否子宮頸內膜細胞/轉化性細胞是否存在，都沒有顯著分別。但在異常細胞的數目上和篩查時間上，兩組則有明顯差異。異常細胞數量在100或以下的，在假陰性一組中佔約79% (34/43)，而真陽性一組則只有11% (5/45) (P<0.001)。真陽性一組中，較多含營養不良細胞，以及含濃染細胞核的異常細胞。

結論 異常細胞的數量、營養不良細胞以及發生核濃染的異常細胞是否存在，似乎是影響高度鱗狀上皮內病變的病人塗片正確檢測的獨立因子。

TABLE. Comparison of patients with false-negative (FN) and true-positive (TP) high-grade squamous intraepithelial lesion smears by liquid-based cervical cytology

	FN (n=43)	TP (n=45)	P value†
Mean (SD) screening time (seconds)	351 (75.6)	490 (133.5)	<0.001
Mean patient age (years)	37.8	36.1	0.394
Mean No. of abnormal cells per smear (slide)	111	600	<0.001
Mean No. of abnormal cell clusters per smear (slide)	1.37	7.02	<0.001
Proportion of smears with endocervical/metaplastic cell present	37 (86%)	43 (96%)	0.121
Proportion of smears with moderate-to-severe inflammation	9 (21%)	6 (13%)	0.344
Proportion of smears with hyperchromatic lesional cells	21 (49%)	33 (73%)	0.018
Proportion of smears having lesional cells with large nuclei*	10 (23%)	11 (24%)	0.896
Proportion of smears with koilocytes present	3 (7%)	23 (51%)	<0.001

* x2 or more the nuclear size of normal intermediate cells in the same smear

† Numerical values were analysed by two-sided *t* test, and proportional values by Chi squared tests

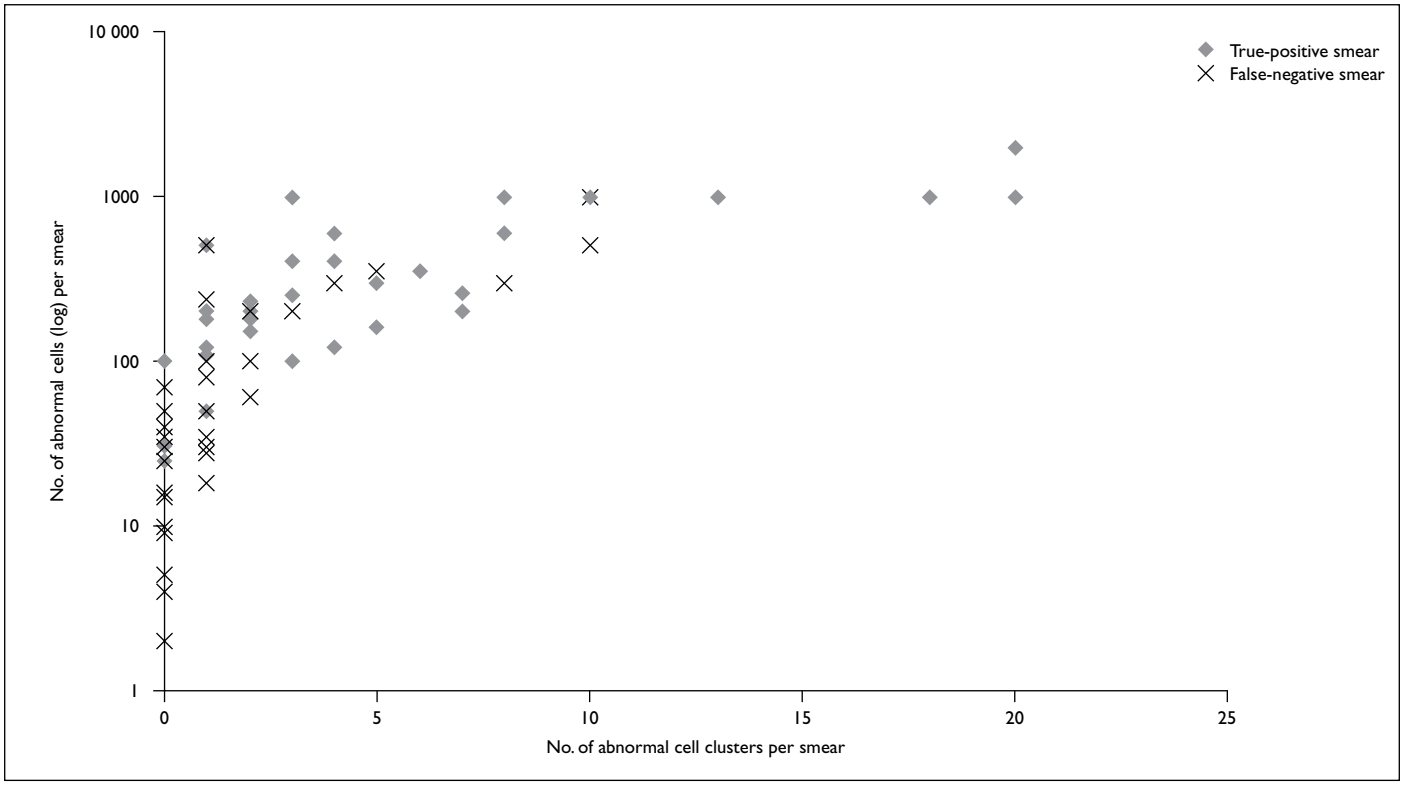

FIG. Number of abnormal cells and abnormal cell clusters in true-positive and false-negative smears

abnormal cell clusters on the slide, while only 29% (13/45) of the TP smears had two or less clusters (P<0.01). Also, 79% (34/43) of the FN smears had 100 or less abnormal cells on the slide, while only 11% (5/45) TP had 100 or less abnormal cells (P<0.01) [Fig]. Since the total number of abnormal cells was related to the number of abnormal cell clusters, multivariate analysis was also performed and showed that only the total number of abnormal cells was a significant independent factor affecting the correct reporting of these smears.

Discussion

In general these results were similar to previous studies on conventional Pap smears,[1-4] there being no significant differences between the two groups in terms of patient age, degree of inflammation, and the presence or absence of endocervical or metaplastic cells. We believe that the longer time spent on screening the smears in the TP group was a result of time spent on marking dots on the slides after abnormal cells were found rather than a factor affecting the chance of detecting abnormal cells. For obvious reasons, the abnormal cells were more easily detectable when a larger number of them were present. Similarly, darker staining nuclei also made the abnormal cells more easily visible. The association between the presence of koilocytes and TP can be understood for the same reason. Koilocytes not only have large and often hyperchromatic nuclei, but also have cytoplasmic halos, making them the easiest abnormal cells to spot. Once seen, the koilocytes draw the attention of screeners to watch out for more sinister lesions. This study and previous studies had pointed out that there is a certain minimum number of abnormal cells that needed to be present before HSIL lesions could be reliably detected by human screening. It has been shown that in conventional smears, FN reporting tended to be associated with fewer than 200 lesional cells.[4] The current study showed that we had a high detection rate, when more than 100 abnormal cells (40/49, 82%) were present. A previous study also pointed out that the presence of abnormal cells in groups may hinder correct interpretation, since such cells were difficult to visualise and could be confused with other cell types such as endometrial cells and endocervical cells with tubal metaplasia.[10] We did not find cellular clusters to be an independent factor affecting the correct diagnosis. The number of abnormal cellular clusters was positively related to the total number of lesional cells; when more cellular clusters were present, more singly dispersed lesional cells could also be seen.

The Food and Drug Administration has approved several devices that use computerised image analysis to assist screening by cytotechnologists. There have been studies indicating that the computer-

assisted screening has the potential to allow the cytotechnologists to detect more disease and reduce the FN rate. Diagnostic rates for all categories, including atypical squamous cells of undetermined significance, cannot rule out HSIL, and the detection rates of LSIL and HSIL were increased.[11-13] However, it is doubtful whether computer-assisted screening can increase detection rate when no lesional cells are present, which was the case in the majority of our negative smears. Similarly, computerisation was unlikely to help when misinterpretation was the cause of FN reporting, although our experience showed that misinterpretation contributed minimally to FN reporting. That leaves only the cases with lesional cells present, but missed because the numbers were small. A study has shown that computer-assisted screening could decrease the FN rate of HSIL lesions by 20%,[11] thus a significant number of cases we missed would very likely have been picked up. However, only physicians, patients, and the society can determine whether the increased cost incurred in computer-assisted screening is justified.

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