Clinical Question

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11-13-08
Morning Report
Clinical Question Search

**Question:** In patients diagnosed with HIV or AIDS, is the modified Wright-Giemsa stain, also known as Diff-Quik(DQ), a reliable method of rapid detection of Pneumocystis jiroveci in respiratory specimens?

**Resource:** Ovid MEDLINE

**Search Terms:** Pneumocystis jirovecii or Pneumocystis pneumonia or Pneumocystis infections (3923) + Diff-Quik(229)

**Limits:** Diagnosis sensitivity(192) + Diagnosis specificity (31)

Study Overview

Background:

• The silver stain has been the traditional method for detecting pneumocystis. While silver stain is considered the “gold standard” each kit runs around $120, and it is designed only to detect a cyst wall--not the trophozoite form of the organism. A very light infection or one with a high trophozoite-cyst ratio could be missed by this technique.

• The Diff-Quik (DQ) stain detects trophozoites and intracystic bodies. It is also inexpensive, fast, and easy to prepare. In most cases a skilled technologist can interpret DQ fairly quickly.
Study Overview

Materials and Methods:

- Respiratory specimens, both IS and BAL, were obtained from VA Medical Center, and San Francisco General Hospital. Diff-Quik (DQ) stain was routinely used at both. Specimens were considered adequate if alveolar macrophages, alveolar epithelial cells, or ciliated columnar epithelial cells were present. Inadequate specimens were not included.

- 100 specimens were collected from 84 individual patients. Multiple slides were prepared for each specimen. Fifty of the 100 specimen samples were IS and fifty were BAL. 47 were negative by DQ and 53 positive. Unstained slides were coded numerically so that once stained, they could be read blindly, without prior knowledge of the diagnosis. Each specimen was then stained with a modified silver stain, a direct IF, and an indirect IF stain according to manufacturer instructions.
Article Review

Positivity:
• A true positive was defined as a smear that was positive by 2/4 stains. Criteria for positivity for the individual tests were defined by recommendations of the manufacturers.
• Sensitivity and specificity were calculated for each staining method, and the statistical significance of differences of the four was calculated by chi-square analysis.
Article Review

Results:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Time (min)</th>
<th>Cost per slide $</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS  BAL</td>
<td>IS  BAL</td>
<td>Prep</td>
<td>Read</td>
</tr>
<tr>
<td>Silver</td>
<td>92  86</td>
<td>92  97</td>
<td>90</td>
<td>10-15</td>
</tr>
<tr>
<td>DFA</td>
<td>97  90</td>
<td>85  90</td>
<td>45</td>
<td>&lt;5</td>
</tr>
<tr>
<td>IFA</td>
<td>97  86</td>
<td>100 100</td>
<td>90</td>
<td>&lt;5</td>
</tr>
<tr>
<td>DQ</td>
<td>92  82</td>
<td>100 97</td>
<td>3</td>
<td>10-30</td>
</tr>
</tbody>
</table>

* = not available in United States
Discussion/Conclusion:

1) The final choice of test must be based on multiple factors, including sensitivity/specificity, predictive value, and likelihood ratios. Clinical suspicion, the patient population, the volume of specimens being processed in the laboratory, and the level of technical expertise available also should factor in.

2) DQ stain continues to be a very useful method, as it is inexpensive, quick, easy

3) While it was the least sensitive of the four methods it approached 100% specificity. No PPV or likelihood ratio calculated here, however other studies have indicated a high PPV\(^1\).

4) DQ highly reliable in light of a positive result → high specificity confers a high degree of confidence that a positive test result is truly positive. But also need high PPV and likelihood ratio.
Clinical Question

• **Limits** – Population characteristics (diagnosis of HIV or AIDS on or off prophylactic antibiotics, as well as those with other immunodeficient states or patients who were immunocompetent, in many cases had limited access to patient’s records → unable to evaluate effect of prophylactic antibiotic tx on sensitivity)

• **Clinically applicable?** Absolutely. Our patient conferred a high suspicion for P. jirovecii infection at the outset of his presentation given his low CD4 count. A positive DQ test serves as a reliable test to “rule in” infection → 1) IFH is well-trained in this method 2) DQ has high specificity thus little indication for further testing via methods such as nucleic acid amplification or real-time PCR 3) importantly, DQ’s low sensitivity among other staining methods tested reminds us that a negative test does not reliably rule out disease
Additional Articles Referenced