LLETZ (Large Loop Excision of the Transformation Zone) Fragmentation: Impact on Margin Assessment and Cervical Biopsy-LLETZ Correlation

Bridget Melley

BSc. (Hons.) in Medical Science

Galway-Mayo Institute of Technology
Cervical Cancer

- 306 new cases and 93 deaths annually in Ireland (NCRI 2015)
- Cervical screening effective in early detection (Arbyn et al 2010)
- Cervical cytology and Papanicolaou staining (Arbyn et al 2010)
- Originates in epithelial cells of transformation zone (Cervical Check 2013)
**Figure 1** Illustration of the opening to the cervix demonstrating the locations of the transformation zone, endocervix and ectocervix (adapted from dreamstime 2016).
Cervical Screening Process Chart

**Letter of invitation (unscreened woman) or re-call (screened woman)**
- Eligible woman presents to smear taker (no letter)

**GP Practice / Clinic:**
- Role - counselling, smear taking
- Smear test

**Laboratory:**
- Role - cytology screening, HPV testing, result & management recommendation

**No abnormality detected (NAD)**
- Post colposcopy surveillance
- Post hysterectomy vault smear
- Smear test in 1 year
- Repeat in 6 months
- Routine screening (3 or 5 years)

**Low grade abnormality (ASCUS or LSIL)**
- HPV not detected
- No further screening

**High grade abnormality**
- HPV detected
- Refer to colposcopy

**Suspicious cervix**
- Unsatisfactory / inadequate
  - 3rd consecutive
  - Repeat in 3 months

**Programme office:**
- Role - call / recall, result recommendation, failsafe

**Recommendation to woman:** Programme issues a letter to woman advising her that her result is available and what the recommendation is.

**Recall:** Programme will issue a letter to woman in advance of the next smear test due date.

**Failsafe:** Programme will issue letters to woman and to doctor after the due date of a recommended early repeat test or a referral to colposcopy, if these do not occur.

**NOTES**

1) **Routine screening:** Women aged 25-44 years -> interval = 3 years. Women aged 45+ years -> interval = 5 years after 2 normal results at 3-year interval.

2) **No further screening:** Woman is or will be over 61 years at next smear test due date and has had 2 successive normal - routine recall results OR woman is over age range and first test has normal result.

**GP Practice / Clinic:**
- Role - smear test results management, follow up / failsafe
- Results management
- Referral to colposcopy
- Follow up / failsafe

**Colposcopy Clinic:**
- Role - investigation, diagnosis, treatment

**Histology laboratory:**
- Role - cervical histopathology

**Return to routine screening**
- Smear test in 3 years

**Smear test in 1 year**
- No further screening

**Recall**

**Recall Failsafe**

**Failsafe**
• Abnormalities associated with increased risk of progression to invasive malignancy termed ‘Cervical Intraepithelial Neoplasia’ (Buckley et al 1982)

• CIN1, CIN2 and CIN3

• Bethesda System
  • Atypical Squamous Cells (ASC)
  • Low Grade Squamous Intraepithelial Lesions (LSIL)
  • High Grade Squamous Intraepithelial Lesions (HSIL)
**Figure 2** Progression of cervical cancer from ‘normal’ cervical epithelial cells to invasive carcinoma (Adapted from Ortoski, R. A. and Kell, C. S. 2011).
What Happens After Screening...?

• Colposcopy – A magnified visual examination aided by application on contrast solutions (Stoler et al 2011)
  • Acetowhite staining
  • Histopathology – samples abnormal tissue in order to obtain diagnosis
  • Punch biopsy
• Large Loop Excision of the Transformation Zone (LLETZ) → aims to excise abnormal lesion (European Commission 2008)
**Figure 3** Image of intact LLETZ specimen in the Histopathology Department of Letterkenny University Hospital (longer demarcations represent centimetres).
Limitations of LLETZ

• Fragmentation

• Diathermy artefact (Bharathan et al 2013)

• Multiple guidelines developed to control quality of screening programme

• National Health Service Cervical Screening Programme (NHSCSP) Guideline

• Recommends removal of 80% of excisional specimens in a single fragment.
• Evidence required to support guideline

• Associations between LLETZ fragmentation and:
  • Indeterminate margins
  • Margin involvement
  • Endocervical margin involvement
    • (Bharathan et al 2013 and Papoutsis et al 2016)

• Cytology – Histopathology correlation used as a measure of quality (European Commission 2008)
Aims and Objectives

• To determine whether the NHSCSP recommendation for removal of LLETZ in a single piece is supported by scientific evidence.

• To investigate relationships between:
  • LLETZ fragmentation and margin assessment
  • LLETZ fragmentation and cervical biopsy-LLETZ agreement
  • Patient age and LLETZ fragmentation
  • Acetowhite grade and grade of subsequent cervical biopsy
Methodology

Sample Selection
N = 416

Cervical Sampling
Prior to study

Data Collection
CoPath LIS

Ethical approval sought and obtained from LUH ethics committee

Statistical Analysis
Excel, Casio fx-85GT, Analyse-It software

Data Analysis
Microsoft Excel
Results

• Age Range: 20-60 years

• Mean age: 32.3 years

• Final Diagnosis:
  • Negative in 15 (3.6%)
  • CIN1 in 54 (13%)
  • CIN2 in 187 (44.9%)
  • CIN3 in 160 (38.5%)
LLETZ Fragmentation and Margin Interpretation

- 43.7% removed intact and 56.3% removed in multiple pieces
- Single fragment → 97% margin interpretability
- Multiple Fragment → 73.4%

*Figure 4* Column chart demonstrating the percentage of interpretable LLETZ specimen margins when the LLETZ biopsy is removed in a single or multiple fragment(s) (N=380).
• Chi-Square Test: p-value < 0.0001
• Rejection null hypothesis
• 7 outliers identified and excluded (N=373)
• Correlation co-efficient (r) = -0.95

**Figure 5** Scatter-plot demonstrating the strong negative relationship between the number of tissue fragments in which a LLETZ biopsy is removed and the percentage of subsequent LLETZ specimens in which the excisional margins are interpretable (N=373).
Causes of Indeterminate Margins

- 62 reports gave reason for indeterminate margins
  - 56 (90.3%) fragmentation
  - 5 (8.1%) artefact
  - 1 (1.6%) poor orientation

Figure 6 Pie chart demonstrating the three causes of indeterminate margins and their associated proportions (N=62).
Patient Age and LLETZ Fragmentation

- Correlation co-efficient \( r = 0.00 \)
- No statistically significant relationship observed

**Figure 7** Scatter-plot demonstrating the absence of a relationship between the patient age (years) and the number of tissue fragments removed during the LLETZ biopsy (\( N=416 \)).
LLETZ Fragmentation and Margin Involvement

- \(\uparrow\) fragments \(\rightarrow\) \(\uparrow\) margin involvement
- \(\uparrow\) fragments \(\rightarrow\) endocervical involvement

**Table 1** The Percentage of LLETZ specimens with margin involvement when the LLETZ is removed in 1, 2 or ≥3 fragments and the proportion of involved margins which are endocervical, ectocervical, both endocervical and ectocervical or unnamed (but true) margins (N=318)

<table>
<thead>
<tr>
<th># LLETZ Fragments</th>
<th>% Margin Involvement</th>
<th>% Endocervical</th>
<th>% Ectocervical</th>
<th>% Both</th>
<th>% Unnamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.4</td>
<td>40</td>
<td>46.2</td>
<td>9.2</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>44.1</td>
<td>53.3</td>
<td>28.9</td>
<td>17.8</td>
<td>0</td>
</tr>
<tr>
<td>≥3</td>
<td>52.7</td>
<td>55.2</td>
<td>37.9</td>
<td>6.9</td>
<td>0</td>
</tr>
</tbody>
</table>
Agreement between Punch Biopsy and LLETZ

- 44.5% single piece and 55.5% multiple piece

- 3 or more fragments $\rightarrow$ perfect agreement

- 3 or more fragments $\rightarrow$ slightly practical agreement

Table 2  Summary of the perfect agreement, the practical agreement and the calculated kappa score for each group of punch biopsy and LLETZ biopsy grades when the LLETZ biopsies were removed in 1, 2 or $\geq$3 fragments (N=416)

<table>
<thead>
<tr>
<th>Group</th>
<th>1 Fragment</th>
<th>2 Fragment</th>
<th>$\geq$3 Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfect Agreement</td>
<td>61.1%</td>
<td>63.7%</td>
<td>51.8%</td>
</tr>
<tr>
<td>Practical Agreement</td>
<td>95.1%</td>
<td>95.2%</td>
<td>92.9%</td>
</tr>
<tr>
<td>Kappa Score</td>
<td>0.38</td>
<td>0.33</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Acetowhite and Punch Biopsy Grade Agreement

- Perfect agreement = 43.5% (163/375)
- Low kappa value of 0.13
- Positive predictive value (PPV) = 93%

Table 3: The acetowhite grade, available for 375 patients, and the grade of the corresponding and subsequent cervical punch biopsy (N=375)

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Acetowhite Grade</th>
<th>Negative</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>8 (14%)</td>
<td>2 (1.9%)</td>
<td>2 (1.2%)</td>
<td>1 (2.2%)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>CIN1</td>
<td>12 (21.1%)</td>
<td>7 (6.7%)</td>
<td>10 (5.9%)</td>
<td>2 (4.4%)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>CIN2</td>
<td>30 (52.6%)</td>
<td>82 (78.9%)</td>
<td>119 (70.4%)</td>
<td>13 (28.9%)</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>CIN3</td>
<td>7 (12.3%)</td>
<td>13 (12.5%)</td>
<td>38 (22.5%)</td>
<td>29 (64.5%)</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57 (100%)</td>
<td>104 (100%)</td>
<td>169 (100%)</td>
<td>45 (100%)</td>
<td>375</td>
<td></td>
</tr>
</tbody>
</table>
To summarise study findings...

- Correct sample chosen for investigation
- Selection criteria introduced a bias
- Rate of intact LLETZ (43.7%) lower than recommended 80%
- Increased LLETZ fragments → Decreased interpretable margins
  - Agreement with other studies
- Fragmentation is the primary cause of indeterminate margins
• No relationship between age and LLETZ fragmentation

• Relationship between LLETZ fragmentation and increased endocervical margin involvement
  • Clinically significant

• Decreased punch biopsy – LLETZ agreement with increased fragments
  • Not statistically significant

• Acetowhite is suitable for confirming presence of CIN and guiding biopsy
  • Not suitable as predictor of histopathological grade
Limitations and Further Study

• Absence of data regarding colposcopist
• Size of sample subgroups
  • Should be larger and relatively equal in size
• 1st prize in Allied Health, in the 6th Annual Multidisciplinary Research Symposium in Letterkenny University Hospital
Conclusion

• NHSCSP guideline supported

• Increased LLETZ fragments;
  • negatively impacts margin interpretation
  • Increases likelihood of endocervical involvement
  • Patient age does not induce LLETZ fragmentation
  • Specific acetowhite grades are not clinically useful
Sources of Reference


Thank you for listening