Urinary Eosinophils in AIN: Farewell to an Old Biomarker?

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Urinary biomarkers of kidney injury are an area of ongoing research in nephrology. For years, urinary eosinophils have been used as biomarkers for acute interstitial nephritis (AIN). The characteristics that would make eosinophiluria a useful biomarker for AIN include that (1) eosinophils are consistently present in the urine of patients with AIN (i.e., AIN is characterized by an eosinophilic interstitial infiltrate), (2) eosinophils are absent in the setting of another etiology of AKI (i.e., other renal and extrarenal diseases are not associated with eosinophiluria), and (3) a predetermined percentage of eosinophils is easily visualized in the urine when present (i.e., excellent stain performance).

As early as 1967, urinary eosinophils were reported in rejection episodes after kidney transplantation, suggesting that they were associated with “kidney inflammation” (1). The earliest description of this test as a biomarker for AIN was by Galpin et al. (2) in 1978. Using the Wright stain, nine of nine cases of methicillin-associated AIN had urinary eosinophils. Six of nine cases had biopsy-proven AIN, with eosinophils within the renal interstitium and occasionally, tubular lumens; 0 of 43 patients with AKI from another diagnosis had eosinophiluria. These data implied that eosinophiluria was a sensitive and specific test for AIN. Linton et al. (3) subsequently described eosinophiluria in six of nine patients with drug-induced AIN. Eight patients had biopsy-proven AIN, with five patients having prominent interstitial eosinophils. Overall, these two small studies suggested that eosinophiluria might be a reasonably good biomarker for AIN.

Additional work on this subject by Corwin et al. (4) examined the clinical correlates of eosinophiluria using the Wright stain in 65 patients. AIN was diagnosed in 9 of 65 patients, with eosinophiluria noted in 8 of 9 of these cases, replicating previous data. However, urinary eosinophils were also shown in 27 of 56 patients with diagnoses other than AIN (Table 1), suggesting that the test was rather nonspecific. Corwin et al. (4) concluded that using a >5% eosinophil cutoff might identify a group with higher probability of having AIN. However, 6 of 56 non-AIN patients and only 4 of 9 AIN patients met this cutoff, which (not unexpectedly) improved specificity but reduced sensitivity. The lack of sensitivity of urinary eosinophils was further emphasized in a review paper based on data from 10 studies examining drug-induced AIN showing that eosinophiluria was present in only 59% (19/32) of biopsy-proven AIN cases (5).

Because low sensitivity was the major concern, Nolan et al. (6) renewed interest in eosinophiluria as an AIN biomarker with a New England Journal of Medicine publication that hailed the Hansel stain as a better method to visualize urinary eosinophils. This stain was chosen based on its use in identifying eosinophils in nasal, bronchial, and ocular secretions of patients with allergy-related diseases; 92 patients with an active urine sediment were identified by the nephrology consult service, of which 11 patients had AIN (proven by biopsy in 2 patients). Hansel stain was positive in 10 of 11 AIN cases, whereas Wright stain was positive in only 2 of 11 cases. Hansel stain was also positive in other diagnoses but negative in all 30 acute tubular necrosis cases (Table 1). Overall, the Hansel stain provided a sensitivity of 91% and positive predictive value of 45%. Nolan et al. (6) concluded that the Hansel stain improved the sensitivity of eosinophiluria in AIN but that the test was not specific. Nolan et al. (6) emphasized that it was useful when the differential diagnosis of AKI boiled down to AIN versus acute tubular necrosis. Additionally, Nolan et al. (6) argued that a positive test in the setting of high pretest probability for AIN tends to confirm the diagnosis, whereas negative eosinophiluria in patients with low pretest probability can reasonably exclude AIN. However, lack of biopsy confirmation of AIN was a major flaw of the paper.

In the face of these data, a study was undertaken to more completely define the significance of eosinophiluria (7). Urine for eosinophils was examined in 183 patients assigned a clinical diagnosis using blinded chart review, with AIN based on biopsy or clinical diagnosis. Eosinophiluria was documented in 20 patients, with Hansel stain positive in 18 of 20 patients and Wright stain positive in 8 of 20 patients. Hansel and Wright stains were positive in five of eight and two of eight AIN cases, respectively. Clearly, Hansel stain improved urinary eosinophil detection compared with Wright stain, confirming the data in the work by Nolan et al. (6). Although Hansel stain was also positive in other diagnoses, it still had a very good specificity and negative predictive value of 98% (Table 1). Corwin et al. (7) concluded that Hansel stain improved detection of urinary eosinophils, but its use for diagnosis of AIN remained unclear.

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A study directed at patients suspected of having an acute kidney process who also had urine eosinophils measured was conducted by Ruffing et al. (8). AIN was noted in 15 patients, of which Hansel stain was positive in 6 patients. Eosinophiluria was also present in 10 of 36 patients with other diagnoses; overall, the test underperformed in this selected group compared with previous studies (Table 1). Ruffing et al. (8) felt that eosinophiluria was not suitable as a stand-alone test to make a diagnosis of AIN. It is also worth noting that, in addition to these studies, numerous case reports have described eosinophiluria in various diseases, including atheroembolic disease, allergic granulomatosis, bladder tumors, ileal conduits, and asthma.

Despite the unclear utility of eosinophiluria, with its varying ranges of sensitivity and specificity, the test has been widely used to evaluate for the possibility of AIN. Our experience is that many clinicians automatically order this test in the workup of hospital-acquired AKI. A positive result is then incorrectly used to support a diagnosis of AIN (and discontinuation of potential culprit medications), whereas negative urinary eosinophils often lead to inappropriate exclusion of AIN as the cause of AKI. A letter to the New England Journal of Medicine highlighted the common and concerning clinical practice of ordering urinary eosinophils for AIN workup (9).

In this issue of CJASN, Muriithi et al. (10) seem to strike, perhaps, the final blow against the use of eosinophiluria in diagnosing AIN. Using data over an 18-year period, Muriithi et al. (10) identified 556 patients with both urinary eosinophil testing and kidney biopsies performed within the same week. Approximately two thirds (63 of 91) of the biopsy-confirmed AIN cases in this cohort tested negative for any urinary eosinophils. When urinary eosinophils ≥1% were used as a cutoff for a positive test, this assay identified only 28 of 91 AIN cases (30.8%), with a similar positive rate in acute tubular necrosis (20 of 69 cases or 29.0%). A 5% urinary eosinophil cutoff only slightly improved specificity but with a concomitant decreased sensitivity.

The study by Muriithi et al. (10) is not without its limitations, chief among them the selection bias inherent in any biopsy-based cohort. Most diagnoses of AIN are made clinically using history, physical examination, and laboratory findings; renal biopsy is generally performed if either the diagnosis of AIN is unclear or the diagnosis is likely but confirmation is needed before modifying therapy or in select cases, starting steroids. In theory, a large number of non-biopsied patients with AIN and urinary eosinophils could have been excluded from this cohort. Nonetheless, such clinical diagnoses of AIN are themselves flawed given lack of confirmation by biopsy, which remains the gold standard for diagnosing AIN. Manual inspection of the urinary sediment by a treating nephrologist is likely the next best option after kidney biopsy in diagnosing AIN. A sediment replete with white blood cell casts and urinary eosinophils, in the setting of an appropriate clinical history, may be sufficient to diagnose AIN. Notably, the results presented by Muriithi et al. (10) highlight the poor performance of the Hansel stain in AIN and do not necessarily apply to manual sediment analysis.

Although the lack of specificity of eosinophiluria for AIN is understandable, why is the test insensitive, even with use of Hansel stain? Possible explanations are that (1) eosinophils are not always shed into the urine at a high enough number to reach >1% of white blood cells, (2) urinary eosinophils lyse before visualization, or (3) the cause of AIN is not characterized by a prominent eosinophilic interstitial infiltrate. The last point is true in many systemic diseases associated with AIN, such as sarcoidosis, Sjogren’s syndrome, and various infections. In contrast, drug-induced AIN is considered to be allergic and marked by an eosinophilic infiltrate. In fact, many pathologists raise the specter of drug-induced AIN when eosinophils are present. However, although β-lactam drugs are classically associated with this finding, not all medications promote an eosinophilic infiltrate (i.e., nonsteroidal anti-inflammatory drugs).

In fact, four studies focusing on drug-induced AIN showed that only 24 of 40 (60%) patients had a significant eosinophilic interstitial infiltrate on biopsy (2,3,11,12). Regardless of the explanation, the paper by Muriithi et al. (10) should signal the end of urinary eosinophils as a useful biomarker for AIN and provide nephrologists with data to definitively recommend against this test.

This end of an era is particularly important, because we have entered a new era of greater use of steroids in confirmed cases of AIN. Although no prospective, randomized controlled trials have emerged to support the use of steroid therapy for AIN, over the last two decades, data showing the

### Table 1. Urinary eosinophils in the diagnosis of acute interstitial nephritis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients (N)/Eosinophil Stain</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Other Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corwin et al. (4)</td>
<td>65/Wright</td>
<td>8/9 (88%)</td>
<td>29/56 (52%)</td>
<td>UTI (12/25), RCIN (3/4), CKD (2/5), GN (2/2), PYN (2/4)</td>
</tr>
<tr>
<td>Nolan et al. (6)</td>
<td>92/Hansel</td>
<td>10/11 (91%)</td>
<td>69/81 (85%)</td>
<td>ATN (0/30), PYN (0/10), GN (1/6), RPGN (4/10), prostatitis (6/10)</td>
</tr>
<tr>
<td>Corwin et al. (7)</td>
<td>183/Hansel</td>
<td>5/8 (63%)</td>
<td>160/175 (93%)</td>
<td>ATN (1/29), UTI (5/37), DN (4/17), PR (1/39)</td>
</tr>
<tr>
<td>Ruffing et al. (8)</td>
<td>51/Hansel</td>
<td>6/15 (40%)</td>
<td>26/36 (72%)</td>
<td>GN (4/6), CKD (2/5), PYN (1/2), PR (1/3)</td>
</tr>
<tr>
<td>Total</td>
<td>391</td>
<td>29/43 (67%)</td>
<td>284/348 (82%)</td>
<td></td>
</tr>
</tbody>
</table>

UTI, urinary tract infection; RCIN, radiocontrast-induced nephropathy; PYN, pyelonephritis; ATN, acute tubular necrosis; RPGN, rapidly progressive GN; DN, diabetic nephropathy; PR, prerenal.
benefits of steroid therapy for AIN have improved from anecdotal case reports to larger cohort series with favorable outcomes in treated versus nontreated patients (13). Appropriate use of steroids, along with removal of the offending agent, can potentially reverse AIN lesions, restore kidney function, and minimize risk of progression to CKD and/or ESRD. This potential, however, seems to rest primarily on how quickly the lesion is identified and how early steroid therapy is introduced. Urinary eosinophils clearly are inadequate biomarkers for such crucial identification.

Disclosures
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References

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