Evaluation of Papanicolaou Smear and Effect of Sample Biopsy in Follow-up of Cervical Dysplasia

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SUMMARY

In a prospective study of women with dysplasia, a superficial sampling biopsy of the cervix was carried out as a periodic check of the cytological findings. The relationship of smear and biopsy results was evaluated, as well as the effect of the biopsy procedure on the subsequent course of dysplasia. We found sufficiently close agreement between smear and biopsy results to conclude that smears and biopsies measure similar aspects of dysplasia. It would appear unwise, however, to rely on Papanicolaou smears exclusively in following women with a history of dysplasia, since negative smears in such women may occasionally show dysplasia in the corresponding biopsy. Periodic corroborative biopsy procedures are therefore indicated in the follow-up care of women with a history of dysplasia. There was no evidence that a superficial sampling biopsy significantly altered the short-term course of dysplasia. There was also no evidence of a cumulative effect of repeated sampling biopsies. These results do not rule out possible effects of other forms of biopsy procedures and schedules on the subsequent course of dysplasia.

INTRODUCTION

The lesions dysplasia and preinvasive cancer of the cervix were first recognized when abnormal cell patterns, detected on Papanicolaou (Pap) smears, were correlated with histopathological changes in the cervix consistent with neoplasia but confined to the mucosa. The in situ change was accepted as an early stage in the pathogenesis of cancer of the cervix, and dysplasia was considered to be a cancer precursor (4). This concept of the natural history of cancer of the cervix carried with it the implication that the Pap smear could be an effective screening test for cancer of the cervix, detecting the disease at an early preinvasive stage when appropriate intervention can effect a cure.

This expectation was expressed by Papanicolaou as follows: "The recently acquired evidence of the existence of distinctive cytologic patterns in the early developmental stages of cervical carcinoma, in contrast to those of the more advanced stages, has provided more sensitive criteria for the diagnosis of early cancer, and possibly a means of prognosis depending on the prevailing cytologic pattern" (6).

Progression from dysplasia to cancer in situ has been reported (1, 12), and it is widely accepted that cancer in situ progresses to the invasive stage. However, the course of dysplasia as a cancer precursor and the rate of progression of preinvasive cancer to the invasive and clinical stages of the disease have not yet been established (5).

The natural history of this disease could be further clarified by prospective study of women with dysplasia kept under observation and tested at regular intervals by means of Pap smears. Because of the limitations of the test, provision for periodic biopsy validation of the cytological findings is desirable. However, if corroborating biopsy is performed, there is the question of altering the very process we wish to observe. Richart (7) has stressed the importance of avoiding biopsy procedures in the prospective study of women with dysplasia. It seems clear, for example, that cone biopsy would almost certainly eradicate dysplasia and that directed biopsy aided by use of the colposcope would also be very likely to excise the entire lesion under view.

In contrast, in this study, we used a multiple, superficial punch biopsy procedure as a periodic check of the cytological findings in a long-term study of women with dysplasia, because of our concern about possible false-negative smear tests. Follow-up of these women provided an opportunity to assess the effect of the biopsy procedure on the subsequent course of the lesion.

As a preliminary task, the relationship of smear and biopsy results was studied over the various degrees of severity of dysplasia and for cancer in situ. We also developed information on the comparability of smear and biopsy results for epithelial modifications in the negative range: squamous metaplasia, cellular atypia, and changes bordering on, but not yet, dysplasia.

MATERIALS AND METHODS

The data base utilized in the analysis is from the prospective study of the effects of an OC on the progress of dysplasia (10). The women with dysplasia were detected by Pap smear screening of subjects, ages 15 to 35, reporting for an initial visit to community family planning clinics. Women whose smears showed dysplasia were followed in a...
special dysplasia clinic. About 65% of the women in the follow-up program used an OC, and the remainder used other methods, mainly the IUD. The women were examined and Pap smears were obtained at regular 6-month intervals after the index test. Biopsies were taken usually after 1 year of follow-up, and annually thereafter. When smear and/or biopsy findings indicated progression to cancer in situ, the patient was referred for diagnostic work-up and treatment. Pathological reports were requested and slides were obtained for comparing this final diagnosis with our findings.

The results used in this report pertain to women whose initial smear was within the dysplasia range. However, a sizable proportion of patients with an initial diagnosis of dysplasia later regressed to the negative range. The patients whose later Pap smears were negative were also biopsied annually in accordance with the protocol. It is thus possible to correlate biopsy and smear findings in women with smears in the negative range, as well as in women with dysplasia and cancer.

Smears are prepared by scraping the squamocolumnar junction of the cervix, using the Ayre spatula. All biopsies were performed by 1 gynecologist as an out patient procedure. Following endocervical curettage, Schiller's iodine test is applied to the cervix. Small, superficial punch biopsies are taken around the circumference of the cervix at the squamocolumnar junction, and from unstained areas. This procedure provides multiple small samples of cervical mucosa. The method of obtaining colposcopically directed punch biopsies from areas with abnormal colposcopic change was not used in our dysplasia clinic. Colposcopically directed biopsy and cone biopsy procedures were carried out when patients were referred for further diagnostic work-up, and provide a means of correlating results of our sampling biopsy with the results of other diagnostic procedures.

In the laboratory, all slides, including those from previous visits, are screened without identification. Slides from previous visits are reread to ensure reliability and to guard against a possible long-term trend in ratings. Slides are reviewed and assigned to a diagnosis by 1 of the authors (E. S.). Smears are read and classified without knowledge of the biopsy result, and conversely.

The classification used is shown in Table 1. Included under cancer were microinvasive and invasive cancer. The results of blind replicate readings of smears by the single observer were available for an analysis of reliability. The reliability was found to be high (11), an indication that the diagnostic categories could be distinguished. A numerical score was applied to the diagnostic categories (see Table 1). In this way, cases could be combined and compared more readily. Our criteria for the diagnostic categories and illustrative examples have been published (9).

In prospective studies of carcinogenesis, it would seem important to include observations over the entire continuum of epithelial change, including epithelial modifications in the negative range. For example, abnormal surface changes in cells from cervical neoplasia have been reported in studies using the electron microscope (3). Williams et al. (13) note that "the stage in the progression from normality through dysplasia to cancer in situ, at which cells lose the ability to form microvilli, is unknown." We found the classification system and numerical scale to be reliable research tools in staging progression from normality through dysplasia to cancer in situ and useful in studying the effect of biopsy on the course of dysplasia.

RESULTS

The study of the relationship between smear and biopsy classification is presented first. We then test the hypothesis that the multiple, superficial sampling biopsy influences the course of dysplasia.

Relation between Smear and Biopsy. Both a smear and a biopsy reading were obtained from a total of 252 patient visits, representing 130 individual patients. The frequency distribution of categories and their corresponding scale values are shown in Table 1. The mean score for smears was 24.8 ± 13.1 (S.D.). The mean score for the biopsies was about 5 units higher, 29.9 ± 16.5. The mean of the difference between smear and biopsy scores was significant at p < 0.01. The subject variability is greater when measured by biopsy than by smear.

The association of smear and biopsy diagnosis is shown in Table 2. The overall relationship is fairly high, with a correlation coefficient of 0.70. It must be remembered, however, that a correlation coefficient (r) of 0.70 means that only 49% (%r²) of the variance in the smear scores is accounted for by their relation to the biopsy scores, leaving considerable room for differences between smear and biopsy scores.

A question of interest was whether the presence of an IUD, and in particular the thread protruding through the cervical os, would inhibit the biopsy procedure and thus affect the smear-biopsy relationship. To explore this problem, a linear regression analysis was performed on the 68 patient visits for patients using the IUD and, separately, on the 184 patient visits for patients using OC. The regression coefficients obtained in the 2 analyses were statistically tested for equality. No evidence of difference in the coefficient was found; therefore, under the linear regres-

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Table 1

<table>
<thead>
<tr>
<th>Numerical scale</th>
<th>Diagnostic categories</th>
<th>Smears</th>
<th>Biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Negative range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>6.7</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>89</td>
<td>35.3</td>
<td>61</td>
</tr>
<tr>
<td>25</td>
<td>59</td>
<td>23.4</td>
<td>59</td>
</tr>
<tr>
<td>Dysplasia range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>56</td>
<td>22.2</td>
<td>43</td>
</tr>
<tr>
<td>45</td>
<td>12</td>
<td>4.8</td>
<td>28</td>
</tr>
<tr>
<td>55</td>
<td>13</td>
<td>5.2</td>
<td>32</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>1.6</td>
<td>7</td>
</tr>
<tr>
<td>65</td>
<td>2</td>
<td>0.8</td>
<td>5</td>
</tr>
</tbody>
</table>

252 100.0 252 100.0
sion model, we conclude that the relationship between smear and biopsy scores is essentially the same under the IUD use and the use of OC. The correlation coefficients between smear and biopsy were also identical under the 2 contraceptive methods (r 0.70), the same value as when the methods were combined. The length of time for which the contraceptive methods were used did not affect the smear-biopsy relationship for either method of contraception.

An estimate of smear-biopsy disagreement for broad diagnostic groups may be of more interest clinically. We therefore collapsed our classification into 3 major diagnostic groups: the negative range (low); those with borderline and minimal dysplasia (intermediate); and those with mild, moderate, and severe dysplasia (high).

It is apparent from Table 3 that smear and biopsy agree in 143 (56.8%) of the 252 comparisons when the broad categories are used. We wish to test the hypothesis that the disagreements are symmetrical. The χ²-test for symmetry was performed (2), and the hypothesis was rejected at the level of significance, p < 0.01. Among the 106 smears in the low range, 7 of the corresponding biopsies were in the high range. Of 31 smears read in the high range, only 1 biopsy was low. Conversely, among 78 cases in which the biopsy score was low, 1 smear was high; and for 72 cases in which the biopsy reading was high, 7 smears were low. Thus, a low reading on a smear coupled with a high reading on a biopsy is more common than a high reading on a smear coupled with a low reading on a biopsy.

In this special population, and based on our definition of 3 broad categories of smears, Table 3 indicates that the probability of the biopsy having a higher reading than the smear is 34%, while the probability of the smear having a higher reading than the biopsy is only 9%. However, only 3.2% of the smear-biopsy pairs show disagreements of more than 1 broad category, i.e., large discrepancies between smear and biopsy are relatively rare. These results are comparable to those of Shingleton et al. (8), who correlated cervical smears with colposcopy-directed biopsy.

Agreement at the upper rather than the lower range of the scale is of more concern clinically. The close correspondence of smear scores in the cancer range with the biopsy findings is demonstrated as follows. There were 16 cases with smears suggestive of cancer in situ, on which concurrent biopsies were obtained. Of the 16 smear-biopsy pairs in which cancer in situ was indicated by the smear, 15 were confirmed as cancer in situ on biopsy, and in 1 the biopsy diagnosis was severe dysplasia.

Table 3
Association of concurrent smears and biopsies (collapsed categories)

<table>
<thead>
<tr>
<th>Smear</th>
<th>Intermedi-</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (negative)</td>
<td>56</td>
<td>43</td>
<td>106</td>
</tr>
<tr>
<td>Intermediate (minimal dysplasia group)</td>
<td>21</td>
<td>58</td>
<td>115</td>
</tr>
<tr>
<td>High (mild, moderate, and severe dysplasia)</td>
<td>1</td>
<td>29</td>
<td>31</td>
</tr>
</tbody>
</table>

Total no. 78 102 72 252

Effect of Biopsy on Subsequent Course of Dysplasia. Our follow-up protocol for dysplasia patients offered a possibility of studying the effect of biopsy on subsequent status, because it provided that the Pap smear taken at 6-month intervals be supplemented by annual biopsy.

Since all women who entered the study with dysplasia of the cervix were biopsied, there was no group without biopsy for comparison. One approach was to use each patient as her own control and to look for evidence of an abrupt change in the trend of dysplasia that could be related to the biopsy procedure. A 2nd approach was to study the cumulative effect of repeated biopsies, comparing patients with varying frequency of biopsy.

Rationale of Analysis for Studying Effect of 1 Biopsy. We assume that on the average there is a uniform trend in the progression or regression of dysplasia within a relatively short interval of time, such as a 12-month period. If this is so, we expect the trend of the average subject in the 1st 6 months to be the same as in the 2nd 6 months. In a 1-year interval, the trend may be represented schematically simply by a straight line (see Chart 1f). The difference between the scores B minus A, would be equal to the difference between the scores, C minus B. That is, (B − A) = (C − B); or (B − A) − (C − B) is zero.

However, if the biopsy removes the pathological material, the uniform trend in the 1-year interval would be disturbed...
because a lower reading would be obtained at B and again at C (see Chart 1/ii). The difference between scores B minus A will then not be equal to the difference between scores C minus B. That is, \((B - A) - (C - B)\) will be less than zero. This would also be true, even if the linear trend is downward, and whether the interruption of the linear trend is temporary or permanent. The difference of the differences \((B - A) - (C - B)\) and its S.E. can be computed and a t-test can be performed.

Since the trends from 12 to 24 months may differ between OC and IUD users, it is desirable to perform the computations separately for OC and IUD users, as well as for both methods combined.

**Effect of 1st Biopsy on Dysplasia Score.** Patients included in this analysis were those who had a 12-month follow-up smear and biopsy, as well as an 18- and 24-month follow-up smear. The protocol required adherence to within ±3 months of the scheduled time. Most of the observations were taken within ±1 month of the scheduled time. There were 61 patients who met the criteria for this analysis. The analysis yields no evidence that the biopsy had an effect on subsequent Pap smear scores. The mean difference in the period 12 to 18 months was not significantly different from the mean differences in the period 18 to 24 months, when all 61 cases were considered. The t value obtained was \(-0.07\) with \(p, 1\)-tailed, of 0.47. Results were similar when contraceptive method was taken into account. Similar results were obtained in the analysis of the 2nd biopsy.

**Effect of Any Biopsy on Subsequent Score: Cases Limited to Those with Dysplasia Present at Time of Scheduled Biopsy.** There was no restriction on the value of the score in the previous analysis. However, in this study, regression from dysplasia toward normality occurred in a large proportion of the women who had dysplasia at entry, and many of the scores at 12 and 24 months of follow-up were below the dysplasia level. It may be argued that scores in the negative range do not constitute appropriate material for the hypothesis under test. We therefore carried out an analysis in which the score at the time of the scheduled biopsy at 12, 24, or 36 months was at least minimal dysplasia. There were 37 biopsies of minimal dysplasia or more for which smear scores were available both 6 and 12 months after the biopsy. The results showed again that there was not a significantly greater drop in score within the 1st 6 months after the biopsy than in the subsequent 6 months. The mean difference score was \(-3.70 \pm 3.06\) (S.E.), yielding a t value of \(-1.21\) and a 1-tailed p of 0.12.

**Rationale of Analysis of Cumulative Effect of Repeated Biopsies.** If each biopsy has a chance of removing the pathological tissue, then the subjects with more biopsies should have had a greater chance of having the lesion removed and their final scores should be lower. This means that, other things being equal, there should be a negative correlation between the final score and the frequency of biopsy. Since the number of biopsies performed in our study is strongly related to length of follow-up, it seemed reasonable to express the frequency of biopsy also as mean interval between biopsies.

**Cumulative Effect of Repeated Biopsies.** This relationship was examined in all patients with at least 2 biopsies, using the following information: score at initial biopsy at 12 months, score at last biopsy, total number of biopsies, length of time elapsed from initial to last biopsy, and method of contraception. There were 95 patients with at least 2 biopsies; 59 of these had only 2 biopsies. The number of biopsies per patient ranged from 2 to 5, and the length of follow-up ranged from 6 to 52 months. Of the patients, 77% showed an average biopsy interval from 10 to 15 months. The mean biopsy interval was 13.7 ± 3.1 months (S.D.).

The correlations of final score with measures of frequency of biopsy are low and are not statistically significant at the 5% level: 0.13 with number of biopsies, and \(-0.14\) with biopsy interval. Since any dysplasia score for a given subject may tend to be positively related to scores obtained at other times, in particular to the initial score, it is desirable to take this initial score into account in the analysis. In addition, if there was an association between final score and use of the contraceptive, it was desirable to partial out method of contraception as well. The correlations are low after the initial score is taken into account (partialled out), as are also the correlations taking into account both initial score and contraceptive method. None of the correlations are statistically significant, and there is no evidence of an effect of biopsy frequency on subsequent score. The 3 sets of correlations are shown in Table 4. Examination of plots of residuals did not suggest the presence of more complex relationships between the variables considered.

An analogous analysis was carried out also for only those 48 individuals whose biopsy score at the time of 1st biopsy (12 months) was at least minimal dysplasia. This was done on the supposition that the effect of the biopsy may manifest itself only if dysplasia is histologically detected (see
L. Youkeles et al.

Table 4
Correlations with final dysplasia score: 95 cases

<table>
<thead>
<tr>
<th></th>
<th>Unconditional</th>
<th>Partial given initial score and contraceptive method</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of biopsies</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Biopsy interval</td>
<td>0.14</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 5
Correlations with final dysplasia score: 48 cases with biopsy at 12 months at least minimal dysplasia

<table>
<thead>
<tr>
<th></th>
<th>Unconditional</th>
<th>Partial given initial score and contraceptive method</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of biopsies</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td>Biopsy interval</td>
<td>0.17</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 5). Again, the results showed no evidence of a biopsy effect on subsequent dysplasia score.

The validity of the analysis depends to some extent on the assumption that the frequency of biopsy in the population from which this sample was drawn is independent of score at initial biopsy. Therefore, this relationship was studied and we found that the correlation between score at initial biopsy and mean length of interval between biopsies was −0.10, a value not statistically significant.

DISCUSSION

There is sufficiently close agreement between smear and biopsy results to conclude that smears and biopsies measure similar aspects of dysplasia. It would appear unwise, however, to rely on Pap smears exclusively in follow-up care of women with a history of dysplasia, since smears in such women may occasionally be negative, with the corresponding biopsy showing dysplasia. Periodic corroborative biopsy procedures are therefore indicated in the follow-up care of women with a history of dysplasia.

In the analysis of the possible effect of a biopsy on the course of dysplasia, the trend of cytological progression in 6-month periods that included a biopsy was compared to subsequent 6-month periods that did not include a biopsy. No significant difference in these trends was found. This result was found in the analysis of the 1st biopsy and of the 2nd biopsy, and was not affected by method of contraception. Also, an examination of the data restricted to patients who had dysplasia on the smear immediately preceding the biopsies taken at 12, 24, or 36 months, did not show an effect of biopsy on subsequent score.

The study of the cumulative effect of repeated biopsies yielded correlations of number of biopsies and biopsy interval with final diagnosis that were not significant. The correlations were small and not significant also, after taking into account initial score, and both initial score and contraceptive method. A similar analysis restricted to those subjects with scores above the normal range at 12 months also did not yield significant correlations or partial correlations.

These results do not rule out possible effects of other forms of biopsy procedures and schedules. We do not see any significant evidence that our practice of annual sampling biopsy alters the course of dysplasia.

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