ABSTRACT. **Objective.** Experimental evidence suggests that neutrophils and their metabolites play an important role in the pathogenesis of pyelonephritis. The aim of this study was to investigate the diagnostic value of polymorphonuclear elastase-a,-antitrypsin complex (E-a1-Pi) for the detection of acute pyelonephritis in children.

**Methods.** Eighty-three patients, 29 boys and 54 girls, 25 days to 14 years of age, with first-time symptomatic urinary tract infection were prospectively studied. Fifty-seven healthy children served as controls. Dimercapto-succinic acid (DMSA) scan and voiding cystourethrography were performed in all patients. Plasma and urinary E-a1-Pi, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neutrophil count, urinary N-acetyl-β-glucosaminidase (NAG), N-acetyl-β-glucosaminidase b (NAG b), and creatinine levels were measured in all patients on admission and 3 days after the introduction of antibiotics. The same markers were also measured in the control subjects.

**Results.** Planar DMSA scintigraphy demonstrated changes of acute pyelonephritis in 30 of 83 children (group A). It was normal in the remaining 53 children (group B). The sex and age distributions were not significantly different between the 2 groups, as well as between the patients and the control subjects (group C). Nineteen of the 33 children with a normal DMSA had body temperature ≥38°C, whereas all but 4 children with abnormal DMSA had temperature ≥38°C. Therefore, the temperature was significantly different between these 2 groups. The sensitivity and specificity of fever (≥38°C) as an indicator of renal involvement based on isotopic findings were 86% and 64%, respectively. Given the significant number of the febrile children with normal DMSA scintiscans, group B was subdivided into B1 with 19 febrile children (14 boys and 5 girls) and B2 with 34 children whose body temperature was below 38°C (8 boys and 26 girls). The sex and age distribution was significantly different between groups B1 and B2. The mean age of group B1 was .78 years (range: 28 days to 9 years; median: .25 years; standard deviation: 2.1). All but 1 child in this group were younger than 1 year of age. In contrast, in group B2, there were only 4 infants, the remaining 30 children were older than 2.5 years (mean age: 6 years; median: 7 years; standard deviation: 3.5; range: 34 days to 12 years). The mean duration of fever before hospital admission was 2.8 days for group A and 1.8 days for group B2. This difference was not statistically significant. Similarly, body temperature was not significantly different between these 2 groups. The distribution of plasma E-a1-Pi values was normal in the control subjects. The sensitivity and specificity of plasma E-a1-Pi, as an indicator of renal involvement, were 96% and 50%, respectively, taking the 95th percentile of the reference range as a cutoff value. However, considering as a cutoff value the level of 72 µg/dL (95th percentile of group B1), its sensitivity and specificity were 74% and 86%, respectively. Plasma E-a1-Pi levels were significantly elevated in group A compared with group B and in both groups, the plasma E-a1-Pi values were significantly higher than in the control subjects. A significant difference also was noticed between group A and each of the subgroups B1 and B2 and also between the subgroups themselves. Plasma E-a1-Pi concentrations correlated significantly with neutrophil count in groups A (r = .3), B (r = .4), and B2 (r = .46), but the correlation was not significant in group B1. ESR levels showed, among the different groups, similar differences with those of E-a1-Pi values. Unlike E-a1-Pi, CRP levels were comparable between groups A and B2, which both consisted of febrile children. Neutrophil count was not significantly different between subgroups B1 and B2. Considering 20 mg/dL as a cutoff level for CRP, its sensitivity and specificity for identifying the urinary tract infection site were 69% and 57%, respectively. The sensitivity and specificity of ESR, using 30 mm/hour as a cutoff value, were 90% and 59%, respectively. The comparison of febrile infants with a normal DMSA scan (all but 1 child of group B2) with those with an abnormal one (a subpopulation of group A) showed significant difference of plasma E-a1-Pi and ESR but not CRP and neutrophils. Urinary E-a1-Pi, as well as NAG and NAG b/creatinine values, showed no significant difference between groups A and B. NAG and NAG b levels were significantly higher in group B2 compared with group B1, but they were similar with those of group A. Reflux was noticed in 16/63 children (19%), 9/30 children with an abnormal DMSA (30%) and 7/53 with a normal DMSA scan (13%); this difference was not statistically significant. The sensitivity and specificity of reflux, as an indicator for renal lesions on the DMSA scan, were 30% and 86%, respectively. The follow-up investigation on the third day revealed that plasma E-a1-Pi levels, as well as CRP, were significantly lower compared with their levels on admission within each group. Despite the fact that ESR levels were lower on the third day, the difference was not significant.

**Conclusions.** Plasma E-a1-Pi is a sensitive but not a specific marker for the detection of acute pyelonephritis. Urinary E-a1-Pi levels cannot be used for this purpose. 

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Urinary tract infection (UTI) is a common clinical problem in infancy and childhood. Prompt diagnosis of the infection and the localization of its level are of great importance in determining the duration of treatment and the appropriate investigation. In the last decade, the dimercaptosuccinic acid (DMSA) scintigraphy has been considered an objective method for the localization of the UTI site, although there are some concerns about its accuracy in infancy.

The clinical parameters and inflammatory markers, which primarily have been used as indices for the differentiation of lower from upper UTI, are fever, age, reflux, leukocytes and/or neutrophil count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), N-acetyl-β-glucosaminidase (NAG), and β2 microglobulin. Several studies tried to correlate these parameters with the UTI site but the results were rather conflicting.

Elastase is a protease stored in the azurophilic granules of neutrophils. While releasing on cell activation, it is capable of degrading connective tissue components. However, it is normally bound and at least partially inactivated by α1 antitrypsin protease inhibitor. The formed complex (elastase-α1-antitrypsin complex [E-α1-Pi]), which is stable and can easily be identified by an enzyme-linked immunosay, has been shown to improve early diagnosis of neonatal septicemia. It has also been found elevated in other inflammatory conditions, such as meningitis, inflammatory bowel disease, etc.

In this study, we investigated the possible use of E-α1-Pi for the diagnosis of pyelonephritis. Taking into account the high molecular weight of α1 antitrypsin (111,000), the presence of neutrophil E-α1-Pi in urine attributable to glomerular filtration is rather unlikely. There was evidence for the pathogenic role of E-α1-Pi in glomerulonephritis, as well as some evidence that the lower urinary tract makes a significant contribution to urinary E-α1-Pi levels. The E-α1-Pi as a response to renal infection has not been assessed in clinical studies. However, there is reason to assume that E-α1-Pi is released during UTI and its plasma and/or urinary levels could be an indicator of the infection site.

The aim of this study was to investigate the diagnostic value of E-α1-Pi for the detection of acute pyelonephritis in children with UTI and to compare it with other inflammatory indices traditionally used for this purpose.

PATIENTS AND METHODS

Eighty-three consecutive patients (29 boys and 54 girls) below 14 years of age (range: 25 days to 14 years; mean: 3.84 years; median: 2.5 years) with first time symptomatic UTI documented by a positive urine culture (>10⁵ colony forming units/mL) of single strain, were enrolled in the study regardless of the presence of fever. The eligibility of patients was determined if there was no previous history of urinary tract obstruction, other chronic inflammatory condition, or other current infectious disease.

Fifty-seven clinically healthy children (20 boys and 37 girls) of similar age and sex of the patients served as controls. All these children had no history of UTI and their urine culture was sterile on admission to the study.

This study was approved by the hospital's ethics committee. Informed parental consent was requested and obtained.

Body temperature, CRP, ESR, neutrophils count, plasma and urinary E-α1-Pi, and urinary NAG and N-acetyl-β-glucosaminidase b (NAG b) levels were measured in all patients on admission before the administration of antibiotics. The same investigation was repeated 3 days after the introduction of treatment. It was also performed in all the control subjects. The treatment consisted of parenteral or oral administration of antibiotics depending on the patient’s age and clinical condition. The route of administration was changed from parenteral to oral within 3-7 days and a 10-day course of therapy was completed in all cases. The total duration of parenteral antibiotic treatment was based on the patient’s age (infants <3 months of age were all given antibiotics intravenously for 7 days), the clinical response to antibiotics and the results of the isotopic investigation.

E-α1-Pi was measured in ethylenediaminetetraacetic acid plasma and in urine supernatant both obtained by centrifugation at 2000 g for 10 minutes within 1 hour of collection. Plasma and urine specimen were kept at −20°C until assayed. Measurements were conducted with a commercially available enzyme-linked immunosay according to the manufacturer's instructions (12580 PMN Elastase, Merck immunosay, Merck, Darmstadt, Germany). NAG and NAG b levels were measured after a fluorometric method, as initially described by Woollen and Walker and subsequently modified by Gnanadurai et al.

Imaging studies of the urinary tract were performed in all patients (DMSA scan, ultrasound, and voiding cystourethrography). Isotopic evaluation was performed within 96 hours of the UTI diagnosis, using a computer system camera equipped with a high-resolution parallel-hole collimator. A dose of 5 MBq/kg99mTc-DMSA (minimum dose of 10 MBq) was administered intravenously. At least 4 hours after the injection, images of 450,000 counts each were obtained in anterior, posterior, and oblique views. A pinhole view was obtained to visualize a particular region of interest in greater detail, using a pinhole collimator fitted with an aperture of 5 mm in diameter. The children, particularly the youngest ones, were adequately immobilized during the period of examination, without using any kind of sedation. The DMSA scan was interpreted independently by 2 consultant radiologists who were aware only of the UTI diagnosis but not of the patient’s clinical findings and laboratory results. When the disclosure of the interpretations revealed inconsistent findings, the 2 specialists jointly reexamined the scans and a final diagnosis was established. Acute pyelonephritic lesions were diagnosed when scintiscan revealed focal (single or multiple) or diffuse areas of diminished 99mTc-DMSA uptake with an intact or slightly bulging contour according to criteria of Majd and Roshston and Patel et al.

The voiding cystourethrography was obtained by standard radiographic technique at a median of 17 days (range: 5-58 days) after the administration of antibiotics. Reflux grades were evaluated according to the International Reflux Study Committee.

Statistics

Mann Whitney U test, Wilcoxon test, χ², and Spearman’s correlation coefficient were used for the data analysis as appropriate. A P value <.05 was considered statistically significant. The SPSS program (SPSS Inc, Chicago, IL) for Windows was used for the analysis.

RESULTS

Planar DMSA scintigraphy demonstrated changes of acute pyelonephritis in 30/83 children (group A). It was normal in the remaining 53 children (group B). Based on the initial evaluation, the interobserver agreement for interpretation of the scintiscans was 96%. Although according to the patients’ history this was the first UTI episode, renal scars were identified...
in 2 children, 1 in an otherwise normal scan and the other in a child with isotopic evidence of acute pyelonephritis.

The sex and age distributions were not significantly different between the 2 groups as well as between the patients and the controls (group C). Nineteen of the 53 children with a normal DMSA had body temperature ≥38°C, although all but 4 children with abnormal DMSA had temperature ≥38°C. Therefore, the temperature was significantly different between these 2 groups (P < .001). The sensitivity and specificity of fever (≥38°C), as an indicator of renal involvement based on isotopic findings, were 86% and 64%, respectively.

Given the significant number of the febrile children with a normal DMSA scintiscan, group B was subdivided into B1, consisting of 19 febrile children (14 boys and 5 girls) and B2 with 34 children (8 boys and 26 girls) whose body temperature was below 38°C. The sex and age distributions were significantly different between B1 and B2 (P < .001).

The mean age of group B1 was 7.8 years (range: 28 days to 9 years; median: 25 years; standard deviation: 2.1). All but 1 child in this group were younger than 1 year of age. In contrast, in group B2, there were only 4 infants, the remaining 30 children were older than 2.5 years of age (mean: 6 years; median: 7 years; standard deviation: 3.5; range: 34 days to 12 years).

The mean duration of fever before hospital admission was 2.8 days for group A and 1.8 days for group B1. This difference was not statistically significant. Similarly body temperature was not significantly different between these 2 groups.

The distribution of plasma E-a1-Pi values was normal in the control subjects. The sensitivity and specificity of plasma E-a1-Pi, as an indicator of renal involvement, were 96% and 50%, respectively, taking as a cutoff value the 95th percentile of the reference range. However, considering as a cutoff value the 95th percentile of group B2, its sensitivity and specificity were 74% and 86%, respectively.

Plasma E-a1-Pi levels were significantly elevated in group A compared with group B (P < .01) and in both groups the plasma E-a1-Pi values were significantly higher than in the control subjects (P < .001). A significant difference was also noticed between group A and each of the subgroups B1 (P = .008) and B2 (P < .001) and also between the subgroups themselves (P = .035; Table 1 and Fig 1). Plasma E-a1-Pi concentrations correlated significantly with neutrophil count in groups A (r = 3; P = .047), B (r = .4; P < .01), and B2 (r = .46; P = .02) but the correlation was not significant in group B1.

ESR levels showed, between the different groups, similar differences with those of E-a1-Pi values (Fig 2). Unlike E-a1-Pi, CRP levels were comparable between groups A and B2 (P = .12), which both consisted of febrile children. Neutrophil count was not significantly different between subgroups B1 and B2. Considering 20 mg/dL as a cutoff level for CRP, its sensitivity and specificity for identifying the UTI site were 69% and 57%, respectively. The sensitivity and specificity of ESR, using 30 mm/hour as a cutoff value, were 90% and 59%, respectively. The sensitivity, specificity, and accuracy of the studied inflammatory indices are summarized in Table 2.

The comparison of febrile infants with a normal DMSA scan with those with an abnormal one (a subgroup of group A) showed significant difference of plasma E-a1-Pi (P = .015) and ESR (P = .003) but not of CRP and neutrophils.

Urinary E-a1-Pi, as well as NAG and NAG b/creatinine values, showed no significant difference between groups A and B. NAG and NAG b levels were significantly higher in group B1, compared with group B2 (P = .001) but they were similar with those of group A.

Reflux was noticed in 16/83 children (19%), grade III to IV in half of them (8/16) and grade I to II in the remaining (8/16). The prevalence of reflux was 30% (9/30) in children with an abnormal DMSA and 13% (7/53) in those with a normal DMSA scan and this difference was not statistically significant. Therefore, 9/16 children with reflux (56%) had renal lesions on the DMSA scintigraphy, but this proportion raised to 75% (6/8) for children with reflux of grade III to IV. The sensitivity and specificity of reflux, as an indicator for renal lesions on the DMSA scan, were 30% and 86%, respectively.

The follow-up investigation on the third day revealed that plasma E-a1-Pi levels, as well as CRP, were significantly lower compared with their levels on admission within each group (Fig 3). Despite the fact that ESR levels were lower on the third day, the difference was not significant.

DISCUSSION

The localization of UTI level is of great importance for the appropriate patient’s management. Variable markers have been used for this purpose but none of these has been considered adequately sensitive and specific for such a differentiation. In this study, we investigated the possible use of E-a1-Pi for the diagnosis of pyelonephritis. It was shown that plasma E-a1-Pi, ESR and CRP were in descending order sensitivity and specificity for the detection of pyelonephritis, considering the DMSA scan as a reference method.

Plasma E-a1-Pi has not previously been used to

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TABLE 1. Plasma and Urinary E-a1-Pi Levels in Different Groups of Patients and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>B1</th>
<th>B2</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>103.5</td>
<td>54.1</td>
<td>72.5</td>
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<tr>
<td>Median</td>
<td>95</td>
<td>44.5</td>
<td>62.1</td>
<td>37</td>
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<tr>
<td>Standard error</td>
<td>9.3</td>
<td>6.5</td>
<td>15.6</td>
<td>3</td>
<td>.7</td>
</tr>
<tr>
<td>Urinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>37.7</td>
<td>653</td>
<td>1446</td>
<td>135.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Median</td>
<td>78.5</td>
<td>90</td>
<td>280</td>
<td>70.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Standard error</td>
<td>35.3</td>
<td>290.2</td>
<td>700.3</td>
<td>38.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

http://www.pediatrics.org/cgi/content/full/105/2/e28
distinguish upper from lower UTI. Experimental studies found that E-a1-Pi release contributed to renal damage pathogenesis in pyelonephritis. It was shown that extracellular granulocyte E-a1-Pi activity was significantly higher in neutrophils stimulated by bacterial strains associated with renal damage, compared with either unstimulated neutrophils or those stimulated by strains not associated with renal damage. These findings were also supported by Matsumoto et al who showed in a rat model the preventive effect of ulinastatin, a strong inhibitor of neutrophil E-a1-Pi, on renal scarring induced by direct or ascending infection with Serratia marcescens or Escherichia coli. Although these studies suggested a possible causal relationship between E-a1-Pi and renal damage in pyelonephritis, they did not explore the possible usefulness of plasma and/or urinary E-a1-Pi measurement in the localization of the UTI site.

Our data suggest that E-a1-Pi sensitivity for the diagnosis of UTI site is high, although its specificity is rather poor. Although a correlation between plasma E-a1-Pi and neutrophil count was found in all but one of the groups, it should be noted that in

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**TABLE 2. Inflammatory Indices for the Diagnosis of Acute Pyelonephritis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-a1-Pi</td>
<td>.96</td>
<td>.50</td>
<td>.65</td>
</tr>
<tr>
<td>ESR</td>
<td>.90</td>
<td>.59</td>
<td>.68</td>
</tr>
<tr>
<td>CRP</td>
<td>.69</td>
<td>.57</td>
<td>.62</td>
</tr>
</tbody>
</table>

---

**Fig 1.** Plasma E-a1-Pi distribution in UTI patients, based on isotopic findings (groups A and B), and their controls (group C).

**Fig 2.** ESR distribution in UTI patients and controls.
individual cases in group A high E-a1-Pi values were found in the presence of a relatively low neutrophil count. In contrast, in individual cases in group B, relatively low E-a1-Pi values were found while neutrophil count was high. This finding indicates that the degree of neutrophil activation may be more important than the actual number of neutrophil count in evaluating the amount of liberated E-a1-Pi, which may also reflect the degree of neutrophil activation in renal parenchyma.

During the last decade, DMSA scintigraphy has been considered the procedure of choice for assessing renal involvement at the early stage of UTI in children and for detecting renal scars. Experimental studies in piglets, which have a multipapillary renal architecture similar to humans, demonstrated that DMSA scan has sensitivity from 80% to 91% and specificity from 99% to 100% for detecting acute pyelonephritic lesions. When the severity of pyelonephritis was graded according to the extent of renal parenchymal involvement, the undetected lesions were minimal. The consistency in interpretation of DMSA findings was another issue raised in literature. However, the criteria developed by Majd and Rushton, as well as the standardized classification proposed by Patel et al., minimize the variability in assessment of renal cortical scan lesions.

In clinical practice, some concerns have been raised about DMSA sensitivity in infancy for revealing acute renal inflammatory changes. Our data showed that 18 febrile infants, up to 1 year of age, had no renal changes on the DMSA scan, although the clinical and laboratory indices were compatible with acute pyelonephritis. It may be attributed, at least to some extent, to the fact that these patients received antibiotics earlier than those with an abnormal scan, because the duration of fever before hospital admission was shorter, although this difference was not statistically significant. Furthermore, generally speaking it is rather common that the younger the patient the greater the alertness of the parents in seeking medical advice; therefore, infants are accustomed to starting treatment earlier. As it is documented in epidemiologic studies, over 90% of infants under 1 year of age with symptomatic UTI are febrile and are more likely to present sooner before renal parenchymal involvement.

Technical difficulties related to this age, such as the limited spatial resolution of the γ camera, might explain a number of possibly false negative results in this age group. It is also well known that DMSA scintigraphy reflects the function of proximal tubular cells and the intrarenal blood flow; consequently, an infection limited to the papilla and the medulla may not be detected on a DMSA scan. A recent study comparing planar DMSA scintigraphy and single photon emission computerized tomography (SPECT) scan in patients under 3 years of age, demonstrated a higher sensitivity of the latter technique, and it was suggested that acute pyelonephritic lesions may occur more commonly than previously believed. However, in an animal model when DMSA scans were obtained by both pinhole and SPECT techniques, the sensitivity of SPECT, for the identification of acute pyelonephritic lesions, was better than pinhole imaging but its specificity was lower, resulting in an overall similar accuracy. The above mentioned remarks imply that in some cases, even using planar DMSA scan, it may be difficult to assess the renal inflammatory involvement and its correlation to laboratory parameters.

It is also of importance that the group of febrile children with a normal DMSA scan had significantly different plasma E-a1-Pi from both group A with abnormal DMSA and group B of nonfebrile children with a normal DMSA scan. Therefore, the febrile children with a normal DMSA are a different population not only from children with renal changes on the DMSA scan but also from nonfebrile patients with a normal renal scan. This assumption is supported by urinary NAG and NAG b levels, which although they are not significantly different between the 2 primary groups (A and B), they are significantly higher in subgroup B1 compared with B2. This finding suggests that the former population may have renal involvement unrecognized by the DMSA scan, because increased NAG excretion indicates a proximal tubular dysfunction. However, the lack of control children with fever of nonrenal origin in our study does not allow such a conclusion, because...
fever of any origin seems to be associated with transient proximal tubular dysfunction with increased excretion of tubular proteins and enzymes in the urine.  

Urinary E-a_1-Pi is of no use in the localization of UTI site. It seems that the kidneys, as well as the lower urinary tract, make a significant contribution to overall urinary E-a_1-Pi. Therefore, there is a wide fluctuation of urinary E-a_1-Pi levels, which does not permit the distinction between upper and lower UTI.

In contrast to previous studies, our data revealed that CRP is not a very sensitive marker for localizing UTI site. However, Melis et al found elevated CRP and ESR in 57% and 83%, respectively, which is in accordance with our results. Although, we found that CRP was less sensitive than the other 2 inflammatory parameters (ESR and E-a_1-Pi), its accuracy was only slightly inferior to that of E-a_1-Pi. ESR was less sensitive than E-a_1-Pi (90% vs 96%) but the overall accuracy of these 2 markers was quite similar. ESR accuracy of 68% was in agreement with that found (70%) by Majd et al, taking 25 mm/hour as the cutoff value.

As far as the presence of reflux is concerned, a lower incidence (19%) than was previously reported was found. However, this difference could be explained by patient selection. Most studies, which demonstrated a higher incidence of reflux (22%-50%), were comprised primarily of febrile patients younger than 5 years of age with clinical manifestations of pyelonephritis. Our results support the view that although the children with reflux, particularly these with reflux of higher grade, are at a higher risk of having an abnormal DMSA scan, there is a significant number of patients with acute renal inflammatory lesions in the absence of reflux.

CONCLUSION

In summary, plasma E-a_1-Pi is a sensitive but not a specific marker for the detection of acute pyelonephritis. Urinary E-a_1-Pi can not be considered a valuable marker for this differentiation. However, the contribution of inflammatory markers for the diagnosis of acute pyelonephritis, particularly in infants, should be reevaluated in case the widespread use of SPECT scan shows that acute pyelonephritic lesions were underestimated by using planar scintigraphy.

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DOI: 10.1542/peds.105.2.e28

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