

Corticosteroids for Children With Febrile Urinary Tract infections

Study Protocol and Statistical Analysis Plan

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STUDY PROTOCOL SUMMARY

Corticosteroids for Children With Febrile Urinary Tract Infections

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A. Objectives and Specific Aims

Urinary tract infection (UTI) is the most frequently occurring serious bacterial infection in young children and accounts for more than 1 million office visits (0.7% of all physician office visits), 5 to 14% of emergency department (ED) visits, and approximately 40,000 admissions per year.¹ UTI involving the renal parenchyma – referred to as acute pyelonephritis (APN) – is one of the principal causes of renal scarring in children. Renal scarring in childhood has been associated with hypertension, preeclampsia and ESRD decades later.²⁻⁴

Current management of children with UTI has focused on the treatment of the subgroup with vesicoureteral reflux (VUR) and on the early treatment of UTI with antibiotics. Although the presence of VUR – backward flow of urine from the bladder to the kidney – increases the likelihood of bacteria gaining access to the kidney, correction of VUR is not sufficient to prevent scarring. Renal scarring frequently occurs in children who do not have VUR,⁵⁻⁸ and the aggressive diagnosis and treatment of children with VUR that started in the 1960's has not been associated with a reduction in the incidence of end stage renal disease.^{9, 10} Similarly, although early antibiotic treatment is clearly necessary, it may not be sufficient to prevent renal scarring in most children with UTI. Because signs of UTI in children are relatively non-specific,¹¹ the diagnosis is often delayed.¹² Furthermore, data from both human and animal studies have convincingly shown that once the infection has localized to the renal parenchyma, treatment with antibiotics alone does not prevent scarring.¹²⁻¹⁶ It is now clear that the current management strategy is not always effective in preventing renal scarring.

The proposed study examines whether an alternate strategy – one that focuses on identification and modulation of the host inflammatory response – is effective in reducing renal scarring.

Aim – To determine whether adjuvant therapy with dexamethasone reduces renal scarring in children treated with antimicrobials for acute urinary tract infection.

Because host inflammatory response is the final and most important step in the formation of renal scars, the use of anti-inflammatory agents may be the best strategy to reduce renal scarring. In animal studies, the use of corticosteroids has been shown to be effective in preventing post-pyelonephritic scarring.¹⁷⁻¹⁹ We will conduct a randomized, double-blind, placebo-controlled trial (n=320 evaluable children, 160 per arm) to determine the efficacy of 3 days of daily adjuvant dexamethasone on the incidence of renal scarring 6 months after a first febrile UTI. We hypothesize that the proportion of children with UTI who develop renal scarring will be lower among children who are treated with both dexamethasone and antibiotics as compared with children treated with antibiotics alone. If corticosteroids are effective in preventing renal scarring, this will have profound consequences on the treatment of children with UTI and on the incidence of ESRD, preeclampsia, and hypertension secondary to childhood UTI.

B Significance

I. Acquired Renal Scarring is Associated with Poor Outcome

Renal scarring in childhood, whether acquired or congenital, is associated with hypertension, preeclampsia, and renal failure later in life.^{2-4, 20} As numerous experimental and longitudinal studies have shown, UTIs cause permanent and significant renal scarring.^{17, 18, 21-27} Furthermore, there is a clear dose-response relationship between the number of UTIs and progression of scarring.²⁸⁻³¹ On the other hand, it is also clear, especially when one looks at referral populations with a large proportion of children with high-grade VUR, that some children are born with small poorly-functioning kidneys. The clinical and radiological presentations of congenital and acquired scarring, albeit overlapping, are often distinct. Dysplasia, which is often detected prenatally,³² is most frequently seen in males with grades IV or V VUR and is characterized by globally atrophied kidney(s) with little or no function.³³⁻³⁶ In contrast, children with UTI-related scarring are most often females who present later in childhood with focal scars.^{3, 22, 37}

Studies have shown that both UTI and dysplasia contribute to ESRD. In her 10 to 41 year follow-up of 226 children with VUR, Smellie noted that those with poor prognosis fell into two categories: boys with severe VUR and scarring at presentation, and girls with mild to moderate VUR with scarring secondary to recurrent

UTIs.³⁸ The studies by Merrick and Wennerstrom show the same pattern, with both dysplasia and UTI contributing to ESRD.^{20, 29, 39}

By examining the outcome of studies that included mostly children likely to have had acquired renal scarring (and not dysplasia) – i.e., studies of females with low-grade VUR or no VUR – one can get a better appreciation for the morbidity associated with childhood UTI. Jacobson reported on a 27-year follow-up of 30 patients (87% females) with non-obstructive focal scarring following APN in childhood.^{4, 40} Approximately 10% of patients developed end-stage renal disease, 23% developed hypertension, and 13% of women experienced preeclampsia. Raz followed 63 adult women hospitalized with uncomplicated APN for 10-20 years; 8% had evidence of impaired renal function (GFR<75 mL/min per 1.73 m²).⁴¹ In a 37-year follow-up study, of 267 children (89% female) with VUR, 9% developed ESRD.⁴² In a long-term study of 100 adults (83% female) with reflux nephropathy, 22 of the 23 kidneys removed showed no histological signs of dysplasia.⁴³

Recent longitudinal UTI studies that were conducted in primary care settings have found that 0% to 2% of children had evidence consistent with dysplasia at enrollment (see preliminary data).⁴⁴⁻⁴⁶ Yet, in these same studies, approximately 15% of children developed permanent renal scarring.^{44, 46}

In summary, there is overwhelming evidence to support the role of APN in the development of permanent renal injury in children. Recent studies confirm the notion that earlier studies likely overestimated the importance of acquired scarring, but that APN remains an important and preventable cause of permanent renal injury.²²

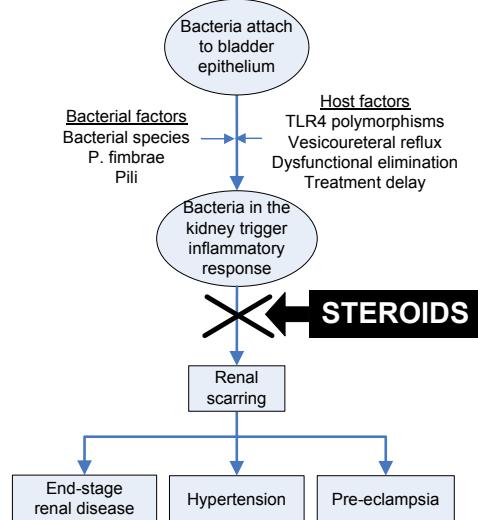
II. Inflammation Leads to Renal Scarring (Figure 1)

APN occurs when bacteria successfully ascend from the bladder to the kidney. Approximately two-thirds of young children with febrile UTI have evidence of renal parenchymal involvement.^{8, 12, 22} VUR, dysfunctional elimination syndrome (DES),^{47, 48, 49-52} and a delay in treatment all predispose to APN. In the kidney, the bacterial inoculum triggers a chemokine response that recruits neutrophils from the blood stream. This intense neutrophilic response results in the accumulation of oxygen radicals and lysosomal enzymes, which, in some individuals, leads to irreversible renal injury. Through a series of animal experiments, Bille,⁵³ Roberts,⁵⁴ and Glauser,¹³ assessed the role of neutrophil response in the pathogenesis of renal injury. Reduction in neutrophil activity through the use of superoxide dismutase,⁵⁴ cyclophosphamide,¹³ or colchicine¹³ was associated with decreased scarring in rats or monkeys with experimentally induced APN. These results were striking because even though animals treated with these agents had higher bacterial counts in their kidneys, reduction of the inflammatory response was associated with less renal damage. The authors concluded that the inflammatory response, rather than direct damage from the bacterial infection, is the most important factor in the formation of renal scars.

III. Treatment of VUR is not Sufficient to Reduce Renal Scarring

VUR provides a mechanism for bacteria to ascend into the kidney and to cause scarring.^{5, 55} Recent evidence, however, suggests that the role of VUR in the pathophysiology of renal scarring has been overstated. First, the majority of children (60%-68%) with UTI have no demonstrable VUR,^{5, 55} and only a very small minority have severe (grade IV, V) VUR (<5%).^{12, 55, 56} Second, VUR is neither necessary nor sufficient for the development of renal scarring.^{5-8, 57, 58} Renal scarring is frequently found in children with no VUR and many children with high-grade VUR do not develop scarring. Thus, it appears that the pathophysiology of scarring is multifactorial, and that VUR only plays a small role in the development of scarring. Third, the common denominator in all cases of post-infectious renal scarring appears to be the inflammatory response to acute infection – not VUR.^{7, 55, 59-61} Once bacteria have invaded the renal parenchyma, the inflammatory response appears similar and the

Figure 1. Pathophysiology of UTI



propensity for renal scarring is equally as great whether or not VUR is present. Fourth, aggressive treatment of VUR has not reduced the incidence of ESRD.^{9, 10, 62} It is now clear that a sole focus on the treatment of VUR is not likely to significantly impact the incidence of renal scarring for the majority of children with UTI.

IV. Prompt Treatment of UTI with Antibiotics is not Sufficient to Eliminate Renal Scarring

Two recent studies suggest that even if early treatment is instituted, once infection is localized to the kidneys, there may be little impact on the rates of scarring (Table 1). In these studies, prevalence of renal scarring on the 12-month DMSA scan was compared in children with UTI treated with antibiotics within 24 hours of onset of fever to children treated later. While early treatment reduced the incidence of APN,¹² it did not reduce scarring,^{12, 14} suggesting that once infection localizes

Table 1. Renal scarring according to timing of treatment

| Author | Early Antibiotics | Late Antibiotics | RR (95% CI) |
|-----------------------|-------------------|------------------|---------------|
| Hewitt ¹⁴ | 15/43 (35%) | 74/244 (30%) | 0.9 (0.6-1.4) |
| Doganis ¹² | 11/24 (46%) | 28/52 (54%) | 1.2 (0.7-1.9) |

to the kidney, there may be little impact of antibiotic treatment on the rates of scarring. Indeed, animal studies of artificially induced APN have confirmed these observations. In a series of elegant studies, Roberts and Glauser independently demonstrated that antibiotic treatment is ineffective in preventing renal scar formation if it is started after 30 hours of the onset of APN.^{15, 13} In a similar study, Slotki showed that treatment begun after 24 hours did not prevent the progression of APN to scarring.¹⁶ Furthermore, because UTI is especially difficult to diagnose in preverbal children,^{11, 63-65} prompt treatment with antibiotics may not always be possible. These findings suggest that antibiotic treatment alone is not sufficient to eliminate scarring.

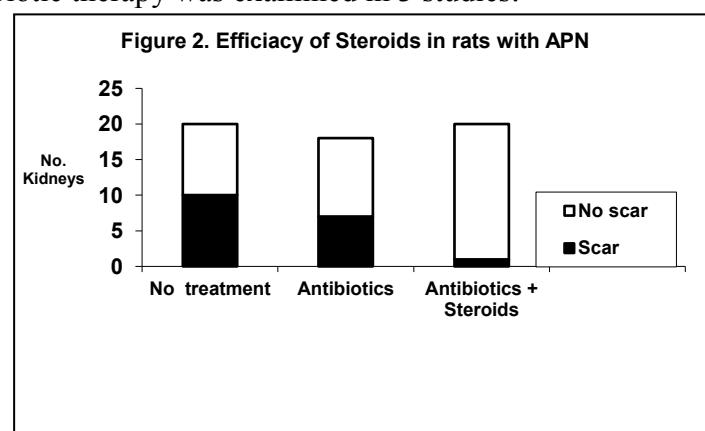
V. Use of Anti-Inflammatory Agents Reduces Scarring from UTI

Altering the inflammatory cascade in animals with UTI has been found to reduce the incidence and severity of renal scarring in multiple studies. Cobra venom was used to deplete complement in both rat and monkey models resulting in the prevention of chronic renal injury.²⁶ Shimamura used nitrogen mustard to induce immunosuppression in rats with UTI; immunosuppressed rats had lower rates of renal scarring when compared with immunocompetent rats.²⁷ Meylan showed that rats treated with dapsone, which inhibits neutrophil oxidant damage, had a 65% reduction in renal scars compared with controls.²⁴ Huang compared rats with APN that were left untreated, treated with antibiotics, treated with anti-inflammatory ibuprofen, or treated with a combination of ibuprofen and antibiotics.²³ Gross renal scarring was highest in the untreated group and lowest in the group receiving combination therapy.

The use of corticosteroids in conjunction with antibiotic therapy was examined in 3 studies:

1. In a study by Haraoka, rats were either left untreated, treated with delayed ciprofloxacin (begun 72 hours after bacterial inoculation), or treated with prednisolone (2 mg/kg/d for 4 days) in combination with delayed ciprofloxacin (Figure 2). Microscopic and/or macroscopic renal scarring was observed in 70% of the untreated rats, 56% of the rats treated with ciprofloxacin alone, and 10% of rats treated with both antibiotics and corticosteroids.¹⁷

2. Pohl evaluated the efficacy of corticosteroids in reducing post-APN renal scarring in a small number of piglets (N= 36). Piglets with experimentally induced APN were randomized to receive ceftriaxone or ceftriaxone plus oral prednisolone. Severity of renal scarring was based on histopathologic findings 2 months after APN. Scarring was observed in 60% of control piglets vs 49% of steroid-treated animals. Given the small sample size, this did not reach statistical significance (p=0.22). Nevertheless, severe APN (defined as involvement of >2/3 of a renal zone) was 3 times more likely to resolve completely with prednisolone. This study suggests that adjunctive corticosteroid treatment may be most effective in moderately and severely affected kidneys.¹⁸



3. In a recent study, 54 children 3 months to 10 years of age with APN were placed on dexamethasone (0.15 mg/kg every 6 hours for 3 days) plus ceftriaxone, or on ceftriaxone alone.¹⁹ Urinary IL-6 and IL-8 were measured at the time of presentation and at 72 hours after initiation of therapy. A significantly larger drop in the levels of urinary IL-6 ($p<0.001$) and IL-8 ($p<0.001$) was noted in children treated with corticosteroids as compared to children treated with antimicrobials alone. Follow-up DMSA scans were not performed.

In contrast, the use of corticosteroids in animals not concurrently treated with antibiotics did not decrease rates of scarring.^{21, 25, 66} In summary, the evidence to date overwhelmingly supports the use of corticosteroids in conjunction with antibiotics for the treatment of UTI. There is a clear need for a randomized controlled trial in a large representative population of children with UTI to test the efficacy of steroids in preventing scar formation.

VI. Efficacy and Safety of Corticosteroids for Other Infectious Diseases

Corticosteroid use during acute infection decreases the inflammatory response by 1) inhibiting cytokine and chemokine production, 2) inhibiting leukocyte migration, 3) inhibiting transcription of inflammatory proteins, and 4) upregulating the transcription of anti-inflammatory proteins.⁶⁷ The efficacy of corticosteroids in reducing morbidity and mortality in patients with infectious diseases has been well documented.⁶⁸ For example, 15 randomized trials have examined the efficacy of corticosteroid therapy for patients with acute bacterial meningitis; most trials demonstrated a reduction in the incidence of sensorineural hearing loss, and some demonstrated a reduction in mortality.⁶⁸ Many studies have examined the use of corticosteroids along with antimicrobials during acute bacterial infection. These studies, which included more than 2000 children, have shown that the use of adjuvant corticosteroids in the context of acute bacterial infection is safe. For example, in children with meningitis, corticosteroid use did not delay clearance of bacteria from the cerebrospinal fluid.

VII. Safety of Corticosteroids for Children with APN

In the previously mentioned study by Sharifian,¹⁹ there were no adverse events attributed to the use of corticosteroids and all 72-hour follow-up urine cultures were negative. These results are important because they provide reassurance that use of corticosteroids in children with UTI reduces kidney inflammation without interfering with bacterial eradication.

VIII. Rationale for Outcome Measure and Timing of the Outcome Measure

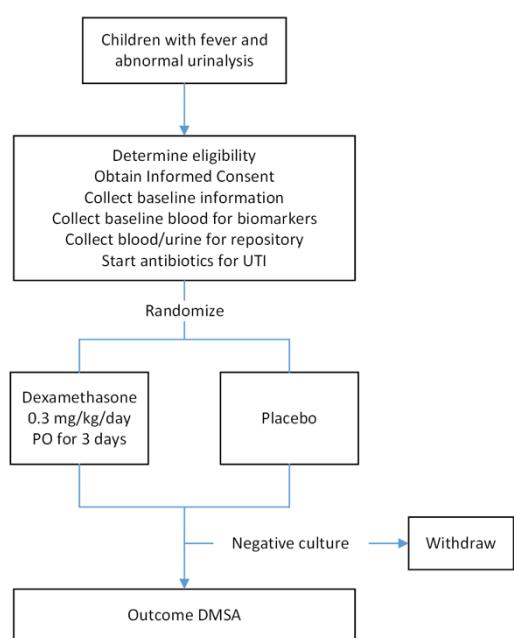
DMSA is the current gold standard for assessing presence of renal scars. Risdon, using a pig model, compared the location and extent of renal scarring on histopathological examination with DMSA scan findings immediately before sacrifice.⁶⁹ DMSA identified 94% of kidneys with pathology and was 100% specific. Approximately 47% of abnormalities noted on scans conducted 2 to 4 months after the index UTI, resolved on DMSA scans obtained at a later date. In contrast, 90% of abnormalities noted on scans conducted ≥ 5 months (20 weeks) after the index UTI were persistent.⁷⁰

C. Research Design and Methods

I. Design

We propose to conduct a multicenter, randomized, placebo-controlled, double-blind clinical trial that will evaluate the efficacy of adjuvant dexamethasone (0.15 mg/kg per dose, twice daily for 3 days) in preventing renal scarring in young febrile children (2 months to 6 years) with a first-diagnosed UTI. 520 participants will be enrolled over a 3.75-year period from 6 sites. Choice of antimicrobial treatment and need for follow-up imaging will be at the discretion of the providing physician. If possible, the study nurse will demonstrate how to give the child the study medication and will administer the first dose to the child, otherwise study medication will be administered to the child no later than 48 hours after the first dose of antibiotic treatment for the UTI. A flavoring syrup will be mixed by the parent before each separate dose

Figure 3. Study Design



with the medication/ placebo to make the study medication flavor more appealing to the child. The parent will draw the prescribed amount of medication/ placebo from the syringed-top medication bottle and push it out into a small medication cup provided by the study team. The parent will then draw an equivalent amount of flavoring syrup from the flavoring bottle using a separate dosing syringe and push the flavoring out into the same medication cup. The parent will then mix the contents in the medication cup. After mixing, the child will be able to drink the contents directly from the medication cup or the parent will re-draw the contents using the syringe and administer the contents to the child with the dosing syringe. The study medication will be given with meals, twice daily to reduce gastrointestinal side effects. If vomiting occurs within 20 minutes of steroid administration, an equivalent second dose will be administered. We will ask that the study medication doses administered to the child be recorded in a medication diary that will be provided by the study. When the child has completed the study, we will ask that the parent return all medication supplies and the medication diary in a postage-paid box that will be provided by the study.

II. Recruitment

The centers participating in this study are: Children's Hospital of Pittsburgh, Nationwide Children's Hospital in Columbus, the American Family Children's Hospital which is a children's hospital within the University of Wisconsin system, Children's National Medical Center in Washington, D.C, Hasbro Children's Hospital in Rhode Island, and Primary Children's Hospital in Utah. At each site, participants will be recruited at the time of UTI diagnosis from the emergency department (ED) and from a network of outpatient/private practice offices. Recruitment from the ED will be supervised by an ED physician (ED Enrollment Coordinator) and a part-time nurse.

III. Eligibility Criteria

Parents of febrile children 2 months (60 days) to 6 years of age (before sixth birthday) who have pyuria on urinalysis or dipstick and who do not meet any of the exclusion criteria will be asked to participate in the study (Table 2). We will exclude children younger than 2 months of age because, 1) the incidence of bacteremia is highest in this age group,^{22, 71} 2) clinical assessment of children <2 months of age is more difficult. Two studies in rats suggested that treatment of near-term premature animals with corticosteroids is associated with increased risk of adverse events; therefore, children between 2 and 3 months of age will only be enrolled if they are born at term (≥ 37 weeks).^{72, 73} We will limit the study to children with their first protocol defined UTI. This will minimize the number of children with preexisting scars secondary to previous UTIs. To minimize the number of children with false positive urinalyses, young children who have urine collected via a bag specimen for culture (without a follow-up

catheterized specimen) will be excluded. Other exclusion criteria are listed in Table 2. See last page of this document for changes made to the protocol after starting patient enrollment.

IV. Randomization

Subjects will be randomized in a 1:1 ratio. Randomization will occur in blocks of 4. Stratification will be based on the duration of fever (either documented or by touch) prior to diagnosis (<48 hours vs

≥ 48 hours) and the site of enrollment. That is, children will undergo site-specific blocked randomization sequences for each of the strata defined by duration of fever. Subjects are randomized into the appropriate strata based on this information. The investigator will be provided with a unique randomization code, which will

Table 3. Summary of Observations

| Observation | Enrollment | Day 2- 4*** | 1 Month | Monthly | 5 - 24 mo |
|---|------------|-------------|---------|---------|-----------|
| Informed Consent | X | | | | |
| Detailed Medical History | X | | | | |
| DES* questionnaire | X | | | | |
| Constipation questionnaire | X | | | | |
| Randomization | X | | | | |
| Blood for biomarkers if parent consents | X | | | | |
| Urine biomarkers/creatinine | X | | | | |
| 1-month study visit/phone call | | | X | | |
| Phone call | | X | X | X | |
| Outcome DMSA** | | | | | X |

* Dysfunctional elimination questionnaire (for toilet trained children only)

** For children with reinfections, DMSA will be performed 4 mo. after last UTI

*** Until the fever has resolved

correspond to a code on one of the bottles of medication available at that site. In this way, only the central pharmacy will be aware of treatment assignment.

Any protocol defined recurrence will be treated with the same therapy initially given to the child. This treatment will be initiated as soon as possible after diagnosis (but no later than 48 hours of starting antibiotic therapy).

V. Data to be Collected (Table 3)

A. Baseline Data

Information on the following potential predictors and confounders of renal scarring will be collected during the baseline evaluation: Demographics (sex, race, ethnicity, age), symptoms (type and duration), past medical history (including result of prenatal ultrasound), medications [including non-steroidal anti-inflammatory drug use (NSAIDS)], and family history (VUR, UTI, scarring, kidney disease). There will be an age-appropriate evaluation for dysfunctional elimination syndrome (DES),⁷⁴ and constipation.⁷⁵ Blood, which will be optional if the parent is reluctant to have their child's blood drawn, and urine for all laboratory tests (Table 3) will be collected before steroids are administered and a portion of the specimen will be saved for future hypothesis-driven studies. If the parent consents to the genetic blood draw at the time of enrollment, this sample can be drawn at the time of the DMSA scan.

B. Follow-up

Day 2-4 Phone Call

To monitor the child's wellbeing and to ensure compliance with medication administration, we will call parents for 3 days following the day of enrollment. If the child's fever has not resolved by day 4, we will continue daily phone calls until the child becomes afebrile. With recurrent UTI, the phone call sequence will begin the day after the study medication is administered and will end after the fever has resolved. At least two attempts each day will be made to contact a parent. Attempts should be made at different times of day or to more than one phone number, leaving a message to call us if there is no answer, with a 24-hour return phone number.

A positive urine culture will be defined as either a:

- 1) Urine specimen obtained by catheterization with:
 - a. The growth of one or more uropathogens at $\geq 50,000$ CFU/mL
 - OR
- 2) Urine specimen obtained by clean catch with:
 - a. The growth of one or more uropathogens at $\geq 100,000$ CFU/mL

For children who have a negative urine culture, corticosteroids will be stopped. All parents will be instructed to arrange a follow-up appointment with their primary care provider irrespective of culture results. Because of theoretical concerns regarding the anti-inflammatory effects of ibuprofen, parents will be asked to refrain from using ibuprofen (or other NSAIDs) during the study.²³

1-Month Visit (+/- 2 weeks)

Approximately one month after enrollment in the study, we will attempt meet parents of enrolled subjects to review 1) the results of the urine culture, 2) the results of any imaging tests ordered by the primary care provider as part of the management of the UTI, and 3) the plan for the remainder of the study period including a tour of the nuclear medicine department where the outcome DMSA will be performed. To save parents from making an extra trip, we will try to coordinate this visit with the routine imaging tests ordered by the child's PCP. If it is very difficult for parents to make this visit in person, we will conduct the visit over the phone.

The research nurse or the PI at each participating site will be available 24 hours a day to parents of enrolled children via a study-dedicated cellular phone or hospital-based beeper. Parents will be instructed to (1) contact

study personnel in the event of intercurrent febrile illness or urinary symptoms, (2) have their child evaluated within 24 to 48 hours and (3) have a urine specimen obtained to evaluate for the presence of UTI. We will call parents monthly to determine whether any interim UTIs have been diagnosed. In case of recurrent UTIs, records from the visit(s) will be reviewed. Children with febrile re-infections will be retreated, in a blinded fashion, with antibiotics and the same study medication (dexamethasone or placebo) that was used at the time of the index UTI.

C. Routine Imaging Tests

All children in the study will be evaluated with currently recommended, age-appropriate imaging tests. Routine imaging tests will be at the discretion of the providing physician. If VUR is identified on VCUG, appropriate treatment for VUR will be instituted. Although current guidelines recommend that children with VUR be treated with prophylactic antibiotics, several studies are underway that may change the way VUR is managed. We will use the best available evidence to manage children with VUR. Although the yield of ultrasounds in this population is expected to be low, in the context of this research study, a normal ultrasound will provide reassurance that few or no children with significant dysplasia are included.

Fig. 4. Scarring



D. Outcome DMSA Renal Scan (5-24 months from the time of enrollment)

The presence and extent of renal scarring is determined using a DMSA scan. When radiolabeled DMSA is given to patients whose tubular cell function is permanently impaired, the scan will show a photon-deficient area(s) with or without a loss of renal contour(s) (Fig. 4). Children with no further UTI episodes (94% of children in the study)²² will have a DMSA scan approximately 5 months (+/- 2 weeks) to 24 months from the time of enrollment) after the index UTI to determine the presence and extent of renal scarring. For children who have a re-infection during the follow-up period, the scan will

be performed at least 4 months after the last UTI. By delaying the outcome scan in these children, photopenia due to the acute infection will have resolved and will not affect the interpretation of the outcome scan. Children will be injected with a dose of 50-75 μ Ci 99m Tc-DMSA per Kg (maximum dose 3 mCi). High-resolution magnified images

of the kidney will be obtained, including posterior and posterior oblique projections using a high-resolution pinhole collimator 1.5 to 3 hours following injection. Scarring will be defined by the presence of cortical defects with or without loss of contours.^{76, 77} These cortical defects will be assessed semi-quantitatively by dividing the renal cortex into 12 equal segments (Table 4). The number of renal segments affected will be determined and the grade of scarring will be assigned based on a system developed by the Imaging Committee of the RIVUR study. DMSA scans will be interpreted by three reference radiologists without knowledge of clinical events or treatment arm. Inter-observer agreement will be assessed. To maximize reliability, we will request that each participating site send 10 de-identified DMSA scans (from children recently seen) to the reference radiologists for review prior to the start of enrollment. If the quality of the scans is suboptimal, appropriate changes will be made.

Table 4. Grading system for renal scarring

| Grade | Description |
|-------|---------------------------------|
| 1 | 1-2 kidney segments affected |
| 2 | 3-4 kidney segments affected |
| 3 | >4 kidney segments affected |
| 4 | Global atrophy/diffuse scarring |



E. Specimen Processing

Urine biomarkers – 1 mL of urine will be filtered, frozen, and processed in batches at CHP or the Luminex Facility at the Pittsburgh Cancer Institute. This method is well-established and is particularly suitable when the sample volume is limited. Concentrations will be measured using multiplex bead technology.

Blood biomarkers – At the time of UTI diagnosis, and if parents' consent to a blood draw, 1 mL of blood will be collected in EDTA plasma tubes for biomarker analysis.

VI. Statistical Analysis

Analysis plan– Efficacy of corticosteroids in the prevention of renal scarring

The proportion of children who have any renal scars on the outcome DMSA scan in each treatment arm will serve as the principal outcome variable. Renal scarring will be defined as decreased uptake of tracer with or without loss of contours. We will also compare the proportion of children with severe scarring between groups. Severe scarring will be defined as the presence of grades 3 or 4 scarring on at least one kidney. Three reference radiologists, blinded to treatment assignment, will make all determinations and the reading endorsed by the majority will be used as the primary outcome measure. The analysis will follow intent to treat principles: subjects will be analyzed according to randomized treatment assignment regardless of adherence. Children who are found to have a negative urine culture will not be included in the efficacy analysis. These children do not have a UTI and are not at risk for developing renal scarring. Up to 6% of children (n=23) are expected to have a recurrent UTI in the follow-up period.²² It is estimated that for most of these children (~85%), the recurrent UTI episode will not result in renal scarring. As such, the effect of re-infections on the primary analysis will be minimal. The incidence and the extent of renal scarring in the two treatment groups will be compared using logistic regression. We will also test for statistical interaction for individual variables (age, gender, race, mother's education, fever duration, infecting organism, CRP, ibuprofen use within 24 hours of enrollment, and 5 preselected urinary biomarkers listed below) and steroid treatment. This is done to investigate the possibility that the relative treatment effect differs among patient subgroups. We are aware that tests for interaction generally have low statistical power and we will likely detect only marked differences in treatment effects in these subgroups. Furthermore, the additional problem of falsely identifying heterogeneity of treatment effect among patient subgroups in clinical trials is well known and therefore the interpretation of subgroup differences is considered exploratory and will be done with caution. Another analysis will be the proportion of children with recurrent febrile UTIs in each treatment group.

Data will be analyzed after approximately one third of enrolled children complete the outcome DMSA scan. If there is a significant difference between groups, the DSMB may consider the possibility of terminating the study. Type I error will be controlled by using the method of Fleming, Harrington and O'Brien. Testing will be conducted at significance levels of 0.005 and 0.048 at the interim and final analysis, respectively.⁷⁸

We will also compare the mean proportion of scarring in the two treatment groups across the three radiologists while adjusting for the correlation resulting because the radiologists evaluated the same set of scans.

Because some of the STARRs participants also participated in the companion “Biomarkers for Pyelonephritis” study, in which early DMSA scan is performed to determine whether pyelonephritis is present, we will examine the efficacy of treatment in children with and without pyelonephritis.

The study will be powered to detect a 10% difference between the two arms. A 10% absolute difference between the two groups translates into a number needed to treat of 10 (i.e., 10 children will need to be treated to prevent one child from developing scars). Assuming a 15% rate of scarring in the control group, a two-sided alpha of 0.05, a beta of 0.8, a 10% attrition rate, and a 7% rate of false positive urinalysis, 190 children will be required per group (i.e., 160 evaluable children per group). However, as the study progressed, we realized that 30% of children enrolled had a false positive urinalysis, and thus increased the required sample size to 260 children per group (to arrive at the 160 evaluable children needed). The 15% rate of scarring in the control group comes from a meta-analysis we conducted.⁷⁹ The attrition rate is based on our previous study of intravenous versus oral antibiotics for the treatment of UTI which was also a randomized multicenter study with a 6-month follow-up DMSA.²²

A recent study described several urinary biomarkers that appear promising in differentiating children with pyelonephritis from children with cystitis.⁸⁰ Because only children with pyelonephritis are at risk for renal scarring, these markers could potentially be used to select children for treatment with adjuvant corticosteroids. As such we will examine for interactions between 5 such biomarkers and treatment effect. Logistic regression

models will be fitted by including treatment by biomarker interaction terms (adjusted as appropriate for other confounders). We will include the following 5 biomarkers, each as a continuous variable (transformed as appropriate): IL-15, MCP1, CXCL1 (also known as GRO-alpha), interferon gamma, and NGAL.

Literature Cited

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