Utility of Urine Eosinophils in the Diagnosis of Acute Interstitial Nephritis

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Summary

Background and objectives Urine eosinophils (UEs) have been shown to correlate with acute interstitial nephritis (AIN) but the four largest series that investigated the test characteristics did not use kidney biopsy as the gold standard.

Design, setting, participants, & measurements This is a retrospective study of adult patients with biopsy-proven diagnoses and UE tests performed from 1994 to 2011. UEs were tested using Hansel’s stain. Both 1% and 5% UE cutoffs were compared.

Results This study identified 566 patients with both a UE test and a native kidney biopsy performed within a week of each other. Of these patients, 322 were men and the mean age was 59 years. There were 467 patients with pyuria, defined as at least one white cell per high-power field. There were 91 patients with AIN (80% was drug induced). A variety of kidney diseases had UEs. Using a 1% UE cutoff, the comparison of all patients with AIN to those with all other diagnoses showed 30.8% sensitivity and 68.2% specificity, giving positive and negative likelihood ratios of 0.97 and 1.01, respectively. Given this study’s 16% prevalence of AIN, the positive and negative predictive values were 15.6% and 83.7%, respectively. At the 5% UE cutoff, sensitivity declined, but specificity improved. The presence of pyuria improved the sensitivity somewhat, with a decrease in specificity. UEs were no better at distinguishing AIN from acute tubular necrosis compared with other kidney diseases.

Conclusions UEs were found in a variety of kidney diseases besides AIN. At the commonly used 1% UE cutoff, the test does not shift pretest probability of AIN in any direction. Even at a 5% cutoff, UEs performed poorly in distinguishing AIN from acute tubular necrosis or other kidney diseases.

Introduction

Acute interstitial nephritis (AIN) is an important cause of AKI, especially in hospitalized patients, and has been reported to occur in approximately 6%–30% of biopsies for AKI (1–3). Since the mid-1980s, when the presence of urine eosinophils (UEs) was shown to correlate with AIN (4), their use has gained widespread acceptance in patients with AKI who are suspected to have AIN. This was initially done using the Wright’s stain until the Hansel stain was first described as a sensitive marker in 1986 (5) and laboratories worldwide have since adopted its use. The four largest series that investigated the test characteristics of UEs (4–8) showed that sensitivity ranged from 40% to 91% and specificity ranged from 52% to 95%. None of these studies, however, used the kidney biopsy as the “gold standard” for diagnosis of AIN.

This wide range in test characteristics reported to date and lack of biopsy diagnoses leave the clinical utility of the UE test in question. In addition, given the wide range of pathologies that can result in the presence of eosinophils in the urine (4,5), the exact application remains uncertain.

Because no previous studies have used the kidney biopsy as the gold standard for diagnosis of AIN in patients who had UEs tested, it remains unclear how the testing of UEs performs as part of the noninvasive work-up for AIN. In this study, our goal is to determine the performance characteristics of UEs as a test for AIN in patients who also had a kidney biopsy done as part of their work-up for AKI.

Materials and Methods

Study Objective and Design

To assess the performance characteristics of UE tests in the diagnosis of AIN as a cause of AKI, we performed a retrospective study of patients with biopsy-proven diagnoses from 1994 to 2011. These patients had UEs checked as part of their work-up for AKI. The indication for biopsy in these patients was AKI either in patients with previously normal kidney function or some degree of CKD as defined by the Kidney Disease Outcomes Quality Initiative criteria (9). AKI was defined by the Acute Kidney Injury Network criteria (10).

The Mayo Clinic’s Institutional Review Board approved the study. No external funding source was
involved and the study complied with Health Insurance Portability and Accountability Act requirements for patient confidentiality.

Patients and Setting
All Mayo Clinic patients who had a UE test and kidney biopsy performed within a week of each other were identified using a comprehensive institution database from 1994 to 2011. Chart review was then carried out for all of these patients with regard to their demographic data, UE test results, presence of pyuria, and renal histology diagnoses. In total, we identified 566 patients with a UE test and kidney biopsy performed during the same episode of AKI who met the inclusion criteria. To ensure complete identification of patients with biopsy-proven AIN, we also searched a separate renal pathology database, from 1994, when the database began to be compiled, up to 2011.

Patients were designated as having AIN if this was the histologic diagnosis in the absence of glomerular or monoclonal disease, which were considered the primary diagnoses. Patients with histologic diagnosis of acute tubular necrosis (ATN) as well as AIN were classified as having AIN.

The control population consisted of patients who had a UE test during the same time period and a kidney biopsy during the same time period but had a primary histologic diagnosis other than AIN.

The study inclusion criteria were adult patients with both a UE test and biopsy of native kidney performed within a week of each other. Exclusion criteria were any patients aged <18 years, patients with a biopsy of allograft kidneys, and patients who had a biopsy done at another institution but were referred to our institution during that episode of AKI.

UE Testing
Urine was tested for the presence of eosinophils using Hansel’s stain in the Mayo Clinic Laboratory (11). The patients had urine collected either by voiding or catheterization.

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**Figure 1.** STARD diagram of patients with urine eosinophils and kidney biopsy. STARD, Standards for the Reporting of Diagnostic Accuracy; AIN, acute interstitial nephritis.

**Figure 2.** Presence of eosinophiluria in various kidney biopsy diagnoses. Number of patients with UE results <1%, 1%–5%, or >5% by biopsy diagnoses. Noninflammatory glomerular disease included minimal change disease, membranous nephropathy, and FSGS. AIN, acute interstitial nephritis; ATN, acute tubular necrosis; TMA, thrombotic microangiopathy; MiDD, monoclonal Ig deposition disease.

This sample was centrifuged to obtain urine sediment that was then used for both the UE test and standard microscopy if requested. Twenty milliliters of freshly voided urine was the recommended amount for collection and 12 ml of this was centrifuged at 1400 rpm for 5 minutes. The
supernatant was discarded and the remaining specimen was examined microscopically for sediment. If this sediment did not show at least one white cell per high-power field, then no further testing was done on it and this was reported as a negative UE test result. If pyuria was present, the specimen was then processed by Cytospin technology and centrifuged again at 10 rpm for 5 minutes, resulting in deposition of the specimen on a glass slide that was then air-dried. The slide was then stained by Hansel’s stain and decolorized using ethanol alcohol. The slide was then magnified ×100 and examined for the presence of eosinophils for every 100 white cells present, and the results were expressed as a percentage.

UEs were considered to be positive when eosinophils were at least 1% of all urinary white cells by Hansel’s stain, as originally described (5). Hansel’s stain easily identifies eosinophils by their bright red-pink granules.

The testing of urine for eosinophils was conducted by laboratory technicians using usual laboratory practices at the institution and reported in patients’ charts. Technicians were all trained in the procedure and had competency assessed annually according to their usual practice. Each test was usually performed by one observer only. The results are reported as a percentage as one of three possibilities: <1%, 1%–5%, and >5%.

### Kidney Biopsy

Kidney biopsies during 1994–2011 are all entered into a database. Of all of the biopsies done during these years, 133 patients had AIN. The indication for biopsy in most of these patients was for AKI. In rare instances, the indication was microscopic hematuria with or without proteinuria.

All biopsy slides were reviewed by renal pathologists in the Division of Anatomic Pathology. Findings on biopsy that led to the diagnosis included the presence of interstitial inflammation, edema, as well as tubulitis as defined by Heptinstall (12). The presence of eosinophils or plasma cells was also noted. The degree of interstitial inflammation was defined according to the Banff working classification that was developed for use for renal allograft pathology (13). Patients with interstitial inflammation and/or tubulitis associated with glomerular disease were classified according to the glomerular disease only.

### Statistical Analyses

Results are expressed as means and S.E.M. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) were computed from these data. The diagnosis of AIN was coded as a binomial variable. The presence of UEs was coded according to the three different possible test results. Statistical analyses were performed using JMP software (version 9; SAS Institute Inc, Cary, NC).

The sensitivity, specificity, PPVs, and NPVs were separately calculated for both 1% and 5% UE cutoffs. In addition, we assessed the performance of the test in all patients and separately in only those with pyuria. We also sought to establish the performance of the test specifically in those with drug-induced AIN, and compared it with all other diagnoses and separately with those with ATN as the biopsy diagnosis.

### Results

#### Patient Characteristics and Spectrum of Diagnoses in These Patients

There were 566 patients (322 men and 244 women) who had a UE test and a kidney biopsy performed as work-up of kidney dysfunction. Their ages ranged from 18 years to 91 years, with a mean of 59 years. Our results showed that 467 patients (82.5%) had pyuria.

Our results showed that 133 of 566 patients had AIN on biopsy. Of the 133 patients with AIN, 91 had UEs checked as part of diagnostic work-up and were included in this study. Of these, 73 patients (80%) were considered by their treating clinicians to have drug-induced AIN (DI-AIN). The prevalence of AIN among all adult native biopsies at our institution was 1.8% (133 of 7575). Among the 91 patients with AIN, 47 patients (51.6%) were men and 44 were women (48.4%). Their mean age was 56.0±16.7 years (range, 18–89 years). The mean baseline serum creatinine was 1.12 mg/dl (range, 0.5–3.9) and peak creatinine was 6.64 mg/dl (range, 1.2–22.8). There were 83 patients (95%) who had some proteinuria (median 1.58 g/24 h; range, 0.07–10.25). Of the 73 DI-AIN cases, 39 (42.9%) were due to antibiotics, 10 (11%) to nonsteroidal anti-inflammatory drugs, and 7 (7.7%) to proton pump inhibitors. Twelve patients (13.2%) were taking multiple drugs and no specific one could be identified as the cause, whereas another five patients were taking various other drugs (e.g., olmesartan, allopurinol, or cimetidine). Of the remaining 18 AIN cases, 8 were due to autoimmune disease (4 were sarcoidosis), 5 were due to various causes (reactive, malignancy), and the rest were unknown.

A total of 179 patients (31.6%) had UEs present at the ≥1% cutoff as a positive test, with the majority of these individuals having biopsy diagnoses other than AIN as

<p>| Table 1. All patients included in analysis by disease category and result of urine eosinophils |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Eosinophils (%)</th>
<th>All Causes of AIN</th>
<th>Drug-Induced AIN</th>
<th>ATN</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Pyuria Only</td>
<td>All Pyuria Only</td>
<td>All Pyuria Only</td>
<td>All Pyuria Only</td>
</tr>
<tr>
<td>&lt;1</td>
<td>63</td>
<td>45</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>1–5</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>&gt;5</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>73</td>
<td>73</td>
<td>58</td>
</tr>
</tbody>
</table>

AIN, acute interstitial nephritis; ATN, acute tubular necrosis.
Table 2. Detailed performance characteristics of urine eosinophils for AIN from all causes and DI-AIN compared with all other diagnoses

<table>
<thead>
<tr>
<th>Test Characteristic</th>
<th>AIN of All Causes Compared with All Other Diagnoses</th>
<th>DI-AIN Compared With All Other Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=566)</td>
<td>Pyuria Only (n=467)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>30.8% (22.2 to 40.9)</td>
<td>19.8% (12.9 to 29.1)</td>
</tr>
<tr>
<td>Specificity</td>
<td>68.2% (63.7 to 72.2)</td>
<td>91.2% (88.3 to 93.4)</td>
</tr>
<tr>
<td>PPV</td>
<td>15.6% (11.1 to 21.7)</td>
<td>30.0% (19.9 to 42.5)</td>
</tr>
<tr>
<td>NPV</td>
<td>83.7% (79.7 to 87.1)</td>
<td>85.6% (82.2 to 89.5)</td>
</tr>
<tr>
<td>Positive LR</td>
<td>0.97</td>
<td>1.1</td>
</tr>
<tr>
<td>Negative LR</td>
<td>0.97</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are presented as the percentage (95% confidence interval) unless otherwise stated. AIN, acute interstitial nephritis; DI-AIN, drug-induced acute interstitial nephritis; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

When the analysis was limited to those with pyuria, the sensitivity improved using either 1% or 5% as the cutoff, as shown in Table 2; specificity declined, PPV remained unchanged, and NPV also remained relatively unchanged.

Performance Characteristics Comparing DI-AIN with All Other Diagnoses

When the analysis was limited to those with DI-AIN only, as shown in Table 2, the sensitivity was 35.6% and 23.3% using 1% and 5% as the cutoffs, respectively. As expected, the specificity remained unchanged because there was no change in the number of patients with other diagnoses apart from AIN. There was little change in the PPV, although the NPV performed better.

Patients with nonsteroidal anti-inflammatory drug–induced AIN were no more or less likely to have UEs present than patients with AIN attributed to other drugs. The cause of DI-AIN did not correlate with the presence of UEs.

Performance Characteristics Comparing AIN with ATN

Table 3 shows the performance of the UE test comparing patients with ATN to those with AIN from all causes or drug-induced AIN. As shown, the sensitivity of the test remains identical, whereas the specificity changes only marginally for each comparison. However, PPV improves significantly from 15.6%, when AIN of all causes was compared with all other diagnoses, to 58.3% compared only with ATN. This is also seen when DI-AIN is compared with ATN with PPV, improving to 56.5% compared with 14.7% (Table 3).

The NPV, however, shows a marked decline, changing from 83.7% comparing AIN of all causes to all other diagnoses using 1% eosinophilia as the cutoff to 43.8%.

In the evaluation of AKI, when trying to differentiate DI-AIN from ATN, a negative UE test at either a 1% or 5% cutoff is hardly helpful and means a patient does not have the disease only 51%–54.4% of the time. When only
Table 3. Performance characteristics of urine eosinophils for AIN from all causes and drug-induced AIN compared with ATN

<table>
<thead>
<tr>
<th>Test Characteristic</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Positive LR</th>
<th>Negative LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1% Cutoff</td>
<td>&gt;5% Cutoff</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>All (n=160)</td>
<td>30.8 (22.2 to 40.9)</td>
<td>35.6 (23.6 to 47.1)</td>
<td>23.3 (15.1 to 34.2)</td>
<td>59.4 (40.2 to 78.2)</td>
<td>1.06</td>
<td>0.97</td>
</tr>
<tr>
<td>Pyuria Only (n=128)</td>
<td>38.4 (28.1 to 49.8)</td>
<td>38.4 (28.1 to 49.8)</td>
<td>24.7 (16.2 to 35.6)</td>
<td>63.6 (44.6 to 82.3)</td>
<td>2.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are presented as the percentage (95% confidence interval) except otherwise stated. AIN: acute interstitial nephritis; ATN: acute tubular necrosis; DI-AIN: drug-induced acute interstitial nephritis; PPV: positive predictive value; NPV: negative predictive value; LR, likelihood ratio.

Discussion

At present, the studies assessing utility of UEs in the diagnosis of AIN have shown sensitivity ranging from 40% to 91% and specificity ranging from 52% to 95% (4–7), with composite values of 67% for sensitivity and 87% for specificity. Despite this wide range of values and the absence of a kidney biopsy in the diagnosis of AIN in these patients, the practice of checking UEs in patients suspected to have the diagnosis has become widespread, especially among general internists (14). In these studies, the diagnosis of AIN was based mostly on the consulting nephrologists’ clinical impression. Given the known discrepancy between the clinically suspected or presumed cause of AKI and actual biopsy diagnosis (15,16), the nonspecific nature of symptoms in AIN (17–19), and the variable performance of UEs, its utility remains in question. Moreover, the presence of UEs is not specific for AIN and can occur in many diseases (4,5). Since the 1994 study by Ruffing et al. (7), there have been scarce data on this issue and we sought to investigate the performance of UEs in patients with biopsy-proven diagnoses. We found that UEs were tested in patients who had a variety of kidney diseases on biopsy and were present in essentially all causes of AKI.

The sensitivity ranged from 19.8% to 44.8%, performing best when limiting the comparison with those with DI-AIN and pyuria at a 1% UE cutoff. The false negative value was quite high at 55.2%–80.2%. The specificity ranged from 61.7% to 91.2%, performing best with a higher UE cutoff of 5%. The false positive value was 8.8%–38.3%. The test may therefore be helpful when it is negative at a 5% UE cutoff, but has a high false positive rate at the usual 1% cutoff. There was a marginal improvement when AIN was compared with ATN only, but not enough to change the implications at the 1% UE cutoff, because the false positive rate still ranged from 28.1% to 36.4%.

At a 16% prevalence of AIN in all patients with both a UE test and kidney biopsy performed, the PPV in this study was low (14.7%–30%) and performed better when AIN was compared with ATN (56.5%–75%). The NPV was 83.7%–89.6% and performed best when comparing DI-AIN to all other diagnoses at a 5% UE cutoff in those with pyuria. Therefore, a negative test may be useful in ruling out the disease at a higher cutoff than is usually used. It should be noted, however, that when AIN was compared with ATN, the NPV performed poorly, ranging from 43.8% to 54.4%. This is particularly significant because AIN (particularly that due to drugs) and ATN are commonly the main competing diagnoses in hospitalized patients who develop AKI. A negative UE test in that scenario would not help distinguish between the two diagnoses.

In the four largest series (4–7), the sensitivity was markedly higher than we report, likely due to the diagnoses of AIN being based on treating nephrologists’ opinions and not histology. The finding of a positive UE test result likely contributed much to this opinion, which may account for the higher sensitivity.

Using the 30.8% sensitivity and 68.2% specificity we derived for a 1% UE cutoff, the positive likelihood ratio...
(LR) is 0.97 and the negative LR is 1.01 (previous studies gave these as 5 and 0.38, respectively). If the 5% UE cutoff is used, the positive LR is 2.2 and the negative LR is 0.9. We show that for any comparison of diagnoses or UE cutoff, no positive LR was >3.0 and no negative LR was <0.8, suggesting that UE testing has no utility in the diagnosis of AIN. Given this poor performance, its continued use is hard to justify.

The main strength of the study is the use of biopsy-proven kidney diagnoses in patients who also had UE tests performed. This enables assessment of UEs as a diagnostic test for AIN diagnosed by kidney biopsy, which remains the accepted gold standard.

This study has some limitations. First, because this is a retrospective study, there is likely some selection bias. Most patients with AIN likely never receive a kidney biopsy diagnosis, likely due to mild presentation or quick resolution of AKI in the case of DI-AIN. The decision to perform a biopsy in the patients in this study was at the discretion of the consultant nephrologist usually in the case of competing diagnoses in severe AKI and/or where steroid therapy is a consideration. Because we can only ascertain the validity of a test compared with the gold standard, no definitive conclusions can be made in cases in which biopsy is not performed.

Second, the retrospective nature of the study precluded the opportunity to independently verify the UE result, which was based on the institution’s usual laboratory practice. The third limitation is one inherent to diagnostic studies and the issue of just how good the “gold standard” test is. When a kidney biopsy is used for diagnosis, one cannot ascertain by other means how many patients with AIN were classified as having ATN or other diagnoses due to focal involvement of the kidney. In addition, we did not specifically exclude those with urinary tract infections, because patients would generally not have been biopsied when actively infected.

Although the UE test itself is seemingly innocuous, it may lead to two specific problems in the work-up of AKI. First, the risk of reliance on a poorly performing test may lead to a false positive diagnosis, which may result in inappropriate treatment of AIN with steroids or inappropriate changes in therapy. Alternatively, a false negative test may lead to a delay in the definitive biopsy diagnosis as well as delays in treatment. The poor performance of the UE test emphasizes the importance of biopsy diagnoses in patients with AKI suspected to be due to AIN.

In conclusion, we found that UEs can occur in a large variety of diseases that cause AKI. The UE test performs poorly at the 1% cutoff, with low sensitivity yielding a high number of false negatives and poor likelihood ratios. More studies using biopsy to determine diagnosis are needed to confirm our findings.

Disclosures
None.

References

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Correction

Because of author error, the following correction has been issued. The statement “Our results showed that 133 of 566 patients had AIN on biopsy” in the second paragraph of the Results section is erroneous and should instead read: “Our results showed that 133 patients had AIN on biopsy.” The authors regret any confusion this may have caused to readers.

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