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# Proprietary non-animal stabilized hyaluronic acid/dextranomer gel (NASHA/Dx) for endoscopic treatment of grade IV vesicoureteral reflux: Long-term observational study

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**Keywords**
Vesicoureteral reflux; Children; Deflux; NASHA/Dx; Urinary tract infection

**Abbreviations**
NASHA/Dx, proprietary non-animal stabilized hyaluronic acid/dextranomer gel; UTI, urinary tract infection; VCUG, voiding cystourethrogram; VUR, vesicoureteral reflux

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## Summary

### Background
Since 1993, children aged >1 year with persistent grade III–V vesicoureteral reflux (VUR) and febrile urinary tract infections (UTIs) attending Uppsala University Hospital have undergone endoscopic injection with proprietary non-animal stabilized hyaluronic acid/dextranomer gel (NASHA/Dx; Deflux®).

### Objective
Investigate long-term incidence of UTI, bladder dysfunction, ureteral reimplantation and overall clinical findings following endoscopic injection of NASHA/Dx.

### Study design
Children with grade IV VUR diagnosed by voiding cystourethrogram (VCUG) and dilating VUR persisting for >1 year were included in this study. 15–25 years after endoscopic treatment, patients' hospital charts were studied. Information on bladder function and UTIs was obtained via questionnaire, 8–18 years after endoscopic treatment.

### Results
185 patients (69 boys, 116 girls) were included in the study; 237 grade IV VUR ureters were treated. All study patients were diagnosed with VUR after a febrile UTI (i.e. pyelonephritis). According to the last voiding cystourethrogram, 69% of ureters showed a positive response (VUR grade 0–I), 7% had VUR grade II and 23% had VUR grade ≥ III. 46 patients (25%) required ureteral reimplantation during follow-up. Among patients treated during the second 5-year period compared with the first (1998–2003 versus 1993–1998), there was a significant decrease in the rate of ureteral reimplantation (31% vs 16%; p = 0.0365). This difference may be attributable to developments over time in the injection technique. UTIs occurred in 30 patients (21% of the evaluable population): 28 females and 2 males. Febrile UTIs were reported in 14 patients (10%), all females. Forty-nine patients (34%) had bladder problems (e.g. underactivity, overactivity, incontinence). Five patients underwent ureteral reimplantation 'late', 6–10 years after the last endoscopic injection. In one male patient, calcification around the NASHA/Dx implantation site was observed during routine examination 2 years after endoscopic treatment; no intervention was required. No safety issues were observed in the remaining 97% of the study population.

### Conclusions
This study represents the longest published follow-up of Grade IV VUR patients undergoing endoscopic treatment. Three-quarters of patients did not need ureteral reimplantation. Optimal injection technique and higher injection volume were associated with a reduced ureteral reimplantation rate. Treatment with NASHA/Dx was durable and well tolerated: long-term risks of UTI, bladder dysfunction and recurrent VUR were low.

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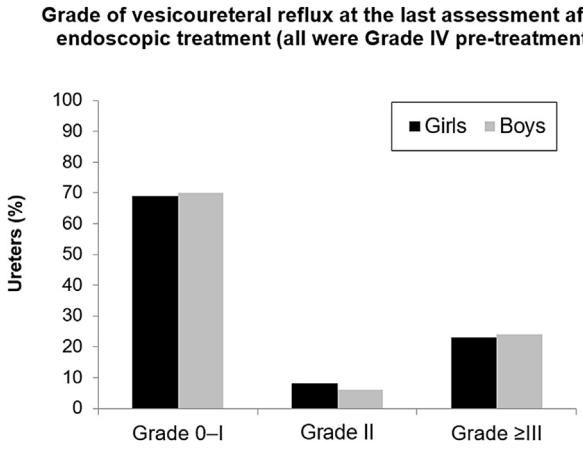


## Introduction

Vesicoureteral reflux (VUR), backflow of urine from the bladder up towards the kidneys, is caused by insufficient closure of the distal ureter and affects ~1% of all children in Europe and North America [1]. VUR is associated with a risk of febrile urinary tract infections (UTIs) and pyelonephritis, potentially leading to renal scarring and increased future risks of hypertension and renal failure [2,3]. The prevention of pyelonephritis is the primary aim in VUR management [4,5].

For patients with high-grade VUR, there are two potentially curative treatment options: endoscopic injection and ureteral reimplantation. Ureteral reimplantation is highly effective, with cure rates exceeding 90% [6,7]. However, there are disadvantages with ureteral reimplantation because it involves open surgery with probable need for a hospital stay, many patients experience post-operative discomfort, and there are risks of complications [8]. Endoscopic injection is a minimally invasive procedure that offers the chance of curing VUR. This treatment approach was first investigated in the 1980s, and polytetrafluoroethylene (PTFE) was initially used as the injection material ('bulking agent') [9,10]. Subsequent improvements in the treatment method (e.g. adjustments to the injection technique; changing the bulking agent to a biocompatible substance) led to its uptake in routine clinical practice [8,11,12]. Unlike open surgery, endoscopic injection allows patients to return to their normal lives rapidly (often within 24 h) [13,14].

Since the early 1990s, endoscopic injection with proprietary non-animal stabilized hyaluronic acid/dextranomer gel (NASHA/Dx) has been routinely offered to VUR patients referred to the University Children's Hospital in Uppsala, Sweden [15]. This treatment has previously been shown to be well tolerated and to provide long-term efficacy [16–21]. There is also evidence to suggest that endoscopic treatment with NASHA/Dx may be effective in high-grade VUR [21–26]. However, cure rates with endoscopic treatment generally decrease with increasing VUR grade [5,17,19,27]. In clinical practice at many centers, all children with persistent grade IV VUR undergo ureteral reimplantation.

We performed a long-term, observational study of children with grade IV VUR undergoing endoscopic injection of NASHA/Dx. The aims were to study the incidence of UTI (particularly febrile UTI), incidence of bladder dysfunction (particularly in girls), late recurrence of VUR, late ureteral reimplantations and overall clinical findings.

## Methods

Endoscopic treatment was performed in patients aged >1 year who had persistent dilating VUR (grade III–V) diagnosed by voiding cystourethrogram (VCUG) before and after a period of antibiotic prophylaxis (>1 year), and breakthrough febrile UTIs or poor compliance with antibiotic prophylaxis. Throughout this study, febrile UTIs were defined as pyelonephritis with fever, a positive urine culture, and a serum C-reactive protein level >70 mg/L. Inclusion criteria for this study were endoscopic treatment between 1 May 1993 and 30 April 2003, and grade IV VUR (diagnosed by VCUG at any time before endoscopic treatment). Exclusion criteria for this study were: endoscopic treatment with agents other than NASHA/Dx; neurogenic bladder dysfunction; previous ureteral surgery; and conditions or anomalies (other than VUR or bladder dysfunction) known to affect renal or bladder function. Eligible patients with bladder dysfunction underwent informal urotherapy (with recommendations on how patients might improve bladder emptying and increase their awareness of the bladder) during the period between VUR diagnosis and endoscopic therapy.

The procedure for performing endoscopic treatment has been described previously [17,28]. Briefly, patients were placed under general anesthesia, and a cystoscope was introduced into the bladder. NASHA/Dx (Deflux®; Palette Life Sciences) was injected below the ureteral orifice (6 o'clock position) via a prefilled syringe and a 3.7 Fr x 23 G x 35 cm needle. Adequate volume was injected to create a prominent bulge, uplifting the distal ureter and the ureteral orifice. For double ureters, NASHA/Dx was injected at the refluxing proximal ureter and under the distal ureter, ensuring that the whole ureteric complex was treated. In addition to treating ureters with grade IV VUR, contralateral ureters with any-grade VUR were treated.

**Table 1** Patients and ureters included in the study.

| | 1993–1998 | 1998–2003 | Total |
|---|---|---|---|
| Number of patients (%) | | | |
| - Girls | 64 (58%) | 52 (70%) | 116 (63%) |
| - Boys | 47 (42%) | 22 (30%) | 69 (37%) |
| - Total | 111 | 74 | 185 |
| Age at first endoscopic treatment, months (median [range]) | | | |
| - Girls | 45 (5–275) | 42 (10–233) | 43 (5–275) |
| - Boys | 26 (3–157) | 23 (4–103) | 26 (3–157) |
| - Total | 32 (3–275) | 30 (4–233) | 31 (3–275) |
| Number of endoscopic treatments per patient (mean ± standard deviation) | 1.50 ± 0.69 | 1.40 ± 0.55 | 1.46 ± 0.63 |
| Time between VUR diagnosis and first endoscopic treatment, months (median [range]) | 16 (3–154) | 17 (2–230) | 17 (2–230) |
| Number of ureters | 143 | 94 | 237 |
| Number of double ureters (percentage of all ureters) | | | |
| - Girls | 23 (29%) | 20 (30%) | 43 (29%) |
| - Boys | 11 (17%) | 2 (7%) | 13 (14%) |
| - Total | 34 (24%) | 22 (23%) | 56 (24%) |
| Mean volume of NASHA/Dx injected per endoscopic treatment, mL/ureter (mean [range]) | | | |
| - Girls | 0.63 (0.1–2.0) | 0.87 (0.5–2.0) | 0.74 (0.1–2.0) |
| - Boys | 0.67 (0.2–1.4) | 0.83 (0.5–1.7) | 0.71 (0.2–1.7) |
| - Single ureters | 0.65 (0.2–1.4) | 0.84 (0.5–1.7) | 0.73 (0.2–1.7) |
| - Double ureters | 0.62 (0.4–1.0) | 0.94 (0.4–2.0) | 0.72 (0.4–2.0) |
| - Total | 0.65 (0.1–2.0) | 0.86 (0.4–2.0) | 0.73 (0.1–2.0) |
| Time between last endoscopic treatment and last VCUG, months (median [range]) | 16 (3–144) | 14 (3–129) | 15 (3–144) |
| Questionnaire analysis: duration of follow-up after the first endoscopic treatment, years (median [range]) | 16 (13–18) | 11 (8–13) | 14 (8–18) |
| Hospital chart review: duration of follow-up after the first endoscopic treatment, years (median [range]) | 23 (20–25) | 18 (15–20) | 22 (15–25) |

Post-endoscopic treatment procedures were as follows. VCUG assessments and renography were scheduled at the patient's local hospital, at 3 and 12 months after endoscopic treatment. For patients treated during the first (but not the second) 5-year period of the study, a third VCUG was recommended at 3 years. Antibiotic prophylaxis was continued until the first VCUG assessment (3 months after endoscopic treatment) had been performed at the patient's local hospital. Patients shown to have persistent VUR (grade $\geq$ III) were retreated endoscopically within 3–6 months, and antibiotic prophylaxis was continued between the VCUG assessment and endoscopic treatment. Individuals demonstrating positive response in their VCUG assessments (bilateral reflux grade 0–I) underwent regular follow-up until the age of 15 years with renal ultrasound, renography and blood/urine tests according to their renal function and bladder status. These patients only underwent further VCUG assessment in case of subsequent UTIs. All VCUG results were checked blindly by a second radiologist before being provided to the study investigators.

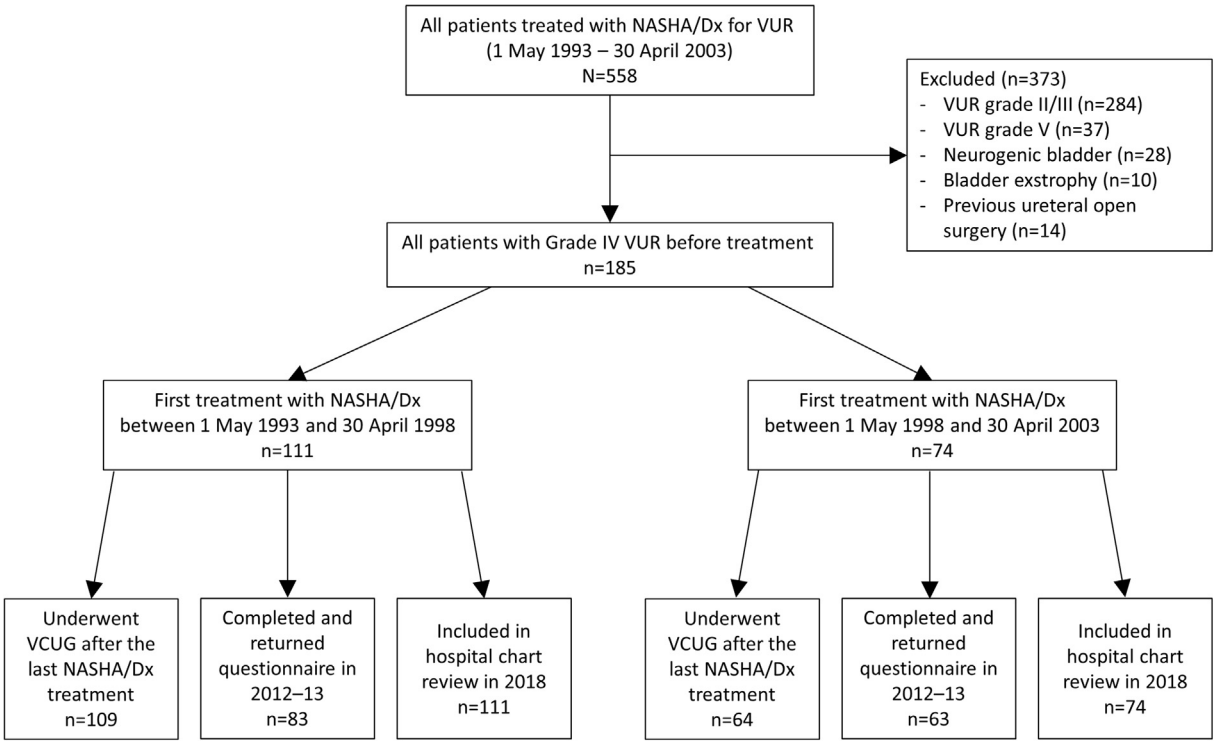


**Fig. 1** Patient disposition.

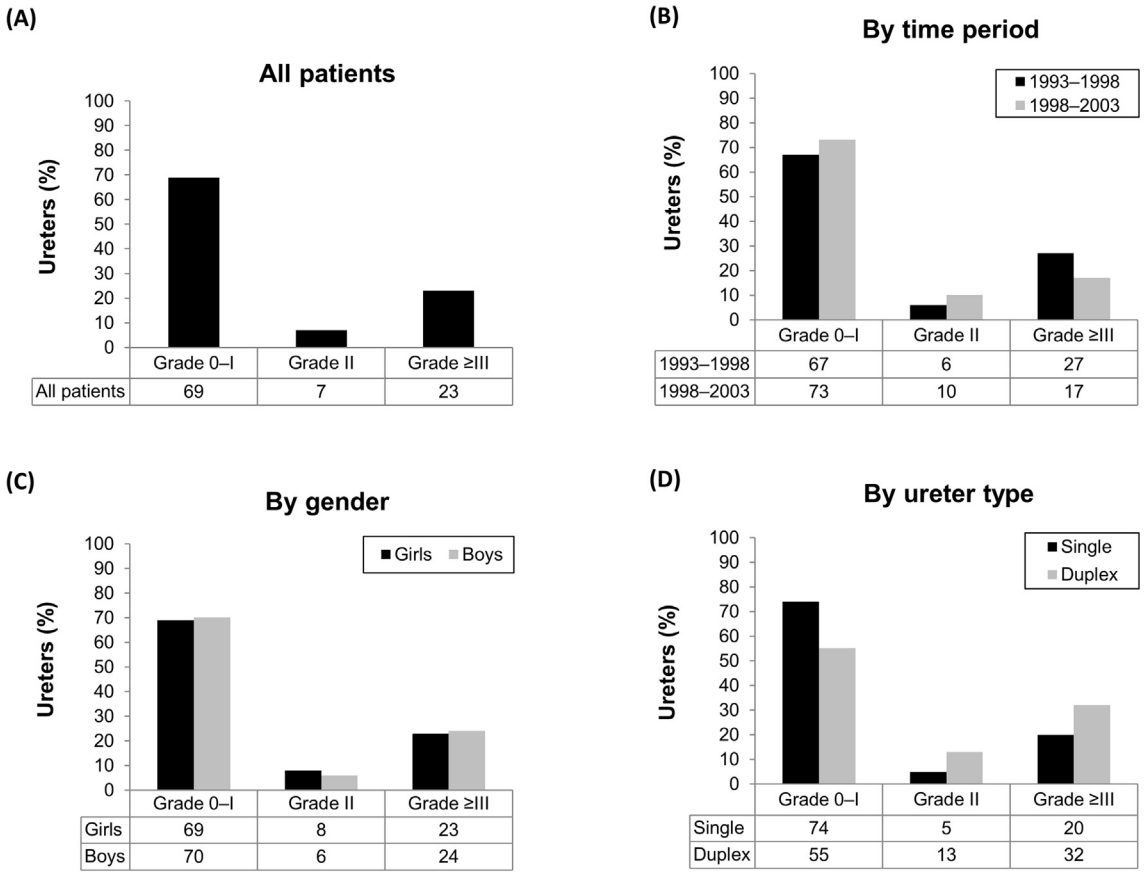


**Fig. 2** Reflux grade at the last VCUG. Data are shown by ureter, for all patients (A), patients treated in the first versus the second 5-year time period (B), for girls versus boys (C), and for single versus double ureters (D). Ureters for which no VCUG test was performed after the last endoscopic treatment were excluded from this analysis.

**Table 2** Questionnaire results. Data are shown by patient, for all patients, patients treated in the first versus the second 5-year time period, for girls versus boys, and for single versus double ureters.[a]

| | All (N = 146) | Results by time period | | Results by gender | | Results by ureteral type | |
|---|---|---|---|---|---|---|---|
| | | 1993–1998 (N = 83) | 1998–2003 (N = 63) | Females (N = 98) | Males (N = 48) | Single (N = 109) | Double (N = 37) |
| Any UTI | | | | | | | |
| - Present | 30 (21%) | 15 (18%) | 15 (24%) | 28 (29%) | 2 (4%) | 22 (20%) | 8 (22%) |
| - Absent | 116 (79%) | 68 (82%) | 48 (76%) | 70 (71%) | 46 (96%) | 87 (80%) | 29 (78%) |
| Febrile UTI | | | | | | | |
| - Present | 14 (10%) | 7 (8%) | 7 (11%) | 14 (14%) | 0 (0%) | 7 (6%) | 7 (19%) |
| - Absent | 132 (90%) | 76 (92%) | 56 (89%) | 84 (86%) | 48 (100%) | 102 (94%) | 30 (81%) |
| Bladder problems | | | | | | | |
| - Present | 49 (34%) | 25 (30%) | 24 (38%) | 36 (37%) | 13 (27%) | 35 (32%) | 14 (38%) |
| - Absent | 97 (66%) | 58 (70%) | 39 (62%) | 62 (63%) | 35 (73%) | 74 (68%) | 23 (62%) |

[a] Patients not responding to the questionnaire were excluded from these analyses.

Patients exhibiting dilating VUR (grade $\geq$ III) after endoscopic treatment were offered repeat endoscopic treatment. Ureteral reimplantation (open surgery) was recommended for patients with persistent VUR (grade $\geq$ III) after two endoscopic treatments. There were some differences between the two study periods in the criteria for performing endoscopic injection. During the second 5-year study period, patients with obstructive refluxing megaureter, and those shown to have a narrow distal ureter, were not considered candidates for endoscopic injection. These patients could receive NASHA/Dx injection during the first study period.

Treatment response by VCUG grade was assessed based on patients' last VCUG assessment after the last endoscopic treatment. Information on bladder function and UTIs was obtained during 2012–13 via a questionnaire and follow-up telephone interviews. The same questionnaire was used in a previous study [29]. The presence of bladder problems was determined from questionnaire responses indicating high or low micturition frequency, urgency, incontinence or voiding difficulty. In 2018 (15–25 years after the first endoscopic treatment), patient charts from Uppsala University Hospital and local county hospitals were studied; these provided details of UTIs that enabled their classification as febrile or non-febrile, as well as VCUG results, renal assessments and other clinical data.

Results were analyzed by treatment period (first or second 5-year period), by gender, by ureter, and for single versus double ureteral systems. P-values for differences between subgroups were calculated using Fisher's Exact test; no adjustments were made for multiple testing.

This study was approved by the Ethical Committee of the Medical Faculty at Uppsala University, with reference number 2010-064.

## Results

Between 1 May 1993 and 30 April 2003, 558 patients with VUR were treated endoscopically with NASHA/Dx. Of these, 185 (69 boys and 116 girls) were included in the current study; 237 ureters with grade IV VUR were treated endoscopically (Fig. 1, Table 1). All the patients were diagnosed with VUR after a febrile UTI (i.e. pyelonephritis). The median time between VUR diagnosis (first VCUG) and first endoscopic treatment was 17 months, and patients' median age at the time of endoscopic treatment was 31 months. The mean number of endoscopic treatments per patient was 1.46. Patients were treated in accordance with the approach described in the Methods, except for 14 patients who underwent a requested third endoscopic injection after two failures and 13 patients underwent requested ureteral reimplantation after one failed endoscopic injection. Across all treatments, the mean volume of NASHA/Dx injected was 0.73 mL per ureter. The injection volume was significantly higher during the second versus the first 5-year period (0.86 vs 0.65 mL per ureter; $p < 0.0001$). VCUG assessment was performed after the last endoscopic treatment in 173 patients (222 ureters). The median period between the last endoscopic treatment and the last VCUG assessment was 15 months and, in 35 patients, this period exceeded 3 years. Questionnaires were completed by 146 patients (79%), median 14 years (range 8–18 years) after the first endoscopic treatment.

According to the last VCUG, 69% of ureters showed a positive response (VUR grade 0–I), 7% had VUR grade II and 23% had VUR grade $\geq$ III (Fig. 2). VCUG assessment was not performed after the last endoscopic treatment in 15 ureters. The positive response rate (VUR grade 0–I) was numerically higher during the second versus the first 5-year time period (67% vs 62%; $p = 0.30$), while minimal differences were observed between male and female patients ($p = 0.50$). Outcomes for single ureters were significantly better than those for double ureters ($p = 0.023$). Similar patterns were observed when the results were analyzed 'by patient' instead of 'by ureter' (Table A.1 [Supplementary data]). Overall, 64% of patients exhibited positive response; 8% had VUR grade II at the last VCUG, and 28% had VUR grade $\geq$ III. There was no significant difference in grade at last VCUG between patients with double versus single ureters ($p = 0.22$).

Forty-six patients (29 girls and 17 boys) required ureteral reimplantation during follow-up. The percentage of patients undergoing ureteral reimplantation (25% overall) was

significantly lower during the second versus the first 5-year study period (16% vs 31%; p = 0.037). Five patients underwent ureteral reimplantation 'late', 6–10 years after the last endoscopic injection. Two of these patients (one male and one female) exhibited persistent VUR after treatment for lower UTI and bladder dysfunction. The other three patients underwent late reimplantation for reasons other than VUR: two females initially had obstructive refluxing megaureter (now considered as a contraindication to endoscopic treatment); and one girl developed a febrile UTI 10 years after her last VCUG – this patient had developed a late obstruction that may have been related to UTIs and bladder dysfunction. There were no other cases of ureteral obstruction.

Forty-eight patients (37 girls and 11 boys) had double ureteral systems. Of these, 15 (31%) required ureteral reimplantation, while the remaining 33 needed no further treatment after endoscopic treatment. None of the ureteral reimplantation procedures performed in the participants of our study were complicated or compromised by previous treatment with NASHA/Dx. In one patient (a boy), calcification around the NASHA/Dx implantation site was observed during a routine intravenous pyelogram 2 years after endoscopic treatment. No obstruction, ureteral dilatation, renal dysfunction or other clinical problems were apparent; no stents were implanted and no other interventions were required. Calcification was not observed in any other patients. Overall, no safety issues were observed in 97% of the study population.

Completed questionnaires were received from 98/116 females (84%) and 48/69 males (70%). The incidence of UTIs after endoscopic treatment was significantly higher in females than in males (28/98 females [29%] versus 2/48 males [4%]; p = 0.0004; Table 2). Febrile UTIs (i.e. pyelonephritis) occurred in 14/98 female patients (14%) and no males (p = 0.0050). Twelve of the 28 females with UTIs had persistent grade III–V VUR after endoscopic treatment and 10 of these underwent ureteral reimplantation. In three of the females reporting febrile UTIs, these occurred after ureteral reimplantation but no UTIs had occurred before the reimplantation procedure. The two males with UTIs had infections of the lower urinary tract; one of these patients had grade I VUR at the last VCUG, while the other exhibited grade III VUR 9.5 years after endoscopic treatment and underwent 'late' ureteral reimplantation. Rates of UTIs and febrile UTIs were numerically higher among patients treated in the second versus the first 5-year period of the study, although these differences were not statistically significant (24% vs 18%, p = 0.42; and 11% vs 8%, p = 0.59, respectively). Patients with double ureters had a slightly higher risk of UTI than those with single ureters (p = 0.82), but the incidence of febrile UTIs was significantly higher in patients with double ureters (p = 0.047; Table 2). Of the 43 patients who underwent ureteral reimplantation for persistent VUR, nine (21%) had UTIs after endoscopic treatment and four (9%) had febrile UTIs. Three of the 43 patients (7%) had UTIs (all of them febrile) after ureteral reimplantation.

Bladder problems were reported in 49 patients (34%). Bladder problems were numerically but not statistically significantly more common in the following groups: patients treated in the second versus the first study period (38% vs 30%; p = 0.38), girls versus boys (37% vs 27%; p = 0.27), and patients with double versus single ureters (38% vs 32%; p = 0.55). Among 38 patients who underwent ureteral reimplantation for persistent VUR with available data, 12 (32%) had bladder dysfunction after endoscopic treatment.

## Discussion

Our study represents the longest follow-up of grade IV VUR patients undergoing endoscopic treatment with NASHA/Dx. This treatment was effective in maintaining low rates of UTIs, with few patients experiencing late failure and long-term sequelae. Endoscopic treatment enabled ureteral reimplantation to be avoided in 75% of children with grade IV VUR, a substantial gain for patients and their parents. Importantly, there was a low risk of late clinical recurrence (UTIs with persistent VUR and a consequent need for ureteral reimplantation) in patients initially responding to endoscopic therapy.

The incidence of UTIs after endoscopic treatment with NASHA/Dx was low, particularly in boys. This may be attributed to successful cure of VUR, preventing UTIs from reaching the upper urinary tract. In addition, successful cure of VUR may potentially enhance bladder function [8]. During the study, the mean duration of antibiotic prophylaxis before endoscopic treatment was 22 months, and antibiotic prophylaxis was administered until the first VCUG (~3 months). However, we now know that long periods of antibiotic prophylaxis could potentially be harmful and are not necessary, provided that UTIs are treated early [30,31]. Therefore, we usually restrict antibiotic therapy to short time periods (i.e. for the treatment of UTIs).

In some patients, endoscopic treatment might help to alleviate bladder dysfunction as well as VUR. Previous observations provide some support for this notion [29,32], although robust data are lacking. Throughout the current study, patients aged >5 years with bladder dysfunction underwent urotherapy between VUR diagnosis and endoscopic therapy. Today, the period available for bladder therapy before endoscopic injection is shorter, as patients undergo endoscopic injection earlier, and urotherapy is performed in accordance with more recent recommendations from the International Children's Continence Society [33].

The percentage of patients undergoing ureteral reimplantation was significantly lower during the second versus the first 5-year study period, and there was a trend towards a higher positive response rate to endoscopic treatment during the second period. These findings could be related to differences between the two study periods in the injection technique; the investigators were learning how best to perform endoscopic injection during the first study period. The standard STING method was used during the first 5-year period, while NASHA/Dx was injected directly into the ureteral mucosa at the orifice (i.e. more proximally) during the second period. Moreover, there was a statistically significant increase of 32% in the mean injection volume during the second versus the first period of the study. Notably, the adjustments to injection technique were not associated with any apparent change in safety or tolerability; the risk of adverse events remained low throughout the study. Our

results are consistent with previous studies showing that injection volume and physician experience can influence outcomes following endoscopic injection [34,35]. Our study predated development of the hydrodistention implantation technique (HIT) and 'double HIT'. These techniques have been reported superior to STING [36,37], although there has been some variability in their success rates in grade IV VUR [16,38,39]. Cross-study comparisons should always be interpreted with caution — apparent differences in response could be related to variations between the study populations, assessment methods or duration of follow-up, aside from the injection technique and physician experience.

Outcomes in the present study were more favourable in patients with single versus double ureters. However, endoscopic treatment should still be considered beneficial for patients with double ureters, as the positive response rate in this group remained above 50% and the majority of patients (69%) were able to avoid open surgery.

The need for curative treatment of VUR has to be considered in context with the tendency for this condition to resolve spontaneously over time. The likelihood of spontaneous resolution tends to decrease with increasing VUR severity, and an annual resolution rate of just 5% has been reported for grade IV—V VUR [40,41]. Patients not undergoing curative treatment may require prolonged antibiotic prophylaxis for UTIs and pyelonephritis. This can be problematic because sub-optimal treatment compliance enables breakthrough infections to occur, and because chronic antibiotic use may encourage antibiotic resistance [8,42]. In addition, significant effects of antibiotic prophylaxis on the gut microbiome and, consequently, on patients' overall health, are increasingly recognized [43—45]. Overall, long-term antibiotic prophylaxis is not as effective or as well tolerated as endoscopic injection.

Most patients in our study (97%) had no safety issues during 15—25 years of follow-up. This reflects previous clinical experience with NASHA/Dx and may be considered unsurprising given the biocompatible nature of the substance [15,17,21,46]. One patient exhibited calcification but this caused no problems and did not require treatment. This finding is not unexpected because calcification has been reported previously with NASHA/Dx and other injectable agents [47—50]. This study did not include assessments specifically designed to assess calcification, although any significant clinical issues would have been detected. Care is needed to ensure that patients previously treated with NASHA/Dx are not mistakenly diagnosed with ureteral stones. We previously reported long-term observational data from VUR patients undergoing endoscopic injection with NASHA/Dx. The NASHA/Dx treatment procedure was perceived as less bothersome than either VCUG or long-term medication [15]. The incidence rate for febrile UTI (3.4%) was lower than that observed in the present study (10%); this is attributable to the study inclusion criteria (VUR grade III—V in the previous study vs grade IV in the present study; patients undergoing ureteral reimplantation were excluded from the previous study but included in the present study) and the duration of follow-up (7—12 years vs 8—18 years). The low rate of obstruction observed in this study is notable; the increased injection volumes used during the second 5-year period did not increase the risk of obstruction or other complications.

Based on the findings of this and other studies, clinicians can confidently use NASHA/Dx as curative therapy for grade III—IV VUR. We suggest early endoscopic treatment (after VUR diagnosis) for girls with high-grade VUR and recurrent UTIs. After endoscopic treatment, boys aged >5 years are likely to be managed conservatively (short-term antibiotic treatment after endoscopic injection and no follow-up VCUG assessments). Treatment of VUR by endoscopic injection rather than ureteral reimplantation provides important benefits, for both patients and the healthcare system. The avoidance of an invasive procedure involving surgical reconstruction and a hospital stay means that the treatment has far less impact on patients' quality of life. Moreover, endoscopic injection has been reported to have lower associated costs than ureteral reimplantation [51].

In Sweden, patients with VUR and febrile UTIs are monitored at their local hospital and few patients are lost from follow-up. In addition, patients and their parents were well informed of the need to attend hospital whenever the patient developed fever, so that UTIs could be detected and treated early. Consequently, the details of practically all febrile UTIs or renal deficiency occurring in the present study population would have been found in the charts that were checked. Other strengths of our study are the long-term duration and the large number of patients. Limitations include the lack of a control group, variability between patients in type/frequency of follow-up assessments, and the non-standardized questionnaire (e.g. bladder function not formally assessed). The study was not designed for formal hypothesis testing and, accordingly, endpoints were not designated as primary or secondary. These limitations are not sufficient to invalidate the study findings.

## Conclusions

Endoscopic treatment with NASHA/Dx showed durable results without significant risk of adverse effects during the longest ever follow-up (8—25 years) of patients treated for grade IV VUR. Low rates of UTIs and late ureteral reimplantation were observed post-treatment. Treatment response rates were similar in males and females, and ureteral reimplantation (open surgery) was avoided in 75% of patients. Comparison of the two 5-year study periods shows that adjustments in clinical practice (e.g. optimal placement of NASHA/Dx, increased injection volume) were associated with a reduced ureteral reimplantation rate. The results of this study suggest that NASHA/Dx can be used with confidence in patients with grade IV VUR.

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Life Sciences reviewed the manuscript for scientific accuracy.

## Conflicts of interest

Göran Läckgren: Speaker at teaching courses for Ferring AB, Sweden; Medical adviser and speaker at instructional courses for Palette AB, Sweden.

All other authors: None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpurol.2020.04.008.