Abstract

Primary ciliary dyskinesia (PCD), a rare cause of chronic respiratory disease, is an important disorder to diagnose at an early stage. The standard method of diagnosis involves transmission electron microscopy at most institutions. There are many limitations with electron microscopy (EM) including necessity of obtaining an optimal sample, and expense. Phase contrast microscopy (PCT), on the other hand, can be done intraoperatively to immediately assess ciliary function following biopsy. We performed a retrospective review of 33 cases that underwent both PCT and EM in the diagnosis of PCD. Only 28.5% of EM-analyzed samples in our study were adequate, while the majority were suboptimal and the ciliary ultrastructure could not definitively be determined. The sensitivity and specificity of PCT was 100% and 83%, respectively. Despite good sensitivity and specificity results, the marks were underscored by the relatively small adequate sample size, making it difficult to formulate a valid conclusion. Future studies could show PCT to be promising if certain steps are made to create a standardized protocol, eliminate confounding factors, and improve the adequacy of samples for EM analysis.

Objectives

- Determine if PCT could compare favorably with EM techniques in the diagnosis of PCD
- Determine if PCT could minimize sampling problems found with EM.

Materials and Methods

Retrospective review of 25 consecutive patients with 33 samples considered for diagnosis of PCD who underwent both PCT and EM analysis between January 2003 and April 2007. Ciliary motility was examined by PCT intraoperatively. Specimens were categorized as having beating cilia or no beating cilia. These specimens were also analyzed by EM and further categorized.

1. Inadequate: EM done, but cilia not evaluable on semi-thin section
2. Suboptimal: EM done and evaluable cilia present, but the state of preservation or number of evaluable cells (at least 25), usually both, precluded definitive interpretation.
3. Adequate: EM done and preservation and number of cilia allowed a definitive interpretation.
4. Deferred: Specimens processed for EM, but since beating had been observed, the surgeon agreed that full EM was not necessary.

Results

Six total samples were not processed by EM because no cilia were found on EM semi-thin sections and deemed inadequate.

Table 1. Comparison of EM thin section samples with phase contrast microscopy results.

<table>
<thead>
<tr>
<th>Phase Contrast Microscopy</th>
<th>Beating Cilia Present</th>
<th>Beating Cilia Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM Suboptimal</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM Adequate</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>EM Deferred</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

**All adequate samples for analysis by electron microscopy were found to have normal ultrastructure.**

Sensitivity = 58 – 83%  
*probability of excluding ciliary defects by the presence of beating cilia on PCT  
Specificity = 1/1 = 100%  
*probability of detecting ciliary defects when presented with absence of beating cilia by PCT  
% of adequate samples = 28.5%

Table 2. A comparison of ciliary ultrastructure by EM with presence of beating cilia by phase contrast microscopy.

<table>
<thead>
<tr>
<th>Phase Contrast Findings</th>
<th>Beating cilia</th>
<th>No beating cilia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ciliary ultrastructure</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Abnormal ciliary ultrastructure</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

Our data showed a significant sensitivity of 83% and specificity of 100%; however, given the large number of inadequate samples and small adequate sample size, the numbers were rather misleading. Therefore, a valid conclusion could not be made from this data. The low incidence of PCD in the population, 1:20,000, was a limiting factor in the outcomes of this research; many more patients were tested than are pathologically positive for PCD. In addition, confounding factors such as inflammation affected the preservation of the cilia and resulted in many suboptimal samples. In fact, the absence of beating cilia by PCT was more likely associated with suboptimal samples when analyzed by EM.

Given our experience, we propose these guidelines for the general otolaryngologist in the workup for PCD:

- Minimizing the presence of inflammation by administering antibiotics and using at least a 4-6 week time frame after the resolution of upper respiratory infections before obtaining samples.
- Emphasis on obtaining biopsy from the most normal appearing area, whether or not intraoperative PCM is done.

REFERENCES