



Clinical impact of perinephric fat stranding detected on computed tomography in patients with acute pyelonephritis: a retrospective observational study

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Abstract

Perinephric fat stranding (PFS) is often detected on computed tomography (CT) in patients with acute pyelonephritis (APN). However, its clinical impact remains unclear. This study aimed to evaluate the clinical impact of PFS detected on CT in patients with APN. This retrospective observational study included patients with APN who underwent CT (median age, 79.5 years). Patients were classified into PFS (patients with PFS observed on CT) and non-PFS (patients without PFS observed on CT) groups, which were further classified into bacteraemia and non-bacteraemia groups. Clinical findings between the groups were compared. Among 194 patients who underwent CT, 111 (57.2%) patients demonstrated PFS. The rate of bacteraemia was significantly higher in the PFS group than in the non-PFS group (55.2 vs. 23.1%, $p < 0.001$). CT findings other than PFS were not associated with bacteraemia. The median peak body temperature was significantly higher in the PFS group than in the non-PFS group (38.8 vs. 38.5 °C, $p < 0.001$); however, the duration of fever and in-hospital mortality rates were not significantly different between the groups. Concordance between blood and urine culture results was observed in 75.0% of the patients; the presence of PFS was not different between patients with concordant and discordant results, regardless of the pre-treatment antibiotic used. Our findings suggest that the presence of PFS in patients with APN predicts bacteraemia; thus, clinicians should consider obtaining blood cultures if PFS is found on CT in patients with APN, even if the patients had received antibiotics prior to admission.

Keywords Pyelonephritis · Perinephric fat stranding · Computed tomography · Bacteraemia · Discordant

Introduction

Acute pyelonephritis (APN) is a common urinary tract infection (UTI) that is usually diagnosed clinically on the basis of fever, chills, malaise, flank pain, costovertebral angle tenderness and symptoms of bladder irritation; the diagnosis is further supported by laboratory abnormalities, such as haematuria and white blood cells (WBCs) in the urine, as well as inflammatory abnormalities observed on blood tests [1]. Computed tomography (CT) is sometimes performed in patients with APN, primarily to evaluate the complications of APN, which include abscess formation, urinary obstruction and necrotising infection [2], especially in case of poor response within 72 h after starting treatment [3]. Perinephric fat stranding (PFS), also called “perirenal infiltration” [4, 5], is a relatively common CT finding and is seen in 29.1–72% [5–7] of patients with APN. However, it is not considered useful for the diagnosis of APN because of its low sensitivity, specificity and positive likelihood ratio [6].

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Bacteraemia is an important complication observed in 29–42% of patients with APN [8, 9]; it potentially leads to increased incidence of other complications and increased mortality rates [10, 11]; these complications include infections caused by antibiotic-resistant strains [12]. Therefore, foreseeing bacteraemia is crucial to predict severity. A previous report indicated that the presence of PFS in patients with APN was associated with bacteraemia [7] and that PFS may be useful to predict bacteraemia before obtaining blood culture results. However, it is not clear whether the presence of PFS provides important clinical information. Therefore, based on this background, we investigated the clinical impact of PFS detected on CT in patients with APN in an observational setting. To the best of our knowledge, this is the first study on the clinical importance of the presence of PFS in patients with APN.

Methods

Study design, setting and patients

We conducted a single hospital-based retrospective observational study between September 2011 and September 2016 and included patients aged 18 years or older diagnosed with APN and admitted to the Nabari City Hospital (NCH), a 200-bed community hospital (all acute medical care beds) in Mie, Japan, which has approximately 80,000 inhabitants.

The enrolment criterion was community-acquired APN patients who underwent CT. The diagnosis of APN was based on the presence of at least one of the three main symptoms (fever, chills, flank pain or costovertebral angle tenderness), and at least one sign of systemic infection (elevated WBC count or C-reactive protein (CRP) levels) [13], together with either positive urine or blood culture or the presence of pyuria, which is defined as a positive leukocyte esterase dipstick test, subsequently confirmed by urinalysis with more than five leukocytes per high-power field in a spun sediment [14]. Two radiologists (one with 17 years' experience in abdominal radiology and the other with 12 years' experience in abdominal radiology) who were unaware of the patient characteristics and clinical outcomes assessed the presence of PFS in the CT scans. All images were viewed in the mediastinal window settings (width, 450 HU; level, 50 HU) of the axial image using a picture archiving communication system.

The following exclusion criteria were applied: (1) patients younger than 18 years old and (2) patients without CT scans.

Data collection

Patient data were collected using an electronic medical record system. Data for the following parameters were collected at the time of admission in NCH: age, sex, patient setting prior to admission (community or nursing home), underlying diseases

(cerebrovascular disease, congestive heart failure, chronic lung disease, chronic kidney disease including haemodialysis, chronic hepatitis, diabetes mellitus and malignancy), immunocompromised status (use of steroids or immunosuppressive agents), complicated UTI (male, use of urinary tract catheter, urinary tract stone, urinary tract carcinoma, renal abscess or emphysematous infection), prior administration of antibiotics within 1 week of admission, septic shock, examination of urine and blood tests and the rates of bacteraemia and positive urine culture. Urine tests were investigated for WBCs (qualitative analysis) and presence of nitrite. Blood tests included WBC count, platelet count as well as serum albumin, blood urea nitrogen (BUN), creatinine and CRP levels. Bacteraemia was defined as a positive blood culture excluding contaminations [15]. A discordant culture result was defined as a positive blood culture with a corresponding urine culture that demonstrated growth of another microorganism, did not show bacterial growth or was contaminated [16].

Peak temperature during treatment, duration of fever (defined as the time until the body temperature fell to below 37.5° after administering the appropriate treatment), treatment duration and rate of in-hospital death were assessed. Vital signs, including body temperature, were checked every 6 to 8 h after admission.

Statistical analysis

Patients were allocated to the PFS group (patients with PFS detected in CT scans) or non-PFS group (patients without PFS in CT scans); these two groups were compared using the Mann-Whitney *U* test or Fisher's exact test (chi-square test when appropriate) for continuous or categorical variables, respectively. Then, we compared patients with and without bacteraemia (bacteraemia and non-bacteraemia groups) using the aforementioned methodology. When analysing BUN and serum creatinine, patients on chronic haemodialysis were excluded. To estimate the risk factors for bacteraemia, a univariate logistic regression model was constructed; then, factors resulting in significant difference on univariate analysis and previously described as risk factors for bacteraemia [7, 17, 18] were included in multivariate analysis. Statistical significance was defined as two-sided *p* values of <0.05. We used odds ratios (ORs) with 95% confidence intervals (CIs) for logistic regression analysis. All statistical analyses were performed using Stata 11 (Stata Corp, College Station, TX).

This study was approved by the Human Research Ethics Committee of Mie University (approval No. 1640) and was conducted according to the guidelines of the Declaration of Helsinki. Due to the retrospective nature of the study, the requirement for informed consent was waived.

Results

During the study period, 250 patients were diagnosed with community-acquired APN. Fifty-six patients were excluded based on the exclusion criteria, and data of the remaining 194 patients were analysed (Fig. 1). PFS was observed in 111 patients; of these, right-sided PFS, left-sided PFS and bilateral PFS were observed in 47 (42.3%), 36 (32.4%) and 28 (25.2%) patients, respectively.

Among the 194 patients, the median age was 79.5 years (interquartile range [IQR], 67–87 years). They were mostly women (73.2%) in community-acquired settings (70.0%). Complicated UTI was observed in 106 patients (54.6%), of which 19 were infections related to devices, including 11, 5 and 3 urethral catheters, ureteral stents and nephrostomies, respectively. A total of 30 (15.5%) patients had prior administration of antibiotics at the moment of presentation. Blood cultures were performed in 161 patients (83.0%), of whom 68 (42.2%) had bacteraemia (Table 1).

Higher body mass index, number of patients in a community-acquired setting and rate of diabetes mellitus were observed in the PFS group. Serum albumin, serum creatinine and CRP levels were higher in the PFS group, while platelet counts were lower in the PFS group. The rate of bacteraemia was significantly higher in the PFS group than in the non-PFS group (55.2 vs. 23.1%, $p < 0.001$), while the positive rate of urine culture was not different between both groups (Table 1).

The most predominant causative gram-negative bacteria (GNR) was *Escherichia coli* for both blood and urine cultures. Four and sixteen *E. coli* isolated from blood and urine cultures, respectively, were of the

extended-spectrum beta-lactamase-producing strain. One *Klebsiella pneumoniae* isolate from a blood culture was a metallo-beta-lactamase-producing strain. Among gram-positive bacteria, the most predominant causative bacteria was *Enterococcus faecalis* for both blood and urine cultures. Two cases of *Staphylococcus aureus* isolated from blood cultures were observed in patients who had a ureteral stent inserted; the urine cultures of these two cases were also positive for *S. aureus*. On investigating the difference in bacterial species obtained from the results of blood culture between the PFS and non-PFS groups, no significant relationships were found among bacterial species. Concordance between blood and urine culture results was observed in 75.0% of the patients (51 of 68 patients) (Table 2). The rate of PFS was not different between discordant and concordant subjects.

The median peak body temperature was significantly higher in the PFS group than in the non-PFS group, while the median fever duration and rate of in-hospital death were not significantly different between both groups (Table 3).

Comparing the bacteraemia group ($n = 68$) and the non-bacteraemia group ($n = 93$), the rate of septic shock and the presence of PFS were higher in the bacteraemia group (septic shock, 10.3 vs. 1.1%, $p = 0.010$; PFS, 77.9 vs. 46.2%, $p < 0.001$).

With respect to patients with CT findings indicative of conditions other than PFS, renal cyst, renal stone, urinary tract stone, urinary tract obstruction not due to stones (e.g., ureteral cancer, gastrointestinal cancer, pelvic tumour and uterine fibroid), hydronephrosis, emphysematous APN and renal abscess were observed in 14, 12, 22, 10, 17, 1 and 2 patients, respectively; the

Fig. 1 Patient enrolment process

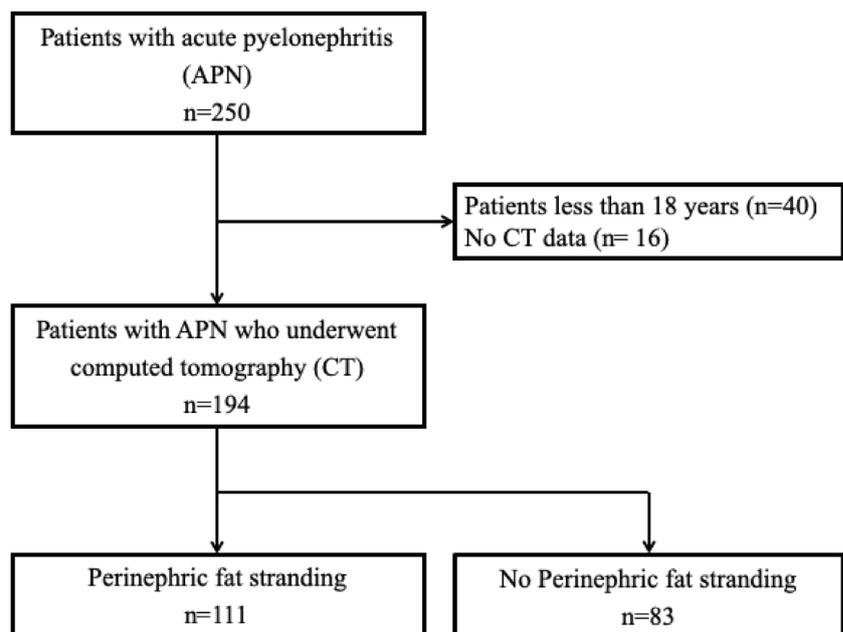


Table 1 Baseline characteristics of the study patients

	All patients (<i>n</i> = 194)	Patients with perinephric fat stranding (<i>n</i> = 111)	Patients without perinephric fat stranding (<i>n</i> = 83)	<i>p</i> value
Age (years) ^a	79.5 (67–87)	77 (63–85)	83 (74–89)	< 0.001
Women (<i>n</i> (%))	142 (73.2)	79 (71.0)	63 (77.8)	0.381
Body mass index (kg/m ²) ^a	20.8 (17.0–23.8)	22.3 (18.8–24.9)	18.2 (15.7–22.8)	< 0.001
Location at onset (<i>n</i> (%))				
Community acquired ^b	135 (70.0)	85 (76.6)	50 (61.0)	0.026
Nursing home	59 (30.0)	26 (23.4)	33 (39.8)	0.018
Underlying disease (<i>n</i> (%))				
Cerebrovascular disease	26 (13.4)	11 (9.9)	15 (18.1)	0.135
Congestive heart failure	23 (11.9)	11 (9.9)	12 (14.5)	0.374
Chronic lung disease	11 (5.7)	4 (3.6)	7 (8.4)	0.210
Chronic kidney disease	17 (8.8)	13 (11.7)	4 (4.8)	0.124
Haemodialysis	4 (2.1)	4 (3.6)	0 (0)	0.137
Chronic hepatitis	8 (4.1)	4 (3.6)	4 (4.8)	0.726
Diabetes mellitus	31 (16.0)	23 (20.7)	8 (9.6)	0.047
Presence of malignancy	18 (9.3)	8 (7.2)	10 (12.1)	0.319
Compromised condition (<i>n</i> (%))	30 (15.5)	14 (12.6)	16 (19.3)	0.232
Complicated UTI (<i>n</i> (%))	106 (54.6)	61 (55.0)	45 (54.2)	1.000
Septic shock (<i>n</i> (%))	9 (4.6)	6 (5.4)	3 (3.6)	0.735
Prior administration of antibiotics (<i>n</i> (%))	30 (15.5)	17 (15.3)	13 (15.7)	1.000
CVA tenderness (<i>n</i> (%))	58 (37.7)	41 (44.1)	17 (22.9)	0.042
Urine test ^c (<i>n</i> (%))				
Positive WBC	168 (86.6)	98 (88.3)	70 (84.3)	0.524
Positive nitrite	112 (58.6)	68 (62.4)	44 (53.7)	0.238
Blood test ^a				
WBC ($\times 10^3/\text{mm}^3$)	11.7 (8.7–15.5)	12.6 (9.1–15.5)	10.5 (7.7–15.2)	0.113
Platelet ($\times 10^4/\text{mm}^3$)	17.8 (13.5–22.3)	16.4 (12.6–21.2)	19.0 (15.0–24.5)	0.007
Albumin (g/dl)	3.6 (3.2–4.0)	3.7 (3.4–4.1)	3.5 (3.1–3.8)	0.003
BUN (mg/dl) ^d	21 (15–31)	21 (15–32)	21 (14–28)	0.300
Creatinine (mg/dl) ^d	0.9 (0.7–1.3)	1.0 (0.7–1.5)	0.8 (0.6–1.1)	< 0.001
CRP (mg/dl)	9.2 (4.2–15.0)	10.1 (5.4–16.3)	7.3 (3.3–13.8)	0.016
Blood culture performed (<i>n</i> (%))	161 (83.0)	96 (86.5)	65 (78.1)	0.176
Bacteraemia (<i>n</i> (%))	68/161 (42.2)	53/96 (55.2)	15/65 (23.1)	< 0.001
Positive urine culture (<i>n</i> (%))	160 (83.8)	95 (85.6)	65 (81.3)	0.434

BUN and creatinine levels were calculated in all except four patients with chronic haemodialysis

BUN, blood urea nitrogen; *CRP*, C-reactive protein; *CVA*, costovertebral angle; *UTI*, urinary tract infection; *WBC*, white blood cell

^a Median (interquartile range)

^b By 2×2 table for community versus non-community acquired (nursing home and healthcare-associated) and by Fisher's exact test for patients with versus those without perinephric fat stranding

^c Positive WBC means the presence of more than five leukocytes per high-power field in the centrifuged sediment, and nitrite was evaluated using a qualitative test

^d BUN and serum creatinine; patients with chronic haemodialysis were excluded

incidence was not significantly different between the bacteraemia and non-bacteraemia groups (Table 4).

Logistic regression analysis was performed for the 194 patients to identify the risk factors associated with bacteraemia. Both univariate and multivariate analyses

demonstrated that PFS (OR, 4.1 (95% CI, 2.11–8.11); $p < 0.001$ and OR, 4.5 (95% CI, 2.19–9.33); $p < 0.001$, respectively) and septic shock (OR, 7.1 (95% CI, 1.43–35.3); $p < 0.001$ and OR, 7.6 (95% CI, 1.39–42.0); $p < 0.001$, respectively) were associated with bacteraemia (Table 5).

Table 2 Frequency of causative bacteria isolated from blood and urine cultures

Causative bacterium ^a	Blood culture	Urine culture	Concordance between blood and urine culture results
Gram-negative bacteria			
<i>Escherichia coli</i>	50	105	41
<i>Klebsiella</i> species	3	12	2
<i>Proteus mirabilis</i>	2	2	0
<i>Citrobacter</i> species	2	6	1
<i>Pseudomonas aeruginosa</i>	2	12	2
<i>Enterobacter</i> species	0	2	0
<i>Serratia</i> spp.	0	1	0
Others ^b	3	8	0
Gram-positive bacteria			
<i>Enterococcus faecalis</i>	3	14	2
<i>Enterococcus faecium</i>	0	3	0
<i>Staphylococcus aureus</i> ^c	2	5	2
Others ^d	5	9	1

^a Multiple species of bacteria were detected in 4 blood cultures and 21 urine cultures

^b Other gram-negative bacteria included two cases of *Peptoniphilus asaccharolyticus* and one case of *Actinobaculum schaalii* in blood culture and three cases of *Morganella morganii*, two cases of *Providencia rettgeri* and two cases of *Alcaligenes* species in urine culture

^c Two cases of *Staphylococcus aureus* included one case of methicillin-susceptible *Staphylococcus aureus* and one case of methicillin-resistant *Staphylococcus aureus*

^d Other gram-positive bacteria included two cases of *Streptococcus agalactiae*, one case of *Streptococcus anginosus*, one case of group G *Streptococcus* and one case of alpha-haemolytic *Streptococcus*

Discussion

We investigated the clinical impact of PFS detected on CT among APN patients in an observational setting. We found that PFS detected on CT was associated with bacteraemia, regardless of bacterial species and irrespective of concordance between blood and urine culture results in patients with APN. However, the presence of PFS did not affect the duration of fever or the rate of in-hospital death. CT findings other than PFS were not associated with bacteraemia.

A previous study also suggested an association between PFS and bacteraemia in patients with APN [7]; however, that

study did not examine the species of bacteria. The present study included not only *E. coli*, the most predominant species causing APN, but also other types of bacteria, suggesting that PFS was associated with bacteraemia, regardless of the species of bacteria. In general, to identify the causative bacteria in patients with APN, obtaining blood and urine cultures are essential. However, culture results may sometimes be false negative, especially in patients who have been administered with antibiotics prior to culture. The present study demonstrated that antibiotics administered prior to admission were associated with a trend toward non-bacteraemia (8.8% in bacteraemia and 18.3% in non-bacteraemia cases) (Table 4),

Table 3 Clinical course of the presence of PFS in patients with APN

	All patients (n = 194)	Patients with perinephric fat stranding (n = 111)	Patients without perinephric fat stranding (n = 83)	p value
Peak temperature (°C) ^a	38.6 (38.0–39.4)	38.8 (38.0–39.5)	38.5 (37.8–39.4)	< 0.001
Fever duration (h) ^a	38.5 (22–58)	42 (25–60)	36 (18–55)	0.252
Treatment duration (days) ^a	13 (10–14)	13 (10–14)	13 (9–14)	0.639
In-hospital death (n (%)) ^a	5 (2.6)	4 (3.6)	1 (1.2)	0.397

APN, acute pyelonephritis; PFS, perinephric fat stranding

^a Median (interquartile range)

Table 4 Characteristics of patients with and those without bacteraemia

	All patients for whom blood culture was performed (n = 161)	Patients with bacteraemia (n = 68)	Patients without bacteraemia (n = 93)	p value
Age (years) ^a	79 (66–87)	78 (65.5–86.5)	81 (66–87)	0.537
Women (n (%))	116 (72.1)	50 (73.5)	66 (71.0)	0.859
Underlying disease (n (%))				
Cerebrovascular disease	24 (15.0)	5 (7.4)	19 (20.4)	0.135
Congestive heart failure	19 (11.8)	8 (11.8)	11 (11.8)	1.000
Chronic lung disease	10 (6.2)	3 (4.4)	7 (7.5)	0.210
Chronic kidney disease	12 (7.5)	4 (5.9)	8 (8.6)	0.124
Chronic hepatitis	3 (1.9)	0 (0)	3 (3.2)	0.726
Diabetes mellitus	24 (14.9)	12 (17.6)	12 (12.9)	0.503
Presence of malignancy	13 (8.1)	7 (10.3)	6 (6.5)	0.319
Compromised condition (n (%))	24 (14.9)	9 (13.2)	15 (16.1)	0.232
Complicated UTI (n (%))	88 (54.7)	38 (55.9)	50 (53.8)	0.873
Septic shock (n (%))	8 (5.0)	7 (10.3)	1 (1.1)	0.010
Prior administration of antibiotics (n (%))	23 (14.3)	6 (8.8)	17 (18.3)	0.112
CT findings				
Perinephric fat stranding	96 (59.6)	53 (77.9)	43 (46.2)	<0.001
Renal cyst	12 (7.5)	4 (5.9)	8 (8.6)	0.562
Renal stone	11 (6.8)	4 (5.9)	7 (7.5)	0.761
Urinary stones	16 (9.9)	8 (11.8)	8 (8.6)	0.597
Urinary tract obstruction not due to stones	9 (5.6)	4 (5.9)	5 (5.4)	1.000
Hydronephrosis	13 (8.1)	5 (7.4)	8 (8.6)	1.000
Emphysematous APN	1 (0.6)	0	1 (1.1)	1.000
Renal abscess	2 (1.2)	1 (1.5)	1 (1.1)	1.000

APN, acute pyelonephritis; CT, computed tomography; UTI, urinary tract infection

^aMedian (interquartile range)

although this was not statistically significant. Antibiotic pre-treatment may sterilise the urine, as many antibiotics are concentrated in urine even when orally administered, while having an insufficient antimicrobial effect in the serum, despite the fact that they may grow on blood culture [19]. Therefore, in addition to false-negative culture results, discordant results

Table 5 Results of univariate and multivariate analyses to estimate risk factors for bacteraemia

	Univariate		Multivariate	
	OR	95% CI	OR	95% CI
Age ^a	1.0	0.98–1.02	1.0	0.98–1.02
Diabetes mellitus	1.2	0.55–2.66	0.9	0.37–2.04
Septic shock	7.1	1.43–35.3	7.6	1.39–42.0
Perinephric fat stranding	4.1	2.12–8.11	4.5	2.19–9.33
Hydronephrosis	0.8	0.25–2.24	1.2	0.37–3.89

OR, odds ratio; CI, confidence interval

^aPer 1 year increase

between blood and urine cultures may also be frequently observed in patients previously treated with antibiotics. In the present study, concordance between blood and urine culture results was observed in 75.0% of the patients with bacteraemia, which was lower than that reported previously [16, 19]. The reasons for the low concordance rates were most often antibiotic pre-treatment [20]; in the present study, concordance between blood and urine culture results was observed in one patient among the six with bacteraemia who received antibiotics previously. The other reasons for the low concordance rate were the presence of an indwelling urinary catheter, the presence of malignancy [20] and advanced age [19]. The median age in the present study population was 79.5 years (IQR, 67–87 years), with a higher proportion of the elderly than in previous studies of discordant culture results [16, 19]. This difference might have resulted in our low concordance rate. In the present study, the proportion of patients with indwelling urinary catheters and malignancies was not significantly different between concordant and discordant patients, although the numbers of patients were small.

Blood culture is important in patients with APN; however, some studies reported that routine blood cultures have little influence on the clinical management and outcome in APN patients in several settings [20–23]. Nevertheless, it should be noted that these studies did not consider discordance between blood and urine cultures. Another recent study also argued that blood cultures were unnecessary in patients with APN admitted to the emergency department [24]; however, patients receiving antibiotics prior to performing blood culture were excluded from the study. Clearly, blood culture is important for the determination of true causative bacteria in patients with APN, especially in light of the risk of negative urine culture results because of prior administration of antibiotics. As the present study demonstrated that the presence of PFS was associated with bacteraemia regardless of the presence of prior administration of antibiotics, the presence of PFS should encourage clinicians to obtain blood cultures in order to determine the offending pathogen, even if patients with APN had already received prior antibiotic therapy.

The presence of PFS may have diagnostic significance in patients with APN; however, these results might not influence treatment planning or decision-making. The treatment regimen for APN is generally a 5-to-7-day course of a fluoroquinolone, a 14-day course of trimethoprim-sulfamethoxazole or a 10-to-14-day course of beta-lactams [3]. Recently, because of increasing concern regarding fluoroquinolone resistance in *E. coli* [25, 26], fluoroquinolones are no longer initially selected; therefore, a 10-to-14-day treatment duration may be given priority in actual clinical settings. Similarly, treatment for bacteraemia due to GNR (except for endocarditis), which is the most predominant causative bacteria of APN, generally requires 10–14 days [27]. Therefore, when starting treatment for patients with APN, if fluoroquinolones could not be initially selected in patients with APN, a 10-to-14-day treatment duration with trimethoprim-sulfamethoxazole or beta-lactams would be initiated, regardless of the presence of bacteraemia [2]. In other words, despite the fact that PFS may be an indicator of bacteraemia in patients with APN, it may seldom affect the duration of antibiotic administration.

Despite the present study's several strengths, including the large number of patients undergoing CT, there are several limitations. First, the study design was retrospective in nature. Second, the number of cases of bacteraemia involving species other than *E. coli* was relatively small, possibly influencing statistical analysis. Third, the median age of our study population was considerably greater than that reported for patients with APN in previous studies [4, 7, 13, 14, 21–24, 28]; therefore, our results may not be generalisable to younger patients. However, the average life expectancy of humans has been increasing worldwide [29], and we believe that our study findings may be applicable in these populations.

In conclusion, the presence of PFS in patients with APN was associated with bacteraemia, regardless of bacterial species, concordance between blood and urine cultures and prior administration of antibiotics. These results suggest that clinicians should perform blood cultures if PFS is found on CT in patients with APN, even if the patients had received antibiotics prior to admission; however, the presence of PFS has limited effect on the clinical outcomes and management. Further large prospective studies are warranted.

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Contributions RT designed the study, supervised the overall data collection process and wrote the manuscript. SI supported statistical data analysis. YT takes responsibility for the manuscript as a whole.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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