Abstract. Aim: To compare two management strategies for cervical intraepithelial neoplasia (CIN) 2, and evaluate reproducibility of the diagnosis. Patients and Methods: Ninety (90) women with biopsy-proven CIN2 diagnosed through the Brazilian public health service were randomly allocated into two groups: 45 in prospective follow-up without treatment, and 45 for radical loop electrosurgical excision procedure (LLETZ). As in the real-life situation, pathology-reviewed diagnoses and HPV genotypes were not available. Results: Excision of the lesion proved to be more effective than prospective follow-up in reaching clearance of CIN2 (hazard ratio=3.66; 95% confidence interval 2.02-6.64). However, 44.1% of the lesions regressed without treatment during the 12-month follow-up. Conclusion: CIN2 lesions regress without treatment in one year, although an ablative procedure is more effective. However, excision of CIN2 may lead to additional morbidity and costs, and tailoring the management on an individual basis may result in better outcome. Misclassification of CIN2 is not a negligible problem.

Cervical intraepithelial neoplasia (CIN) grade 2 has been listed as a precursor of cervical cancer, despite the fact that the literature covering studies published between 1950 and 1992 showed that 43% of such lesions regress spontaneously, 35% persist, 22% progress to CIN3 and only 5% to invasive cancer (1). In fact, CIN2 has a biological behavior intermediate between CIN1 and CIN3 but the natural history of CIN2 is closer to that of CIN1 (2). However, the distinction between the different grades of CIN is subjective and only modestly reproducible among pathologists. For CIN1 and CIN3, the diagnoses seem consistent but the biological significance of CIN2 is much more ambiguous because these lesions could represent a mixture of human papillomavirus (HPV) infections, i.e. an intermediate step in the transition from HPV infection to cervical cancer or simply a result of misclassification of by the pathologist (3).

Despite these problems in diagnosis of CIN2, histological diagnosis of CIN2 and CIN3 have similar clinical importance in many countries; both diagnoses usually trigger surgical or ablative treatment (4). The recommendation to excise all CIN2 lesions may cause unnecessary morbidity and, because of this, CIN2 diagnosis and treatment decisions are problematic for pathologists and gynecologists, respectively.

Optimally, quality control (i.e. pathology-reviewed diagnosis), HPV genotyping and use of biomarkers (such as p16) would help gynecologists in their treatment decisions. However, in many developing countries and under normal conditions, these adjunct tools are not available in public health services. In the present study, we simulated the daily routines at Brazilian public health services and evaluated two management options for biopsy-proven CIN2: i) excision and ii) conservative follow-up. We monitored evolution of the lesions during a one-year follow-up and considered the reproducibility of the diagnoses.

Patients and Methods

The present randomized comparative study was carried out at the Leonor Mendes de Barros Maternity Hospital, State Secretariat of Health, São Paulo, Brazil. The research protocol was approved by the Institutional Review Board. The women were selected from among the patients referred for colposcopy due to abnormal Pap smear, and who had no previous excision of their cervical lesion. Criteria for inclusion in the study were: i) complete visibility of the...
abnormal colposcopic features; and ii) diagnosis of CIN2 in the biopsy. Under normal conditions, HPV test and marker analysis are not used in the Brazilian public health services.

Women were randomly allocated into two groups: i) prospective follow-up (FU) without treatment, or ii) prompt radical loop electrosurgical excision procedure (LLETZ) of the lesion. Of the 90 women included in this analysis, 45 were allocated to the FU arm and 45 to the LLETZ arm.

Specimens obtained from colposcopy-directed biopsies or in surgery were fixed in formalin, embedded in paraffin, and cut into 4-μm-thick hematoxylin-eosin (HE)-stained sections according to routine procedures. The histological diagnosis of CIN2 was confirmed in all cases. As under normal circumstances in Brazilian public health care, the choice of pathologist was blinded, i.e. the gynecologists do not know the pathologists. The CIN2 diagnosis of each patient was analyzed by only one pathologist and the diagnosis of all 90 women was analyzed by the same number of distinct pathologists. The initial CIN classification was accepted without revision.

These women were scheduled for follow-up visits at 3, 6, 9 and 12 months after the biopsy or ablative procedure. Two women in the FU group and one in the LLETZ group withdrew their consent to participate. Cytology and colposcopy was performed at every follow-up visit, and cervical biopsies were taken when colposcopy indicated lesion progression or when cytology suggested CIN3 or worse. At the end of the 12-month follow-up, all women with any abnormal colposcopy were subjected to LLETZ.

Total regression was defined as i) the absence in Pap smear of colposcopic abnormality, or ii) a negative biopsy. A case was defined as partial regression when i) cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) or low squamous intraepithelial lesion (LSIL) was detected, or ii) the biopsy disclosed CIN1. Persistence was defined as continuous presence of CIN2, while progression to CIN3 or carcinoma was always based on biopsy results.

Cox’s proportional hazards regression was used to calculate hazard ratios (HR) with respective 95% confidence intervals (95% CI). Values of p<0.05 were considered statistically significant.

**Results**

In the two groups, the mean age was 30 (range 18-61) years and 31 (range 18-67) years, and age at first sexual intercourse was 16 (12-47) years and 17 (12-24) years, respectively, in the FU and LLETZ groups. Twenty-six women (57.7%) were white in color and 19 (42.2%) non-white in the FU group, and 26 (57.7) and 18 (40.0), respectively, in the LLETZ group. The percentage of smokers was similar in the two groups: smokers 31.1% and non-smokers 68.8%.

The disease outcome is shown in Table I. Three patients were lost to follow-up, two in the FU and one in the LLETZ group. Among the 43 women in the FU group, 44.1% experienced spontaneous regression, 20.9% had partial regression to CIN1 or ASCUS, 23.2% progressed to CIN3, and 11.6% showed CIN2 persistence. Among the 44 women whose the lesion was excised, the regression rate was 90.9%, while 4 (9%) presented progression to CIN3.

The excision of the lesion proved to be 3.36 times (HR) more effective at resulting in regression of CIN2, with 95% CI of 1.89-6.00 (p<0.01) as compared with prospective FU.

CI of 1.89-6.00 (p<0.01) as compared with prospective FU. When adjusted for potential confounders, HR=3.66 (95% CI= 2.02-6.64) (p<0.01) (Table II).

Analyzing only the women allocated to the FU group, total regression was observed in 44.1% of the patients at the 12-month FU visit (Table III), within a mean interval of 8.3 months, while partial regression to CIN1 took place in 20.9% of the cases. Progression to CIN3 occurred in 23.3% of the cases, whereas 11.6% persisted as CIN2. The regression of CIN2 increased from 27.3% at the 3-month visit to 44.2% at the 12-month. Most of the cases with partial regression (54.5%) were recorded during the first 3 months, and the highest percentage of progression to CIN3 (11.5%) occurred at 9 months. All progressive lesions were identified by colposcopy, and one additional case was also detected by cytology.

**Discussion**

This study confirmed that the excision of CIN2 lesion is three times more effective in achieving disease regression than prospective FU alone. However, despite this impressive HR, we must consider that a substantial proportion of
biopsy-confirmed CIN2 lesions regress without treatment, within a relatively short (12-month) period of observation. This is important to keep in mind because a rigid recommendation to excise all CIN2 lesions will inevitably lead to substantial unnecessary morbidity and increasing (avoidable) costs for health services.

Reports in the literature confirm that CIN2 regression is more frequent than progression. Most of these large cohort studies were carried out in patients with cytology suggesting moderate dysplasia, followed up by repeat periodic cytology and biopsy whenever cytological progression was detected (Table IV; 5-13). These data imply that behavior of CIN2 is far closer to that of CIN1 than that of CIN3. The results of the present study are in perfect agreement with most of these studies, although based on only 12-month prospective follow up of the patients.

Another important point is the frequent misclassification of CIN lesions. It is possible that some of the CIN1, 2 or 3 lesions reported at baseline and as endpoints measuring regression or progression might be subject to misclassification by pathologists. In the medical community, histopathological interpretations are generally considered as the reference gold standard upon which treatment of cervical disease is based. In fact, histopathology of cervical biopsies is not more reproducible than monolayer cytology, and, given the degree of non-reproducibility that exists among well-trained pathologists, realistic performance expectations should guide the use of interpretations (14).

Usually, no reproducibility problems arise in making the distinction between CIN1 and CIN3, but more difficulty is experienced in differentiation of CIN1 and the mildest forms of CIN2 from CIN3. As the these problems inherent to CIN classification are not adequately solved, however, by any of the recent attempts to classify these precursor lesions into two categories only (high-grade and low-grade) as in the

---

**Table III. Outcome of cervical intraepithelial neoplasia grade 2 in women allocated to the follow-up group.**

<table>
<thead>
<tr>
<th>Evolution</th>
<th>Follow-up visit (months)</th>
<th>Total</th>
<th>Mean interval for event</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Progression</td>
<td>6.1</td>
<td>9.4</td>
<td>11.5</td>
</tr>
<tr>
<td>Persistence</td>
<td>12.1</td>
<td>21.9</td>
<td>3.8</td>
</tr>
<tr>
<td>Partial regression</td>
<td>54.5</td>
<td>40.6</td>
<td>34.6</td>
</tr>
<tr>
<td>Total regression</td>
<td>27.3</td>
<td>28.1</td>
<td>50</td>
</tr>
<tr>
<td>Women attending the follow-up visit</td>
<td>33</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>Women missing the follow-up visit</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total number of women scheduled per visit</td>
<td>34</td>
<td>34</td>
<td>29</td>
</tr>
</tbody>
</table>

Percentage based on the women who attended the follow-up visit.

**Table IV. Selected cohort studies on natural history of cervical intraepithelial neoplasia grade 2 (CIN2).**

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Total women</th>
<th>Diagnostic criteria</th>
<th>Follow-up length</th>
<th>Regression to normal (%)</th>
<th>Persistence (%)</th>
<th>Progression to CIN 3 or worse (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasiell et al. (1983) (5)</td>
<td>894</td>
<td>Cytology</td>
<td>50-78 months</td>
<td>54</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>De Brux et al. (1983) (6)</td>
<td>762</td>
<td>Cytology and biopsy</td>
<td>42 months</td>
<td>39</td>
<td>44</td>
<td>17</td>
</tr>
<tr>
<td>Bibbo et al. (1989) (7)</td>
<td>78</td>
<td>Cytology, and biopsy</td>
<td>1-17 years</td>
<td>26</td>
<td>60</td>
<td>14</td>
</tr>
<tr>
<td>Weaver et al. (1990) (8)</td>
<td>21</td>
<td>Cytology, biopsy and HPV test</td>
<td>27 months</td>
<td>43</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Kataja et al. (1992) (9)</td>
<td>67</td>
<td>Cytology, and biopsy</td>
<td>60 months</td>
<td>54</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Holowaty et al. (1999) (10)</td>
<td>4645</td>
<td>Cytology</td>
<td>13 months</td>
<td>33 within 2 years</td>
<td>-</td>
<td>16 within 2 years</td>
</tr>
<tr>
<td>Yokoyama et al. (2003) (12)</td>
<td>71</td>
<td>Cytology, biopsy, and HPV test</td>
<td>3.1-57 months</td>
<td>39</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>Castle et al. (2009) (13)</td>
<td>397</td>
<td>Colposcopy, and HPV test</td>
<td>24 months</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Bethesda System (TBS). Most importantly, setting the cut-off between the low- and high-grade disease, with the latter comprising CIN2 and CIN3 lesions, leads to inevitable failure to correlate the lesion grade with its biological behavior in a longitudinal setting (3).

In the present study, we cannot exclude the possibility that borderline CIN2/CIN3 cases might have been included in the group followed-up without treatment. The fact that only a few cases of CIN3 were detected during the follow-up suggests that these borderline CIN2/CIN3 cases may have a regression rate similar to that of classical CIN2, and therefore do not represent any have worse cytological or colposcopic features. In the present study, the few cases of CIN3 detected during the follow-up of women who did not undergo any treatment should represent true progression since their colposcopic pattern showed evidence of progressive disease in every case.

Some lesions classified as CIN2 could probably be reclassified as CIN1 or CIN3 because the initial histological diagnosis of CIN2 was not revised by the reviewing pathologists. However, under normal circumstances, a second or third opinion is not available in public health services in our country. Because of this, CIN2 diagnosis and treatment decisions are problematic for pathologists and gynecologists.

CIN3 diagnosis seems to be more reproducible and should be validated more frequently with HPV tests and cytological interpretations than CIN2 because of its higher inter-observer reproducibility. Taken that HPV testing has a high sensitivity but fair specificity and positive predictive value, which makes the role of colposcopy in the accurate identification of patients requiring treatment even more important, the inaccuracies of the colposcopically directed biopsies must be carefully addressed, being responsible for the low sensitivity in detection of CIN3 (15). In the other research, there was evidence that approximately 40% of undiagnosed CIN2 will regress over 2 years, but CIN2 caused by HPV16 may be less likely to regress than CIN2 caused by other high-risk HPV genotypes (13). In average, HPV DNA seems to persist longer than related cytological abnormalities. It appears that the natural history of HPV includes periods before and after cytological abnormality, during which the HPV test is the most sensitive indicator of disease (16).

However, when predictors (cytological interpretation, pathology review, HPV results, and colposcopic impressions) of precancerous lesions were examined among women with CIN2 diagnosis in the ASCUS LSIL Triage Study (ALTS), clear evidence was found that HPV16 detection helped clarify whether a biopsy specimen diagnosed as CIN2 represents HPV infection or true cervical precancer, although this relationship was not sufficiently robust to be clinically useful in reducing the overtreatment of women with HPV infections (17). Comparison among cytology, colposcopy, HPV typing and biomarker analysis in cervical neoplasia showed that the most efficient combination, increasing sensitivity and negative predictive value was HPV genotyping associated with colposcopy (18). A satisfactory follow up is the main requirement for conservative management and HPV typing is important to detect persistent types to identify women at risk of developing cervical abnormalities (19).

With the above reservations, our results suggest that CIN2 lesions regress without treatment during a short (12-month) observation period, albeit the ablative procedure is more effective in producing disease clearance. Careful follow up with no treatment could be considered as a safe enough option, however. The recommendation to invariably treat CIN2 may lead to unnecessary morbidity and increasing costs that could be avoided by tailoring the management decisions on individual basis.

Furthermore, the inherent problems in reproducibility of CIN2 diagnosis should be considered in all studies using CIN2 as the endpoint. In such cases, HPV genotyping and quality controlled review of histopathological diagnoses would be important adjunct measures. However, in the health services of developing countries where these measures are not available, it might be better to abandon this intermediate category (CIN2) and classify all CIN lesions as low (CIN1) or high grade (CIN3).

Acknowledgements

This study was funded by the São Paulo State Foundation for the Support of Research, Brazil (FAPESP, 03/08180-6).

References


Received December 2, 2009
Revised April 1, 2010
Accepted April 13, 2010