Peer Review File

Reviewer Comments

Comment 1: It would be helpful to have the "radioactivity count" converted to mSv, which is another common measure for radiation (line 100).

Reply 1: Thank you for your comments. According to the comments, we have converted the "radioactivity count" to mSv **(See Page 3, line 91 - 92)**.

Comment 2: Reference 15-16 do not represent clinical guidelines for DMSA (as stated on line 102). A recommended ref suggestion would be the paper by Piepsz et al from 2001 in Eur J Nucl Med.

Reply 2: Thank you for your comments. According to the comments, we have modified the reference 15 to "Piepsz A, Colarinha P, Gordon I, Hahn K, Olivier P, Roca I, Sixt R, van Velzen J; Paediatric Committee of the European Association of Nuclear Medicine. Guidelines for 99mTc-DMSA scintigraphy in children. Eur J Nucl Med 2001; 28(3): 37-41." **(See Page 10, line 304 - 306)**. The reference 16 by Farhat W et al from 2000 in J Urol represent the clinical guideline of the BBD.

Comment 3: The definition of renal impairment as 10 % or greater difference in differential renal function could need some nuancing (line 106). What about the situation with bilateral renal damage? Or with a duplicated collecting system on one side?

Reply 3: Thank you for your comments. According to the manuscript by "Keren R, et al Risk Factors for Recurrent Urinary Tract Infection and Renal Scarring. Pediatrics 2015", we have modified the definition of renal impairment as ①a kidney with a differential function < 45%, or ② eGFR < 90 ml/(min·1.73m^2), and added those definition "The percentage of renal function on each side (differential renal function) was calculated by delineating the kidney regions of interest and background through computer. A kidney with a differential function < 45% was considered renal impairment. In addition, the eGFR < 90 ml/(min·1.73m^2) was also defined renal impairment, which calculated by the Schwartz formula [2,16]" in the manuscript **(See Page 3 - 4, line 96 - 100)**. In this study, there was no patient with a duplicated collecting system on one side.

Comment 4: Is it previously known that LUTD, with or without bowel dysfunction, is associated with an increased risk of recurrent UTI and lower rates of VUR resolution. Therefore, it is recommended that bladder function should be evaluated even before potty training, for example with a free voiding observation, for a better risk grading and individualized management of the smallest children, who are at greatest risk for further renal damage. Did the authors consider this? In this study, BBD was evaluated in only 54/256 children, only on potty trained and only by a questionnaire. Still, it turned out as a risk factor for UTI. That shows how important the bladder function is. I still miss a subgroup analysis of the 54 children – girls or boys? High- or low-grade reflux?

Reply 4: Thank you for your comments. We agree with your opinion that bladder

function should be evaluated even before potty training, and we also had a consideration about this. However, our study was a single-center, retrospective cohort. Although the children were regularly followed up in our hospital, while during reviewing data there still had some information defects. In that way, we would like to do a multicenter prospective clinical study to evaluate the bladder function before potty training and further confirm the reliability of the results. Furthermore, according to the comments, we have reanalyzed clinical features of the 54 children. A total of 54 VUR children who received potty training during the follow-up were evaluated with the Dysfunctional Voiding Symptom Score (DVSS) questionnaire, including 33 males and 22 females. There were 21 (38.89%) children with BBD, including 10 males and 11 females. Among these 21 patients with BBD, 2 were grade I-II VUR and the other 19 were grade III-V VUR. Meanwhile, 12 were bilateral VUR. Chi-Square test found that bilateral VUR was correlated with BBD ($P < 0.05$), while there was no difference in other parameters (sex, age and VUR grade) between the BBD and without BBD groups ($P > 0.05$) (please see the table 1). In addition, we have added this part in the "Result" **(See Page 6, line 165 - 172)** and summarized in the table (**See table 3 in the manuscript**). Table 1: Risk factor analysis of BBD in children with VUR during prophylactic antibiotic intervention.

Characteristic	BBD (N = 21)	No BBD (N = 33)	P
Sex			0.135
Male	10 (18.52)	22 (40.74)	
Female	11 (20.37)	11 (20.37)	
Age group			0.381
≤ 12 mo	13 (24.07)	23 (42.59)	
> 12 mo	8 (14.81)	10 (18.52)	
VUR grade			
I-II	2 (3.70)	5 (9.26)	0.437
III-V	19 (35.19)	28 (51.85)	
VUR grade			
I-III	10 (18.52)	18 (33.33)	0.414
IV-V	11 (20.37)	15 (27.78)	
VUR			0.028
Bilateral	12 (22.22)	9 (16.67)	
Unilateral	9 (16.67)	24 (44.44)	

Note: VUR: vesicoureteral reflux; BBD: bladder and bowel dysfunction

Comment 5: The Kaplan-Meier curve only shows the difference between age < or > 12 months. A similar curve with the difference between boys and girls would be interesting.
Reply 5: Thank you for your comments. We have also made a Kaplan-Meier curves analyze in VUR children by sex. However, there had no difference between boys and girls. According to the comments, we have added the "The differences in other

parameters such as the factor of sex between the BT-UTI and without BT-UTI groups were not significant" (See Page 5, line 148 -150).

Comment 6: The boys with BT-UTI – how old were they? And the girls? It would also be interesting to see the details on the 19 children with BT-UTI who had their diagnosis > 12 months – were they mainly girls? Potty trained or not? This information would be interesting in the discussion regarding CAP management in boys vs. girls and the timing of when to end prophylaxis.

Reply 6: Thank you for your professional comments. In this study, among 19 children with BT-UTI who had their diagnosis > 12 months, 4 were males (13 months, 18 months, 18 months and 32 months) and 15 were females (median age of 32 months, range: 14 - 56 months). According to the review's comments and previous studies, we modified the "Discussion" part to read that "In our study, there were only 4 boys who had BT-UTI beyond 12-month old, while 15 girls with BT-UTI more than 1-year old. The risk of BT-UTI decreases significantly after the first year of life in boys [24]. In that way, discontinuation of antibiotic prophylaxis can be discussed, especially in boys with a low-grade reflux and normal renal parenchyma in DMSA scan. For girls, the risk for BT-UTI remains higher overall and the new scars were acquired and found to be related to severe inflammatory processes, while in boys the renal damage was often congenital [24]. Thus, prophylaxis also had a protective effect against new renal scarring in girls during 2 years of follow-up, but there were no effects on UTI recurrence or renal damage in boys [25]. For girls the timing for CAP discontinuation should be extended after the patient is toilet-trained" (See Page 6 - 7, line 195 - 204) and added the references 24 - 25 (See Page 11, line 327 - 330). We didn't collect the potty train information in this study. We would like to pay more attention on this issue in the clinical practice.

Comment 7: You cannot stress enough this: the fact that you didn´t find a relation between high-grade VUR and BT-UTI in this study doesn't mean that there is none. It only means that you were not able to show it. Possibly because of the high proportion of high-grade VUR as you mentioned in the discussion. Or the limited follow-up. This is one of the reasons why it is hard to perform a high-quality study with all grades of VUR and ages mixed.

Reply 7: Thank you for your comments. Taking the high proportion of high-grade VUR and limited follow-up into consideration, it is hard to stress that there was no relation between high-grade VUR and BT-UTI. Therefore, according to your comments, we have modified sentences in the "Results" (See Page 5 - 6, line 157 - 164) as "High-grade VUR did not show any significant effect on the occurrence of BT-UTI on univariate analysis (P = 0.152) in our group. Multivariate regression was performed, and no significant difference was observed neither (HR: 1.062, 95% CI: 0.197 - 5.714, P = 0.945). Moreover, when children were divided into grade IV-V and grade I-III groups, univariate analysis showed that grade IV-V VUR did not show significant effect on BT-UTI occurrence (P = 0.221). Multivariate regression again showed no significant difference (HR: 1.194, 95% CI: 0.460 - 3.099, P = 0.716)". In the future we would like

to do a multicenter prospective clinical studies to further verify the reliability of our results.