Association Between Escherichia coli Bacteriuria and Renal Function in Women

Long-term Follow-up

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Background: We sought to investigate whether Escherichia coli bacteriuria is associated with a decline in renal function or with the development of end-stage renal failure after long-term follow-up.

Methods: We performed a full cohort analysis for women who participated in 2 population-based studies. The baseline cohort consisted of women who collected morning midstream urine samples that were stored. In the cohort study, the presence of E coli bacteriuria was subsequently determined by real-time polymerase chain reaction. After a mean±SD follow-up of 11.5±1.7 years, blood samples were drawn from 490 women. In the nested case-control study, cases comprised all women who underwent kidney therapy (hemodialysis or renal transplantation) between participation in the baseline cohort study and a mean±SD of 13.8±7.4 years later.

Results: The mean±SD age at baseline was 45.0±3.2 years, and 48 women (10%) had E coli bacteriuria. After 11.5 years, the mean±SD creatinine clearance (Cockroft-Gault formula) was similar between the 2 groups (87±21 mL/min [1.5±0.4 mL/s] and 85±18 mL/min [1.4±0.3 mL/s] for women who had and those who did not have bacteriuria, respectively). In the nested case-control study, the prevalence of E coli bacteriuria was 14% among cases and control subjects. The odds ratio corrected for age for the development of end-stage renal failure in the presence of E coli bacteriuria at baseline was 1.1 (95% confidence interval, 0.4-2.8; \( P = .86 \)).

Conclusion: Escherichia coli bacteriuria is not associated with a decline in renal function or with the development of end-stage renal failure in a population of generally healthy women during 12 to 14 years of follow-up.

Arch Intern Med. 2007;167:253-257

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CHRONIC KIDNEY DISEASE IS an increasing public health problem. In the United States, the prevalence is estimated to be approximately 11% of the adult population.¹ Chronic kidney disease may progress to end-stage renal failure, a condition associated with high morbidity and mortality. In the Netherlands² and in the United States,³ the prevalence rates of end-stage renal failure are 65.8 and 144.6 cases per 100 000 population, respectively.

Although the urinary tract is normally sterile, asymptomatic bacteriuria is a common phenomenon, especially in women. Studies⁴ ⁵ report a prevalence of approximately 5% among healthy young women, increasing to more than 20% in elderly women. Escherichia coli is the most prevalent causative microorganism in symptomatic and asymptomatic bacteriuria, accounting for more than 80% of uncomplicated urinary tract infections.⁶ Most uropathogenic E coli possess type 1 fimbriae that have been shown to cause renal scarring in animal investigations.⁷ Previous findings indicate that patients with renal scarring due to pyelonephritis are at increased risk for the development of hypertension and chronic kidney disease.⁸ For asymptomatic bacteriuria, this association is less clear.⁹ ¹⁰ In a previous study,¹¹ it was reported that bacteriuria might affect renal function of women with type 1 diabetes mellitus. The present study addressed whether E coli bacteriuria in women is associated with an increased risk of a decline in renal function or the development of end-stage renal failure during long-term follow-up.

METHODOLOGY

To study the different endpoints (a decline in renal function and the development of end-stage renal failure), 2 separate studies were performed, a cohort study and a nested case-control study, using the same baseline cohort (Figure 1). This study was approved by the Medical Ethics Committee of the University Medical Center Utrecht, Utrecht, the Netherlands.
Baseline measurements performed between 1974 and 1986 included extensive questionnaires, a short medical examination, and the collection of a morning midstream urine sample. Data obtained through the questionnaires in-cluded blood pressure monitoring. Nonfasting blood samples were administered questionnaires and a medical examination that in-
cluded information on future morbidity and mortality. Baseline measurements performed between 1974 and 1986 included extensive questionnaires, a short medical examination, and the collection of a morning midstream urine sample. Data obtained through the questionnaires included age, diet, parity, drug use, marital status, smoking habits, and menopausal status. During the medical examination, weight and height were measured. Approximately 200 mL of urine was collected in plastic polypropylene jars without preserving agents and was stored at −20°C for future analyses. All women gave oral consent to use their data and urine samples for future scientific research.

Figure 1. Using the same baseline cohort, a cohort study and a nested case-control study were performed. To select the cases, data from the baseline cohort and the Renal Replacement Registry Netherlands (RENINE) were matched on maiden and married names combined with date of birth. A group consisting of 4 times the number of cases was randomly selected from the baseline cohort to form the control group. Prospect-EPIC indicates European Prospective Investigation Into Cancer and Nutrition.

Consortium & Study on Relation Between Nutrition and Cancer

European Prospective Investigation Into Cancer and Nutrition

Between January 1974 and December 1986 all women born between 1911 and 1945 who lived in Utrecht, the Netherlands, and the surrounding area were invited for a breast cancer screening program, with a participation rate of 68% to 72%. A total of 38 994 women aged 39 to 68 years at intake participated (the baseline cohort). Baseline measurements performed between 1974 and 1986 included extensive questionnaires, a short medical examination, and the collection of a morning midstream urine sample. Data obtained through the questionnaires included age, diet, parity, drug use, marital status, smoking habits, and menopausal status. During the medical examination, weight and height were measured. Approximately 200 mL of urine was collected in plastic polypropylene jars without preserving agents and was stored at −20°C for future analyses. All women gave oral consent to use their data and urine samples for future scientific research.

Cohort Study

To address the association between E coli bacteriuria and renal function, we performed a full cohort analysis for women who participated in the baseline cohort and in a follow-up cohort. Between January 1993 and December 1997, 50 313 women living in Utrecht and the surrounding area who were scheduled for breast cancer screening during this period received an invitation by mail to join an additional study (the European Prospective Investigation Into Cancer and Nutrition) to assess the association between nutrition and cancer and other chronic diseases (the follow-up cohort). A total of 17 357 women (participation rate, 35%) agreed to take part. Participants were aged 49 to 70 years at enrollment. Information was collected on the basis of 2 self-administered questionnaires and a medical examination that included blood pressure monitoring. Nonfasting blood samples were successfully drawn from 98% of the women and were stored under liquid nitrogen at −196°C. Approximately 88% of the women signed a detailed informed consent, enabling the researchers to use their blood samples for future analysis and to obtain information on future morbidity and mortality.

Five hundred six women were selected randomly from among the women who participated in the baseline cohort and the follow-up cohort. Sixteen women were excluded for the following reasons: missing urine sample (n = 13), missing blood sample (n = 2), or kidney transplantation before blood withdrawal (n = 1). Therefore, 490 women were included in the prospective study to assess the association between bacteriuria and renal function. The mean±SD duration of follow-up was 11.5±1.7 years, ranging from 8.1 to 18.6 years from baseline until participation in the follow-up study.

Nested Case-Control Study

To obtain follow-up information on end-stage renal failure, we obtained data from the Renal Replacement Registry Netherlands (RENINE) that were available in May 2002; RENINE is a foundation in which all Dutch nephrologists participate and where patients are registered who at any time used kidney therapy (hemodialysis or renal transplantation), with a coverage rate throughout the years of almost 100%. To select the cases, data from the baseline cohort and RENINE were matched on maiden and married names combined with date of birth (Figure 1). A group consisting of 4 times the number of cases was randomly selected from the baseline cohort to form the control group. After excluding 4 cases with missing urine samples, 49 cases and 206 control subjects were included. Among the cases, the mean±SD duration until the date of kidney therapy was 13.8±7.4 years, with minimum and maximum durations of 1.6 and 25.5 years, respectively. Among the controls, the mean±SD follow-up (ie, the time from participation in the baseline cohort until the study end point in May 2002) was 27.0±0.2 years.

Escherichia coli Bacteriuria

Escherichia coli bacteriuria was defined as the presence of 10^5 colony-forming units (CFU) of E coli per milliliter of urine. It was diagnosed by a real-time polymerase chain reaction (PCR) assay that was developed and validated previously and has been used by others. Polymerase chain reaction primers and probes complementary to regions of the gadA gene specific for E coli were designed for the real-time PCR assay. The laboratory sensitivity and specificity of the real-time PCR assay tested with 30 E coli strains and 41 non-E coli strains (including the most prevalent uropathogens and many members of the vaginal and anal flora) were 100% (50/50) and 98% (40/41), respectively. For clinical evaluation, 42 urine specimens (12 with and 30 without E coli) were tested, and the results were compared with those of a clinical conventional urine culture. The sensitivity and specificity of the real-time PCR in these clinical samples were 92% and 87%, respectively. The test results were quantitative and allowed distinguishing between significant bacteriuria (ie, 10^5 CFU/mL) and low-count bacteriuria that might have been due to contamination.

To test a study urine sample, 1 mL of urine was centrifuged at 16,250g for 5 minutes. The pellet was washed twice, it was suspended in 1 mL of sterile injection water, and 100 µL of the suspension was heated for 2 minutes at 100°C in a microwave oven for DNA preparation. Five microliters was added to the real-time PCR reaction volume as a DNA template. Each 25-µL reaction volume consisted of 12.5 µL of Taqman Universal PCR Master Mix (Applied Biosystems, Branchburg, NJ) that contains AmpliTaq Gold DNA polymerase, 300-nm forward primer (5'-ACCGACAATCTGTGGTATGC-3'), 300-nm reverse primer (5'-AGCAGACGTTTCAACAAGTCCA-3'), and 175-nm probe (5'-CATTATGTGTCTGGGCTTCCAGA-3'); the DNA template was the last ingredient added. The sequence detection system (ABI PRISM 7700; PE Biosystems, Nieuwerkerk aan de IJssel, the Netherlands), which detects and quantitates nucleic acid, was used for the real-time PCRs. Cycling variables included the uracil-N-glycosylase reaction at 50°C for 2 minutes, then AmpliTaq Gold activation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds and combined annealing and extension at 60°C for 1 minute. Fluorescence emitted from
RENAL FUNCTION

We used the Cockroft-Gault formula to estimate the creatinine clearance (in milliliters per minute) as a proxy for the glomerular filtration rate. In addition, the calculated glomerular filtration rate was categorized as 1 of the following 5 stages (according to the National Kidney Foundation classification): at least 90 mL/min [1.5 mL/s] (stage 1), 60 to 89 mL/min [1.0-1.5 mL/s] (stage 2), 30 to 59 mL/min [0.5-1.0 mL/s] (stage 3), 15 to 29 mL/min [0.3-0.5 mL/s] (stage 4), or less than 15 mL/min [<0.3 mL/s] (stage 5).

STATISTICAL ANALYSIS

The present results are based on a cohort study of 490 women who were followed up for a decline in renal function and on a nested case-control study on the development of end-stage renal failure, both relative to the presence of E coli bacteriuria at baseline.

Baseline characteristics are compared between women who had and those who did not have bacteriuria. Comparisons between means were performed using the t test, and comparisons between nominal or categorical data were performed using the chi-square test. Linear regression analysis was used to calculate the difference in creatinine clearance between women who had and those who did not have bacteriuria. The distribution among 5 stages of renal function (based on the glomerular filtration rate) according to women who had and those who did not have bacteriuria was tested using the Mann-Whitney test. In the nested case-control study, the relative risk of the development of end-stage renal failure in the presence vs the absence of bacteriuria was estimated by the odds ratio and 95% confidence interval as calculated using logistic regression analysis. Adjustments were made for potential confounding factors (i.e., age and weight).

RESULTS

COHORT STUDY

The baseline characteristics of the follow-up cohort are given in Table 1. Forty-eight (10%) of 490 women were classified as having E coli bacteriuria at baseline. No correlation was found between the presence of bacteriuria and age. At baseline, the use of antibiotics was the only factor that significantly differed between women who had and those who did not have bacteriuria (33% vs 11%, \( P = .047 \)).

At study end point, the mean ± SD creatinine clearances among 48 women who had bacteriuria at baseline and among 442 women who did not have bacteriuria at baseline were 87 ± 21 mL/min and 85 ± 18 mL/min, respectively.

Table 1. Cohort Study Baseline Characteristics Among Women With and Without Escherichia coli Bacteriuria at Baseline*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Absent (n = 442)</th>
<th>Present (n = 48)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>45.0 ± 3.2</td>
<td>45.4 ± 3.2</td>
<td>.35</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>102 (23.1)</td>
<td>12 (25.0)</td>
<td>.77</td>
</tr>
<tr>
<td>Body mass index, mean ± SD†</td>
<td>24.2 ± 3.5</td>
<td>24.9 ± 3.6</td>
<td>.21</td>
</tr>
<tr>
<td>Married or living with partner (n = 377)</td>
<td>303 (89.6)</td>
<td>37 (94.9)</td>
<td>.30</td>
</tr>
<tr>
<td>Given birth to ≥1 living child</td>
<td>392 (88.7)</td>
<td>45 (93.8)</td>
<td>.28</td>
</tr>
<tr>
<td>Use of antibiotics (n = 113)</td>
<td>11 (10.6)</td>
<td>3 (33.3)</td>
<td>.047</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) or mean ± SD among 490 subjects.
†Calculated as weight in kilograms divided by height in meters squared.

Table 2. Cohort Study Follow-up Results on Renal Function Among Women With and Without Escherichia coli Bacteriuria at Baseline (Univariate Analysis)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Absent (n = 442)</th>
<th>Present (n = 48)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine level, mean ± SD, mg/dL</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>.68</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD, mL/min</td>
<td>85 ± 18</td>
<td>87 ± 21</td>
<td>.69</td>
</tr>
<tr>
<td>Stage†</td>
<td></td>
<td></td>
<td>.55</td>
</tr>
<tr>
<td>1</td>
<td>153 (34.6)</td>
<td>19 (39.6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>273 (61.8)</td>
<td>26 (54.2)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 (3.2)</td>
<td>3 (6.3)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 (0.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) or mean ± SD.
†Stage 1, 90 mL/min or higher (>1.5 mL/s); stage 2, 60 to 89 mL/min (1.0-1.5 mL/s); stage 3, 30 to 59 mL/min (0.5-1.0 mL/s); stage 4, 15 to 29 mL/min (0.3-0.5); and stage 5, less than 15 mL/min (<0.5 mL/s).

SI conversion factor: To convert creatinine to micromoles per liter, multiply by 88.4; creatinine clearance to milliliters per second, multiply by 0.01667.

Figure 2. At study end point in the cohort study, the mean ± SD creatinine clearances among 48 women who had bacteriuria at baseline and among 442 women who did not have bacteriuria at baseline were 87 ± 21 mL/min and 85 ± 18 mL/min, respectively.
In this prospective cohort study in a population of healthy women, no association was found between the presence of E coli bacteriuria at baseline and a decline in renal function after a mean follow-up of 12 years. Similarly, there was no association between the presence of E coli bacteriuria at baseline and the development of end-stage renal failure after a mean follow-up of 14 years.

Among the strengths of our study are its size and the length of follow-up. Almost 500 women were evaluated for the development of a decline in renal function during a mean follow-up of 12 years. The baseline cohort consisted of more than 38,000 potential study subjects, which allowed us to identify 49 women who developed end-stage renal failure in the presence of E coli bacteriuria at baseline and the development of end-stage renal failure after a mean follow-up of 14 years.

The limitations of the study include the fact that we had to rely on only 1 urine sample per subject to define the presence or absence of bacteriuria. However, this method was validated previously. We assumed that bacteriuria might be transient in a proportion of the study subjects and that the presence of bacteriuria at a single point reflects a higher susceptibility to recurrent and persistent bacteriuria in general, even after antimicrobial therapy. Previous findings support this assumption. A Swedish study of 1462 women showed that women who had bacteriuria at study entry had an increased risk of having bacteriuria 6 and 12 years later compared with women who did not have bacteriuria (odds ratio, 6.9 and 3.1 after 6 and 12 years, respectively; P < .01 for both). Other investigators showed a strong correlation between a history of urinary tract infections and current urinary tract infection among sexually active women. Another limitation of our methods is that some of the urine samples may have become contaminated before storage; however, this most likely affected cases and controls in the same proportions. Moreover, contamination usually leads to the growth of more pathogens, often non–E coli, with low CFU counts that are not detected by real-time PCR. In urine specimens stored since the 1970s, the presence of pyuria can no longer be determined. To our knowledge, there are no published data to support the test characteristics of real-time PCR on urine samples stored for more than 30 years. We tested urine samples that had been stored for up to 5 years and compared the results with those of the conventional urine cultures that were performed at the time the samples were fresh, with similar results (R.M., unpublished data, 2002). Therefore, it seems plausible that the gada gene for E coli should still be present after a long period.

Although the urinary tract is normally sterile, bacteriuria is common, especially among women and elderly populations. Previous studies reported prevalences of asymptomatic bacteriuria of 5% among sexually active young women and of more than 20% among ambulatory elderly persons; E coli is the most common infecting uropathogen. Similarly, the present study showed prevalences of E coli bacteriuria of 10% and 14%. American and European guidelines do not recommend screening for or treating asymptomatic bacteriuria in premenopausal nonpregnant women or older persons living in the community. The results of the present study confirm these recommendations.

Results from previous in vitro and in vivo studies indicate that urinary tract infections with E coli can lead to renal damage by the microorganism or by the subsequent host response. For instance, it has been shown that type 1 fimbrae (the adhesive organelles at the outer surface of the bacterial membrane) can cause scarring in the renal parenchyma of rats, with large foci of inflammation. This might be because of the activation of polymorphonuclear leukocytes by type 1 fimbrated strains, which leads to the release of tissue-destructing enzymes. Although neutrophils are important in bacterial clearance, mice models have shown that they can cause renal damage. In a recent follow-up study, renal scarring was detected in 29 of 63 women 10 to 20 years after hospitalization for pyelonephritis. In contrast, no findings have convincingly shown that asymptomatic bacteriuria can lead to a clinically relevant decline in renal function in otherwise healthy women. To our knowledge, our study is the first to demonstrate that a (minor) decline in renal function and the development of end-stage renal failure in the same cohort of women in the age range of our cohort are similarly unaffected by the presence of bacteriuria. Our longitudinal findings in this large cohort of women strongly support the absence of such an association. As an explanation, Wullt et al found that certain E coli strains,
which do not lead to symptomatic UTIs, but are only responsible for asymptomatic bacteriuria, stop expressing adherence factors once they have established bacteriuria. Therefore, these strains can remain present in the bladder without triggering an inflammatory response from the host and without causing adverse effects. 23

We assessed the presence of E coli bacteriuria and cannot exclude the presence of other uropathogens in urine that can lead to renal scarring. For instance, Klebsiella species possess type 1 fimbriae that are antigenically related to the fimbriae found in E coli. 23 However, Klebsiella is the causative organism in only a small proportion of urinary tract infections.

Studies 4,5,26 have evaluated factors predisposing to asymptomatic bacteriuria and to symptomatic urinary tract infections such as female sex, older age, and the presence of diabetes mellitus. In accord with Hooton et al, 8 we found no association between age and the presence of bacteriuria at baseline, possibly owing to the small age range among the subjects. In the baseline cohort, the presence of diabetes mellitus was established in only a few participants. In the cohort study and in the nested case-control study, the few patients with diabetes mellitus made it impossible to draw conclusions on the possible effect of the presence of bacteriuria on renal function in this subpopulation. We found a higher use of antibiotics among the women who had bacteriuria compared with those who did not have bacteriuria. This is suggestive of the presence of symptomatic urinary tract infections in these women at the time of study inclusion, but data on urinary tract infection symptoms are lacking.

In conclusion, the findings of our follow-up study among a large cohort of healthy women support the view that the presence of E coli bacteriuria at a single point does not lead to a decline in renal function. Similarly, the presence of E coli bacteriuria is not associated with the development of end-stage renal failure in the long term.

Accepted for Publication: October 9, 2006.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by the Foundation De Drie Lichten, the Netherlands (Dr Meiland).

Acknowledgment: We thank Stichting RENINE for giving us the opportunity to use their data.

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