

INTRAOPERATIVE ULTRASOUND-ASSISTED ENDOSCOPIC TREATMENT OF PRIMARY INTERMEDIATE AND HIGH-GRADE VESICoureTERAL REFLUX IN CHILDREN IN A LONG-TERM FOLLOW-UP.

--Manuscript Draft--

Manuscript Number:	JPUROL-D-23-00305R2
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Abstract:	Background: Endoscopic treatment of vesicoureteral reflux (VUR) is the first an important minimally invasive surgical approach in patients undergoing surgical treatment of VUR . In our past experience, we observed that a bulking agent mound sagittal diameter of 10 mm is the main predictor of effectiveness of the procedure. Moreover we noticed that the use of intraoperative ultrasound, allows the surgeon to better identify the site, volume and shape of the bulking agent injected, finally reducing operative time. Objective: We aimed to evaluate if the intraoperative ultrasound assistance could definitively improve effectiveness of the endoscopic procedure. Methods: we retrospectively compared two series treated with endoscopic procedures for intermediate and high risk primary VUR, respectively without (series A) and with (series B) intraoperative ultrasound (IO-US). In all patients VCUG was performed to assess VUR grade and to verify resolution or VUR downgrading during the follow-up. Results: A total of 177 ureteric units were treated. Endoscopic procedures globally were effective in 68/96 ureters (70,8%) in series A and in 68/81 ureters (83.9%) in series B. No significant differences in effectiveness were observed comparing the series with regard to VUR grades, but a significant difference is shown ($p < 0.05$) when grouping grades III-V VUR when the series are globally compared . No significance in differences of volume injected were detected, but operative time was significantly lower in series B (27.5 min vs 19.6 min , $p < 0.05$). Mean sagittal mound diameter measured during cystoscopy in series B was 10.45mm (range 8.5 - 14.2mm) . Discussion: The intraoperative ultrasound assistance during endoscopic treatment of VUR could represent a valid tool for surgeons to better identify location, volume and shape of the bulking agent. Furthermore, the use of an objective parameter of evaluation of the implant can overcome the subjective intraoperative evaluation of the implant itself, improving results for experienced surgeons and reducing the learning-curve for inexperienced ones. Conclusions: Results of endoscopic injection of bulking-agent can be improved with intraoperative ultrasound, allowing at the same time a significant reduction of operative

	time.
Suggested Reviewers:	



Azienda Ospedaliera Universitaria
Policlinico Paolo Giaccone



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DI PALERMO

**DIPARTIMENTO MATERNO-INFANTILE
U.O. DI CHIRURGIA PEDIATRICA
Chirurgia Pediatrica
Resp.: Prof.ssa Maria Rita Di Pace**

Palermo, 26/08/2023

TO:

The Editor **"The Journal of Pediatric Urology"**

Dear Editor,

The paper JPUROL-D-23-00305:

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Has been revised according to Reviewer commentaries and suggestion. The title should be modified as follows:

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INTERMEDIATE AND HIGH-**GRADE** VESICoureTERAL REFLUX IN CHILDREN IN A LONG-TERM
FOLLOW-UP."**

I would like to kindly ask you to make a change in the order to the Authors list, as specified and highlighted in yellow in the new title page.

I hope the modified manuscript will be worth for publication on your very prestigious Journal.

Please, do not hesitate to contact me for further inquires.

My best regards,

Marco Pensabene

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Azienda Ospedaliera Universitaria
Policlinico Paolo Giaccone



UNIVERSITÀ
DEGLI STUDI
DI PALERMO

**DIPARTIMENTO MATERNO-INFANTILE
U.O. DI CHIRURGIA PEDIATRICA
Chirurgia Pediatrica
Resp.: Prof.ssa Maria Rita Di Pace**

Palermo, 03/10/2023

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I would like to kindly ask you to make a change in the order to the Authors list, as specified and highlighted in yellow in the new title page.

According to the latest suggestions and requests of the reviewers some changes have been made to the paper. You can find all new modifications highlighted in green, as the previous ones have been left highlighted in yellow.

I hope the modified manuscript will be worth for publication on your very prestigious Journal.

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My best regards,

Marco Pensabene

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Journal of Pediatric Urology – Author response form for Manuscript Revisions

Manuscript Title: INTRAOPERATIVE ULTRASOUND-ASSISTED ENDOSCOPIC TREATMENT OF PRIMARY INTERMEDIATE AND HIGH-RISK **GRADE** VESICOURETERAL REFLUX IN CHILDREN IN A LONG-TERM FOLLOW-UP.

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We thank the reviewers for their interesting contributions and commentaries, which gave us the opportunity to clarify our research and improve our paper. In the manuscript, as well as in the title, you will find all changes highlighted in yellow.

COMMENTS <i>Reviewer's question here.</i>	RESPONSE <i>Answer to reviewer's question here. Please respond to each individual comment in a separate row. Responses should be comprehensive and should explain why you do or do not agree with the reviewer's comment, and what if any action you are taking in response. If a reviewer asks for more information or clarification, you should assume that this new information/clarification should be added to the manuscript (in other words, do not respond in this document but leave the manuscript unchanged). If text is changed to answer this question, <u>please insert the relevant revised text in your answer</u>, and indicate what text is new with bold or highlighted font.</i>	REFERENCE PAGE <i>Page and line number where change can now be found.</i>
Reviewer 1		
<i>Methods - Can the authors explain where the need for a 10 mm sagittal diameter mound comes from?</i>	Thanks for this observation that allows us to better describe our experience. In our previous experiences we showed that the sagittal diameter of the mound of injected Dx/HA could be considered as an important and independent predictor of success. More precisely, we observed that a diameter of 9.97 +/- 1.61 mm on postoperative evaluation is significantly associated with a successful endoscopic procedure. This result has been confirmed in a subsequent paper which describes the intraoperative US assisted procedure, highlighting how in successfully treated patients mean sagittal diameter was > 10mm (10.62 +/-1.36 mm) [See Ref 7, 8, 9].	/
<i>Discussion - In the discussion there are many paragraphs discussing open, laparoscopic and pneumovesicoscopic correction of VUR, while accurate I do not see the relevance to this paper, the paper is assessing if intraoperative USS can improve endoscopic results. For the sake of brevity those paragraphs could be removed without distracting from the article</i>	Thank you for your observation. We aimed to offer the reader a wide and exhaustive description of available surgical options. Nevertheless, we evaluated other papers focusing on endoscopic treatment. If you deem it necessary, we are available to summarise the discussion.	
<i>The authors hypothesized that a larger study would show a difference between grades, could the authors do a power calculation so that we could know how</i>	According to our results we could hypothesise that a significant power calculation could be obtained with an amount of 3x patients for each VUR grade.	

<i>much larger the study would need to be.</i>		
Reviewer 2		
<i>In the introduction and several points in the discussion, the authors indicate more consensus about the approach to VUR management than presently exists. For example, they suggest that surgery is no longer performed for grades 1 and 2 VUR, and that antibiotics are only indicated for treatment of infections. Further, they indicate that endoscopic management is as effective as other techniques, and that traditional open reimplant is only reserved for complex cases. At present, reflux management is much more variable. There are many urologists who still gave prophylactic antibiotics are performed surgical procedures for lower grade reflux in the setting of urinary tract infections, and not all urologists agree that minimally invasive approaches are the best treatment for VUR. Would recommend adjusting the most declarative statements in the introduction and discussion to reflect current variability and practice</i>	In the introduction we added some statements about CAP based on EAU - ESPU Guidelines 2022, and the most declarative statements have been modified in discussion also (all new sentences and words are highlighted in yellow).	Page 1 in yellow
<i>The authors rightfully acknowledge that inclusion of only one surgeon and one radiologist is a limitation period it would be helpful to further contextualize this limitation by indicating how long the surgeon had been performing and endoscopic correction of VUR prior to 2010. This will allow the reader to more accurately assess the possibility of learning curve being responsible for the difference between the two groups related to success rates and operative time.</i>	According to ref number 28 in the paper, the same surgeon in 2000 published his experience, reporting the results in endoscopic procedures for VUR since 1991.	/
<i>Recommend adding recurrent UTI rates</i>	In Series A 22/67 (33%) patients underwent open ureteral reimplantation. Six of them (29%)	Page 7 in yellow

<i>and proportion undergoing secondary procedure (ureteral reimplant) for both groups.</i>	presented recurrent UTI under CAP In Series B 11/56 (19%) patients underwent open ureteral reimplantation. Four of them (36%) presented recurrent UTI under CAP. It has been inserted in the results section	
<i>Paragraph 6 of the discussion (beginning with "The choice of surgical treatment...") Seems repetitive with much of the introduction and beginning of discussion. Recommend consolidating.</i>	The paragraph has been shortened	
<i>The authors state that, "The introduction of different surgeons and radiologists in the study would have strengthened or weakened our conclusions." Would recommend rewording this, simply stating that the potential effect of including more surgeons or radiologist is unknown.</i>	The sentence has been changed	Discussion, last page (13) in yellow
<i>Minor: the authors alternate between abbreviating and fully spelling out dextranomer/hyaluronic acid. Would pick one version.</i>	Thank you, modified.	
Reviewer 3		
Abstract		
<i>Endoscopic injection is one of the minimally invasive options for correcting VUR, but not the only one, and not considered superior to other minimally invasive methods such as robot assisted or laparoscopic reimplantation.</i>	Thank you for your observation. We agree, endoscopic treatment is not the only one option for minimally invasive treatment of VUR, but according to guidelines (ESPU - EAU 2022) laparoscopic approaches <i>"cannot be recommended as a routine procedure"</i> . Nevertheless, we changed the sentence in the abstract	Page 1, line 1 in yellow
<i>Please clarify the aim. By "effectiveness" do the authors mean an increased rate of radiographic resolution of VUR?</i>	By effectiveness the Authors mean observation of rights principles during the procedure to allow an optimal result in terms of reflux downgrading or resolution. Having good results, radiographic examinations would also be reduced.	
<i>The background references a 10mm minimum diameter for successful VUR correction. The abstract should reference the average implant diameter noted in the series.</i>	We refer to our previous experience (Ref 7-9). In the series B, the mean mound sagittal diameter measured during cystoscopy was 10.45mm (range 8 - 14.2mm). This has been added in the abstract and in the Results sections.	Abstract: results, in yellow. Results, page 6 in yellow
<i>The authors should choose different</i>	With "globally compared" authors mean that resolution rates are significantly different when	Abstract: results,

<i>wording in the abstract when series were "globally compared." What does globally compared mean? The authors should consider rephrasing this as "when grouping grades III-V VUR"</i>	ureteres are compared together, and not grade by grade. The sentence has been changed.	highlighted in yellow
<i>How much less time was required for series A and B</i>	In series B a mean of 7.9 minutes less were required for the procedure. Values have been inserted in the Abstract	Abstract: results, highlighted in yellow
	Introduction	
<i>The last sentence of the second paragraph, the authors state "surgical treatment is recommended in patients with symptomatic grade 3 VUR." Treatment is recommended, however surgery is only one option as per the AUA guidelines. Antibiotic prophylaxis is frequently recommended in children with a high rate of spontaneous resolution based on a variety of risk stratification tools such as distal ureteral diameter, VUR index, ureteral diameter ratio, VUR timing, and timing of upper tract drainage to name a few. The authors should revise this statement.</i>	The introduction has been changed, some sentences canceled and some others added. You can find all new sentences highlighted in yellow.	Introduction: page 1, in yellow
<i>The authors use the phrase "high-risk" VUR, are you referring to high-GRADE? If risk, please refer to the risk stratification tool used. There seems to be interchangeable use of risk and grade. The convention is grade. This is an issue throughout the paper.</i>	Thank you for your precious observation. We referred to the GRADE, as conventional established. In all sections of the paper you will find the grades, not risk.	
<i>The third paragraph in the introduction refers to "intraoperative tools to predict the effectiveness." The authors should reference the importance of technique, specifically the consistently improved success rate of double HIT vs Sting</i>	The purpose of this article is not to compare endoscopic "techniques" but to emphasise the role of ultrasound examination during the endoscopic procedure. We adopted both HIT and STING related to a specific endoscopic anatomical aspect	
	Methods	
<i>Was fluoroscopic VCUG used?</i>	Yes it was.	
<i>The risk stratification should be Please clearly define inclusion and exclusion</i>	We specified that patients included in the study were not affected by secondary reflux and anatomical abnormalities of the ureters as you can find in M&M section ("patients treated with	M&M section, page 4

<i>criteria. Specifically define patients with secondary VUR. We're patients with anatomical abnormalities of the ureter included - paraureteral diverticulum, ureterocele, duplicated collecting system or ectopic ureter?</i>	<i>endoscopic injection of (Dx/HA) between January 2010 and December 2019 for <u>primary</u> VUR" - "Patients with secondary VUR or with a follow-up shorter than 3 years were excluded".)</i>	
<i>The authors switch between different terminology for describing reflux severity - risk, grade. The convention in the literature is the international grading system for VUR and I recommend the authors consistently use this for clarity. Lebowitz, R. L., et al. "International system of radiographic grading of vesicoureteric reflux." Pediatric radiology 15 (1985): 105-109.</i>	We thank the Reviewer for his observation. The terminology has been changed from "risk" to "grades" according to the International system of radiographic grading of vesicoureteric reflux, as you can find highlighted in yellow in the entire paper	All sections
<i>The authors reference ureteral hydrodistension. The typical description of ureteral hydrodistension involves endoscopic assessment of the orifice, with more hydrodistension associated with more severe VUR, and complete absence of hydrodistension as an endpoint of injection with the double HIT technique. What is ultrasound hydrodistension? If this is a new type of hydrodistension, I recommend including ultrasound images and/or a diagram to help explain.</i>	We simply refer to the endoscopic hydrodistention view.	
<i>Did the authors perform endoscopic assessment of hydrodistension as described by Cerwinka and Kirsch? Cerwinka, Wolfgang H., Hal C. Scherz, and Andrew J. Kirsch. "Dynamic hydrodistention classification of the ureter and the double hit method to correct vesicoureteral reflux." Archivos Espanoles de Urologia 61.8 (2008): 882-887.</i>	We paid attention to hydrodistention during the procedure, but did not follow the mentioned classification.	
<i>What needle and endoscope were used for the procedure?</i>	We used the needle included in the "Deflux®". The endoscope was a "Stortz" operative 9Fr cystoscope	

<i>The authors describe a second injection if persistent VUR was noted on VCUG at 3 months after the first injection. We're patients who proceeded to second injection considered a success after the second category? These have classically been considered a failure. Please clarify</i>	Thanks for your observation. Some patients have been successfully treated after a single procedure, while some others needed a second bulking-agent injection. In both cases patients have been considered successfully treated. As we stated in the “Discussion” section, we believe that “The endoscopic procedure, however, should not be considered as a single stage therapy. It is well described how the reiteration of the procedure concurs to reach satisfying success rates, although each procedure appears to be less effective than the previous one”. Thus, after the second procedure, as we described in the “Materials and Methods” section “In case of VUR persistence or recurrent UTI after the second procedure, it [the procedure] was considered ineffective, even in cases of VUR downgrading...”.	
<i>The study seems to focus on radiographic success of endoscopic injection. The authors have extensive followup, mean 46-82 months, and consideration should be given to include clinical success in this series, specifically recurrent pyelonephritis after VUR treatment. For many, the primary driver in VUR treatment has become symptom-oriented, where clinical success, meaning the absence of recurrent pyelonephritis after VUR treatment, is considered a stronger marker of success as opposed to radiographic success.</i>	Thanks for your observation. This could become an important focal point for a subsequent paper. Based on your comment we added the incidence of symptomatic UTI among patients with persistent, radiographic VUR in the “result” section	Results: Pages 6 and 7 in yellow
	Results	
<i>The 50/50 distribution of male and female is unusual in VUR demographics, as VUR with UTI is far more common in girls due to their higher UTI risk, with 10:90 or 20:80 distribution more typically reported in the literature. Please comment in the discussion.</i>	We are aware that the distribution is unusual, but a similar distribution of patients have been reported in our previous papers. Nevertheless it has been added as a limit of the study in the discussion section.	
<i>Series A second paragraph - See comment #7 in methods section. Was persistent VUR measured after the first or second injection.</i>	According to the “Materials and Methods” section, the VUR was considered persistent and the procedure ineffective after the second injection (“In case of VUR persistence or recurrent UTI after the second procedure, it [the procedure] was considered ineffective, even in cases of VUR downgrading...”). The difference and the success rates for the first and the second procedures are resumed in tables 2 and 3 respectively for series A and B.	/
<i>Series A last sentence - the authors should be more specific. Consider saying the endoscopic procedures resulted in radiographic resolution of VUR</i>	A specific sentence have been added	Results: Page 6 in yellow
<i>Please reference Tables 2 and 3 at the point</i>	References for tables 2 and 3 have been moved to the suggested points.	Results: Page 6,

<i>in the results where the data is relevant</i>		at the end of “Series A” and “Series B” paragraphs, in yellow
<i>The sentence regarding a postoperative catheter should be moved to the methods section</i>	The sentence has been moved to M&M	
<i>We're contralateral ureters with evidence of hydrodistension but no radiographic reflux injected? If so, what was the rate of de novo contralateral VUR as the risk is approximately 10-15% when treating unilateral VUR.</i>	In the presented cohort of patients we treated only ureters showing VUR on VCUG	
<i>The authors report a reduction in operative time with ultrasound. Consider including a graph of operative time on the Y axis, and individual procedures in chronological order on the X axis. Did the operative time improve because of increased surgeon experience?</i>	We considered the impact of the increasing learning curve in effectiveness rates negligible because the surgeon (MC), started his experience in paediatric cystoscopy in 1991 (Ref 28 in the paper: <i>De Grazia E, Cimador M. Long-term follow-up results of vesico-ureteral reflux treated with subureteral collagen injection (SCIN). Minerva Pediatr. 2000 Jan-Feb;52(1-2):7-14.PMID: 10829588</i>). Consequently, we attributed the reduction in operative time to the role and aid the US can offer in choosing the injection site, resulting in an easier, more accurate and more rapid injection of the bulking agent	
<i>Most of the data presented in the results section is present in the tables. The authors do not need to reiterate this data in text and can refer to the tables as needed.</i>	o make it easier for the reader to understand the data, we prefer to leave the written text as well. However, as you requested in your comment #4, references of tables 2 and 3 have been moved to the suggested points.	
	Discussion	
<i>The first 3.5 paragraphs of the discussion should be removed- A 3.5 paragraph review of VUR treatment techniques is not necessary. How does ultrasound guided deflux injection improve surgical outcome? How does this compare to the current literature? Are there other procedures in other fields with ultrasound guided bulking agent injection?</i>	The discussion has been shortened. As discussed in the section we believe that IOUS could concur to standardise the endoscopic technique by two aspects: • Help the surgeon to better find the correct submucosal plane where the bulking agent should be injected • Measures the increase in mound height till a 10 mm in sagittal diameter is reached <i>“We hypothesize that the higher volume injected may be related to the attempts to reach the value of 10mm in sagittal diameter of the bulking agent, even in those cases in which the endoscopic evaluation alone would suggest concluding the procedure.”</i> <i>“intraoperative ultrasound (IO-US) may represent a valid tool for pediatric urologists in</i>	

	determining the exact location, shape and volume of the Dx/HA mound, concurring to standardize the technique and to overcome the lack in surgeon experience, allowing to improve the effectiveness of the procedure”	
Reported radiographic resolution rates for endoscopic injection are as high as 90% after a single endoscopic injection for grade I-IV VUR. Kaye JD, Srinivasan AK, Delaney C, Cerwinka WH, Elmore JM, Scherz HC, Kirsch AJ. Clinical and radiographic results of endoscopic injection for vesicoureteral reflux: defining measures of success. J Pediatr Urol. 2012 Jun;8(3):297-303. doi: 10.1016/j.jpurol.2011.02.006. Epub 2011 May 4. PMID: 21543259.	Thanks for this observation. The rates of success (71 - 83%) are reported by EAU-ESPU guidelines 2022. This new reference, which refers to a single experience, has been inserted, and the others renumbered.	Discussion: page 9, last paragraph, in yellow
The authors reference mound height as an independent marker of success and effective outcome, but there is no data in the results section or tables on mound height. This should be moved to the results section with a sub-analysis of group b patients that failed and their mound height compared to those that were successful.	This consideration moves from a previous study (ref 7 in the paper), in which we found the mound height was the main predictor of success in a cohort of 32 patients. In this paper we compared two populations of patients treated with endoscopic injection of bulking agent. Comparing patients with “success” vs “persistence of reflux” the mound height emerged as the main parameter correlating with resolution of VUR on VCUG. (respectively 9.97 mm +/- 1.61 vs 7.29 mm +/-1.74; p< 0.0005) [Zambaiti E, Pensabene M, Montano V, et al. Ultrasonographic mound height as predictor of vesicoureteral reflux resolution after endoscopic treatment in children. J Pediatr Surg. 2016 Aug;51(8):1366-9. doi: 10.1016/j.jpedsurg.2016.01.021. Epub 2016 Feb 14. PMID: 26882868.]	

Journal of Pediatric Urology – Author response form for Manuscript Revisions

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COMMENTS <i>Reviewer’s question here.</i>	RESPONSE <i>Answer to reviewer’s question here. Please respond to each individual comment in a separate row. Responses should be comprehensive and should explain why you do or do not agree with the reviewer’s comment, and what if any action you are taking in response. If a reviewer asks for more information or clarification, you should assume that this new information/clarification should be added to the manuscript (in other words, do not respond in this document but leave the manuscript unchanged). If text is changed to answer this question, please insert the relevant revised text in your answer, and indicate what text is new with bold or highlighted font.</i>	REFERENCE PAGE <i>Page and line number where change can now be found.</i>
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<i>The authors hypothesized that a larger study would show a difference between</i>	According to our results we could hypothesise that a significant power calculation could be obtained with an amount of 3x patients for each VUR grade.	

<i>grades, could the authors do a power calculation so that we could know how much larger the study would need to be.</i>		
Reviewer 2		
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<i>The authors rightfully acknowledge that inclusion of only one surgeon and one radiologist is a limitation period it would be helpful to further contextualize this limitation by indicating how long the surgeon had been performing and endoscopic correction of VUR prior to 2010. This will allow the reader to more accurately assess the possibility of learning curve being responsible for the difference between the two groups related to success</i>	According to ref number 28 in the paper, the same surgeon in 2000 published his experience, reporting the results in endoscopic procedures for VUR since 1991.	/

<i>rates and operative time.</i>		
<i>Recommend adding recurrent UTI rates and proportion undergoing secondary procedure (ureteral reimplant) for both groups.</i>	In Series A 22/67 (33%) patients underwent open ureteral reimplantation. Six of them (29%) presented recurrent UTI under CAP In Series B 11/56 (19%) patients underwent open ureteral reimplantation. Four of them (36%) presented recurrent UTI under CAP. It has been inserted in the results section	Page 7 in yellow
<i>Paragraph 6 of the discussion (beginning with "The choice of surgical treatment...") Seems repetitive with much of the introduction and beginning of discussion. Recommend consolidating.</i>	The paragraph has been shortened	
<i>The authors state that, "The introduction of different surgeons and radiologists in the study would have strengthened or weakened our conclusions." Would recommend rewording this, simply stating that the potential effect of including more surgeons or radiologist is unknown.</i>	The sentence has been changed	Discussion, last page (13) in yellow
<i>Minor: the authors alternate between abbreviating and fully spelling out dextranomer/hyaluronic acid. Would pick one version.</i>	Thank you, modified.	
Reviewer 3		
Abstract		
<i>Endoscopic injection is one of the minimally invasive options for correcting VUR, but not the only one, and not considered superior to other minimally invasive methods such as robot assisted or laparoscopic reimplantation.</i>	Thank you for your observation. We agree, endoscopic treatment is not the only one option for minimally invasive treatment of VUR, but according to guidelines (ESPU - EAU 2022) laparoscopic approaches <i>"cannot be recommended as a routine procedure"</i> . Nevertheless, we changed the sentence in the abstract	Page 1, line 1 in yellow
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<i>The background references a 10mm minimum diameter for successful VUR correction. The abstract should reference the average implant diameter noted in the</i>	We refer to our previous experience (Ref 7-9). In the series B, the mean mound sagittal diameter measured during cystoscopy was 10.45mm (range 8 - 14.2mm). This has been added in the abstract and in the Results sections.	Abstract: results, in yellow. Results, page 6 in yellow

series.		
<i>The authors should choose different wording in the abstract when series were "globally compared." What does globally compared mean? The authors should consider rephrasing this as "when grouping grades III-V VUR"</i>	With “globally compared” authors mean that resolution rates are significantly different when ureteres are compared together, and not grade by grade. The sentence has been changed.	Abstract: results, highlighted in yellow
<i>How much less time was required for series A and B</i>	In series B a mean of 7.9 minutes less were required for the procedure. Values have been inserted in the Abstract	Abstract: results, highlighted in yellow
	Introduction	
<i>The last sentence of the second paragraph, the authors state "surgical treatment is recommended in patients with symptomatic grade 3 VUR." Treatment is recommended, however surgery is only one option as per the AUA guidelines. Antibiotic prophylaxis is frequently recommended in children with a high rate of spontaneous resolution based on a variety of risk stratification tools such as distal ureteral diameter, VUR index, ureteral dimeter ratio, VUR timing, and timing of upper tract drainage to name a few. The authors should revise this statement.</i>	The introduction has been changed, some sentences canceled and some others added. You can find all new sentences highlighted in yellow.	Introduction: page 1, in yellow
<i>The authors use the phrase "high-risk" VUR, are you referring to high-GRADE? If risk, please refer to the risk stratification tool used. There seems to be interchangeable use of risk and grade. The convention is grade. This is an issue throughout the paper.</i>	Thank you for your precious observation. We referred to the GRADE, as conventional established. In all sections of the paper you will find the grades, not risk.	
<i>The third paragraph in the introduction refers to "intraoperative tools to predict the effectiveness." The authors should reference the importance of technique, specifically the consistently improved success rate of double HIT vs Sting</i>	The purpose of this article is not to compare endoscopic “techniques” but to emphasise the role of ultrasound examination during the endoscopic procedure. We adopted both HIT and STING related to a specific endoscopic anatomical aspect	
	Methods	

Was fluoroscopic VCUG used?	Yes it was.	
<i>The risk stratification should be Please clearly define inclusion and exclusion criteria. Specifically define patients with secondary VUR. We're patients with anatomical abnormalities of the ureter included - paraureteral diverticulum, ureterocele, duplicated collecting system or ectopic ureter?</i>	We specified that patients included in the study were not affected by secondary reflux and anatomical abnormalities of the ureters as you can find in M&M section (" <i>patients treated with endoscopic injection of (Dx/HA) between January 2010 and December 2019 for <u>primary</u> VUR</i> " - " <i>Patients with secondary VUR or with a follow-up shorter than 3 years were excluded</i> ".)	M&M section, page 4
<i>The authors switch between different terminology for describing reflux severity - risk, grade. The convention in the literature is the international grading system for VUR and I recommend the authors consistently use this for clarity. Lebowitz, R. L., et al. "International system of radiographic grading of vesicoureteric reflux." Pediatric radiology 15 (1985): 105-109.</i>	We thank the Reviewer for his observation. The terminology has been changed from "risk" to "grades" according to the International system of radiographic grading of vesicoureteric reflux, as you can find highlighted in yellow in the entire paper	All sections
<i>The authors reference ureteral hydrodistension. The typical description of ureteral hydrodistension involves endoscopic assessment of the orifice, with more hydrodistension associated with more severe VUR, and complete absence of hydrodistension as an endpoint of injection with the double HIT technique. What is ultrasound hydrodistension? If this is a new type of hydrodistension, I recommend including ultrasound images and/or a diagram to help explain.</i>	We simply refer to the endoscopic hydrodistention view.	
<i>Did the authors perform endoscopic assessment of hydrodistension as described by Cerwinka and Kirsch? Cerwinka, Wolfgang H., Hal C. Scherz, and Andrew J. Kirsch. "Dynamic hydrodistention classification of the ureter and the double hit method to correct vesicoureteral reflux."</i>	We paid attention to hydrodistention during the procedure, but did not follow the mentioned classification.	

Archivos Espanoles de Urologia 61.8 (2008): 882-887.		
What needle and endoscope were used for the procedure?	We used the needle included in the “Deflux®”. The endoscope was a “Stortz” operative 9Fr cystoscope	
The authors describe a second injection if persistent VUR was noted on VCUG at 3 months after the first injection. We're patients who proceeded to second injection considered a success after the second category? These have classically been considered a failure. Please clarify	Thanks for your observation. Some patients have been successfully treated after a single procedure, while some others needed a second bulking-agent injection. In both cases patients have been considered successfully treated. As we stated in the “Discussion” section, we believe that <i>“The endoscopic procedure, however, should not be considered as a single stage therapy. It is well described how the reiteration of the procedure concurs to reach satisfying success rates, although each procedure appears to be less effective than the previous one”</i> . Thus, after the second procedure, as we described in the “Materials and Methods” section <i>“In case of VUR persistence or recurrent UTI after the second procedure, it [the procedure] was considered ineffective, even in cases of VUR downgrading...”</i> .	
The study seems to focus on radiographic success of endoscopic injection. The authors have extensive followup, mean 46-82 months, and consideration should be given to include clinical success in this series, specifically recurrent pyelonephritis after VUR treatment. For many, the primary driver in VUR treatment has become symptom-oriented, where clinical success, meaning the absence of recurrent pyelonephritis after VUR treatment, is considered a stronger marker of success as opposed to radiographic success.	Thanks for your observation. This could become an important focal point for a subsequent paper. Based on your comment we added the incidence of symptomatic UTI among patients with persistent, radiographic VUR in the “result” section	Results: Pages 6 and 7 in yellow
	Results	
The 50/50 distribution of male and female is unusual in VUR demographics, as VUR with UTI is far more common in girls due to their higher UTI risk, with 10:90 or 20:80 distribution more typically reported in the literature. Please comment in the discussion.	We are aware that the distribution is unusual, but a similar distribution of patients have been reported in our previous papers. Nevertheless it has been added as a limit of the study in the discussion section.	
Series A second paragraph - See comment #7 in methods section. Was persistent VUR measured after the first or second injection.	According to the “Materials and Methods” section, the VUR was considered persistent and the procedure ineffective after the second injection (<i>“In case of VUR persistence or recurrent UTI after the second procedure, it [the procedure] was considered ineffective, even in cases of VUR downgrading...”</i>). The difference and the success rates for the first and the second procedures are resumed in tables 2 and 3 respectively for series A and B.	/

<i>Series A last sentence - the authors should be more specific. Consider saying the endoscopic procedures resulted in radiographic resolution of VUR</i>	A specific sentence have been added	Results: Page 6 in yellow
<i>Please reference Tables 2 and 3 at the point in the results where the data is relevant</i>	References for tables 2 and 3 have been moved to the suggested points.	Results: Page 6, at the end of "Series A" and "Series B" paragraphs, in yellow
<i>The sentence regarding a postoperative catheter should be moved to the methods section</i>	The sentence has been moved to M&M	
<i>We're contralateral ureters with evidence of hydrodistension but no radiographic reflux injected? If so, what was the rate of de novo contralateral VUR as the risk is approximately 10-15% when treating unilateral VUR.</i>	In the presented cohort of patients we treated only ureters showing VUR on VCUG	
<i>The authors report a reduction in operative time with ultrasound. Consider including a graph of operative time on the Y axis, and individual procedures in chronological order on the X axis. Did the operative time improve because of increased surgeon experience?</i>	We considered the impact of the increasing learning curve in effectiveness rates negligible because the surgeon (MC), started his experience in paediatric cystoscopy in 1991 (Ref 28 in the paper: <i>De Grazia E, Cimador M. Long-term follow-up results of vesico-ureteral reflux treated with subureteral collagen injection (SCIN). Minerva Pediatr. 2000 Jan-Feb;52(1-2):7-14.PMID: 10829588</i>). Consequently, we attributed the reduction in operative time to the role and aid the US can offer in choosing the injection site, resulting in an easier, more accurate and more rapid injection of the bulking agent	
<i>Most of the data presented in the results section is present in the tables. The authors do not need to reiterate this data in text and can refer to the tables as needed.</i>	o make it easier for the reader to understand the data, we prefer to leave the written text as well. However, as you requested in your comment #4, references of tables 2 and 3 have been moved to the suggested points.	
	Discussion	
<i>The first 3.5 paragraphs of the discussion should be removed- A 3.5 paragraph review of VUR treatment techniques is not necessary. How does ultrasound guided deflux injection improve surgical outcome? How does this compare to the current literature? Are there other procedures in other fields with ultrasound guided bulking agent injection?</i>	The discussion has been shortened. As discussed in the section we believe that IOUS could concur to standardise the endoscopic technique by two aspects: • Help the surgeon to better find the correct submucosal plane where the bulking agent should be injected • Measures the increase in mound height till a 10 mm in sagittal diameter is reached	

	<i>“We hypothesize that the higher volume injected may be related to the attempts to reach the value of 10mm in sagittal diameter of the bulking agent, even in those cases in which the endoscopic evaluation alone would suggest concluding the procedure.”</i> <i>“intraoperative ultrasound (IO-US) may represent a valid tool for pediatric urologists in determining the exact location, shape and volume of the Dx/HA mound, concurring to standardize the technique and to overcome the lack in surgeon experience, allowing to improve the effectiveness of the procedure”</i>	
<i>Reported radiographic resolution rates for endoscopic injection are as high as 90% after a single endoscopic injection for grade I-IV VUR. Kaye JD, Srinivasan AK, Delaney C, Cerwinka WH, Elmore JM, Scherz HC, Kirsch AJ. Clinical and radiographic results of endoscopic injection for vesicoureteral reflux: defining measures of success. J Pediatr Urol. 2012 Jun;8(3):297-303. doi: 10.1016/j.jpuro.2011.02.006. Epub 2011 May 4. PMID: 21543259.</i>	Thanks for this observation. The rates of success (71 - 83%) are reported by EAU-ESPU guidelines 2022. This new reference, which refers to a single experience, has been inserted, and the others renumbered.	Discussion: page 9, last paragraph, in yellow
<i>The authors reference mound height as an independent marker of success and effective outcome, but there is no data in the results section or tables on mound height. This should be moved to the results section with a sub-analysis of group b patients that failed and their mound height compared to those that were successful.</i>	This consideration moves from a previous study (ref 7 in the paper), in which we found the mound height was the main predictor of success in a cohort of 32 patients. In this paper we compared two populations of patients treated with endoscopic injection of bulking agent. Comparing patients with “success” vs “persistence of reflux” the mound height emerged as the main parameter correlating with resolution of VUR on VCUG. (respectively 9.97 mm +/- 1.61 vs 7.29 mm +/-1.74; p< 0.0005) [Zambaiti E, Pensabene M, Montano V, et al. Ultrasonographic mound height as predictor of vesicoureteral reflux resolution after endoscopic treatment in children. J Pediatr Surg. 2016 Aug;51(8):1366-9. doi: 10.1016/j.jpedsurg.2016.01.021. Epub 2016 Feb 14. PMID: 26882868.]	

REVISIONS – SECOND ROUND

	Reviewer 2	
The authors responded appropriately to most of the reviewer comments, but did not include corresponding edits to all of them. Specifically, recommend that the authors address the following in the text: 1. The power calculation question raised by reviewer 1 2. The point related to HIT vs sting raised by reviewer 3	All requested modifications to the text have been done, and specifications added. You can find it highlighted in green.	Introduction: Page 4, in green; Discussion: page 12, in green
Also, the fact that the authors did not use the hydrodistension grading system should be added as a limitation.	A new limitation of the study has been added in the discussion section and the relative reference	Discussion: page 15 in green; References: new reference n°33, in green

INTRAOPERATIVE ULTRASOUND-ASSISTED ENDOSCOPIC TREATMENT OF PRIMARY INTERMEDIATE AND HIGH-RISK GRADE VESICoureTERAL REFLUX IN CHILDREN IN A LONG-TERM FOLLOW-UP.

Pensabene M ^a, Cimador M ^a, Spataro B ^a, Serra G ^b, Baldanza F ^a, Grasso F ^a, Corsello G ^b, Salerno S ^c, Di Pace MR ^a, Sergio M ^a

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Research Article

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Research Article

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Summary

Background: Endoscopic treatment of vesicoureteral reflux (VUR) is ~~the first~~ **an important** minimally invasive surgical approach in patients undergoing surgical treatment of VUR. In our past experience, we observed that a bulking agent mound sagittal diameter of 10 mm is the main predictor of effectiveness of the procedure. Moreover we noticed that the use of intraoperative ultrasound, allows the surgeon to better identify the site, volume and shape of the bulking agent injected, finally reducing operative time.

Objective: We aimed to evaluate if the intraoperative ultrasound assistance could definitively improve effectiveness of the endoscopic procedure.

Methods: we retrospectively compared two series treated with endoscopic procedures for intermediate and high risk primary VUR, respectively without (series A) and with (series B) intraoperative ultrasound (IO-US). In all patients VCUG was performed to assess VUR grade and to verify resolution or VUR downgrading during the follow-up.

Results: A total of 177 ureteric units were treated. Endoscopic procedures globally were effective in 68/96 ureters (70,8%) in series A and in 68/81 ureters (83.9%) in series B. No significant differences in effectiveness were observed comparing the series with regard to VUR grades, but a significant difference is shown ($p < 0.05$) **when grouping grades III-V VUR** ~~when the series are globally compared~~. No significance in differences of volume injected were detected, but operative time was significantly lower in series B (**27.5 min vs 19.6 min**, $p < 0.05$). **Mean sagittal mound diameter measured during cystoscopy in series B was 10.45mm (range 8.5 - 14.2mm)**

Discussion: The intraoperative ultrasound assistance during endoscopic treatment of VUR could represent a valid tool for surgeons to better identify location, volume and shape of the bulking agent. Furthermore, the use of an objective parameter of evaluation of the implant can overcome the subjective intraoperative evaluation of the implant itself, improving results for experienced surgeons and reducing the learning-curve for inexperienced ones.

Conclusions: Results of endoscopic injection of bulking-agent can be improved with intraoperative ultrasound, allowing at the same time a significant reduction of operative time.

		Endoscopic injection of Dx/HA		US-assisted Endoscopic injection of Dx/HA		p-value
VUR -Grade		Global effectiveness		Global effectiveness		
	III	31/35 (88.6%)		32/33 (97%)		0.357
	IV	26/40 (65%)		29/37 (78.4%)		0.217
	V	11/21 (52.4%)		7/11 (63.6%)		0.712
VUR - Global	III-V	68/96 (70.8%)		68/81 (84%)		0.049
		Mean	Range	Mean	Range	
Operative time	III-V	27.64 min	10 - 70 min	19.6 min	8 - 40 min	0.007
Volume injected	III-V	0.7923 ml	0.3 - 2 ml	0.8516 ml	0.5 - 1.3 ml	0.273

Key words: Vesicoureteric reflux, Bulking agents, Endoscopy, Intraoperative Ultrasound, Children.

List of abbreviations

VUR: Vescicoureteral reflux

UTI: Urinary tract infections

Dx/HA: dextranomer/hyaluronic acid copolymer

US: Ultrasound

VCUG: voiding cystourethrography

HIT: Hydrodistention injection technique

CAP: continuous antibiotic prophylaxis

IO-US: intraoperative ultrasound

Introduction

Vesicoureteral reflux (VUR) is a well-known condition involving 1% to 3% of children, often presenting with febrile urinary tract infections (UTIs) [1]. VUR is considered the most important risk factor for acute pyelonephritis in children and, in untreated patients, it could determine a progressive loss of renal function, due to renal scarring, especially in intermediate and high grade VUR [2,3].

The surgical treatment has evolved in the last 20 years, an increasing number of patients being treated conservatively but recommendations regarding VUR treatment are still different and controversial. There are several unresolved questions about what is the best management in patients with VUR; this could be related to many variables that can increase the risk of UTI and persistence of VUR, such as patient's age, sex, circumcision, presence of LUTS and acquired toilet training. Most—Some Authors recommend continuous antibiotic prophylaxis as the initial management, as a spontaneous resolution or downgrading of reflux is expected within the first year of life; other Authors, conversely, do not use antibiotic prophylaxis according to low evidence of effectiveness in preventing renal scarring and its undefined impact on antimicrobial resistance. A persistent low-risk low-grade VUR ~~should~~ could only be managed with *on-demand* antibiotics if UTI occurs, while some Authors prefer to offer surgical treatment also in low-grade VUR in order to prevent UTI [1,4,5]. Nevertheless, surgical treatment is recommended in patients with symptomatic intermediate and persistent high-grade reflux ~~risk reflux (grade III) and in those with persistent high-risk reflux (grade IV and V VUR)~~ [6].

However, the best surgical option for primary intermediate and high-risk ~~high-risk~~ high-grade VUR is still questionable, and results are often controversial. The endoscopic procedures have

gained more and more acceptance, proving to be quite effective as other open or miniminvasive procedures, with the well-known benefits of minimally invasive surgery in terms of cost/effectiveness, patient's tolerance, hospital stay and pain killer's administration [1]. However the only intraoperative tools to predict the effectiveness of the procedure is the endoscopic appearance of the bulking-agent mound and the ureteral orifice modifications. Thus effectiveness rates have been strongly related to the surgeon-experience, VUR grades and patient's selection. In a recent paper we suggested that the effectiveness of endoscopic injection of bulking agents can benefit from intraoperative ultrasound, allowing the surgeon to optimize site, shape and volume of the bulking-agent mound introducing moreover a more objective parameter for evaluation of procedure's effectiveness[7,8] . Moreover, we observed that an adequate size of the bulking-agent mound can predict effectiveness of the procedure and VUR resolution, potentially avoiding postoperative cystourethrogram introducing a standardization in the treatment and allowing a greater reliability of the procedure[9]. In order to confirm our previous observations the aim of this paper is to compare two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA), respectively without and with intraoperative US-guidance in terms of general aspects of the implant, intra-operative time, and DX/Ha amount.

Materials and Methods.

The present retrospective study compares two series of patients treated with endoscopic injection of ~~dextranomer/hyaluronic acid copolymer~~ (Dx/HA) between January 2010 and December 2019 for primary VUR. The first series (A) refers to the period January 2010 -

December 2014 and includes patients treated with endoscopic procedures without the intraoperative US guidance. The second series (B) refers to the period January 2015-December 2019, and all endoscopic procedures were integrated with intraoperative US, as previously described [7-8]. Patients presenting with VUR under 1 years of age were initially managed conservatively with continuous antibiotic prophylaxis, and thereafter re-evaluated with VCUG. According to the EAU/ESPU guidelines [10], all patients with primary symptomatic ~~intermediate-risk~~ **III grade** vesicoureteral-reflux (VUR) and primary ~~high-risk~~ **IV-V grade** VUR, even if preserved renal function, were treated. In all patients VUR was detected by voiding cystourethrography (VCUG), and assessed according to the International Reflux Study Committee parameters [11-13]. Low/moderate grade reflux was treated only if a contralateral concomitant higher grade reflux was treated, but ureteric units with second grade VUR were not included in the study. Patients with secondary VUR or with a follow-up shorter than 3 years were excluded.

Ureteric units were divided into 3 main groups according to the VUR grades. All procedures were carried out by the same surgeon (MC) for both series. In the series B, the intraoperative ultrasound was performed contemporary to the endoscopic procedure, in order to visualize the bladder trigone, the ureteral dilatation, the meatus hydrodistention before and after injection, the distribution of material along the course of ureter, allowing an optimal location and size of the implant [7-9]. The intraoperative US was performed by a single pediatric radiologist (SS).

For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable .

In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US.

In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned. In case of VUR persistence or recurrent UTI after the second procedure, it was considered ineffective, even in cases of VUR downgrading, and patients were managed with a classical open cross-trigonal Cohen ureteral reimplantation.

Patients had an indwelling catheter for an average 24 hours (18-36) in order to reduce intravesical pressure, allowing the early bulking-agent stabilization in the first postoperative period, and were discharged the day after the procedure.

All patients have been followed-up with clinical and US examination on postoperative months 1,3, 6, 9, then every year.

Significant differences in effectiveness of techniques were tested with the Fisher exact test, while the Student test was used for comparison of operative time and bulking-agent volume injected. Significance was identified with $p < 0.05$.

Results.

A total of 123 patients matching inclusion criteria have been treated with endoscopic procedures for primary VUR at our institution, 59 males and 54 females between January

2010 and December 2019; of them, 59 presented with bilateral VUR, and a total of 177 ureteric units were treated (Table 1).

Series A. It includes 67 patients treated without intraoperative US-assessment of endoscopic injection of Dx/HA, respectively 32 males and 35 females. Mean age at surgery was 20 months (range 14 - 44 months), and mean follow-up was 82 months (range 75 – 114 months). Twenty-nine patients, 19 females and 10 males, presented with a bilateral VUR. Thirty-five ureters presented with III grade VUR, 40 with grade IV, and 21 with grade V . A total of 96 ureteric units were finally included.

Persistent VUR was present in 28 ureters, respectively, 4 ureters (11.4%) with grade III VUR, 14 ureters (21.6 %)with grade IV , 10 ureters (47,6%)with grade V VUR.

Endoscopic procedures resulted in radiographic resolution of VUR globally were effective in 68/96 ureters (70,8%). Results are resumed in Table 2.

Series B. 56 patients were included, 27 males and 29 females; the mean age was 23 months (range 18-55 months). Mean follow-up was 46 months (range 35 – 76 months). In 30 patients, 12 males and 19 females, a bilateral VUR was observed. Thirty-three ureters showed a grade III VUR, 37 a grade IV, and the remaining 11 a grade V. VUR persistence was detected in 13 refluxing ureters after the second procedure, namely 1 ureter with (3%) grade III VUR, 8 ureters (21,6%) with grade IV VUR, and 4 ureters (36,4%) with grade V VUR.

Endoscopic procedures globally were effective in 68/81 ureters (83.9%), and Mean sagittal mound diameter measured during cystoscopy in series B was 10.45mm (range 8.5 - 14.2mm). Results are resumed in table 3.

Mean operative time was 21,5 minutes (range 8-70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$). Mean Dx/HA volume injected per ureter was 0.834 ml, respectively 0.7923 (range 0.3 - 2ml) in series A and 0.8516 in series B (range 0.5 - 1.3 ml); the difference was not significant ($p= 0.2733$). No intraoperative or postoperative complications were reported. ~~Patients had an indwelling catheter for an average 24 hours (18-36) in order to reduce intravesical pressure, allowing the early bulking agent stabilization in the first postoperative period, and were discharged the day after the procedure.~~ In 6 patients a minor self-limiting hematuria was observed. In Series A 22/67 (33%) patients underwent open ureteral reimplantation. Six of them (29%) presented recurrent UTI under CA. In Series B 11/56 (19%) patients underwent open ureteral reimplantation. Four of them (36%) presented recurrent UTI under CAP.

Globally, a bilateral condition was observed in 15/33 patients with persistent VUR, respectively, 6/22 in series A, and in 9/11 patients (Series B). Endoscopic procedures were effective in 136/177 ureters, respectively 68/96 (70.8%) ureters in series A, and in 68/81 ureters (84%) in series B. ~~Results are resumed in tables 2 and 3.~~

As for Unilateral or Bilateral condition, no statistically significant difference was found between the two series, as well as for the severity of VUR (categorized in Grade III, IV and V) and the clinical outcome when results are compared according to the VUR grade.

Nevertheless, a significant difference is shown when grouping grades III-V VUR if series are globally compared ($p= 0.049$, Table 4).

Discussion

Indications for surgical treatment of VUR have progressively reduced in the last 20 years, but, at the same time, technical options have widened. Only in selected cases low-grade VUR ~~usually does not~~ undergo surgical treatment. Furthermore, ~~and~~ continuous antibiotic prophylaxis (CAP) ~~becomes less used~~ ~~is no more indicated,~~ as on-demand antibiotic therapy could be administrated when UTI occurs. ~~since these patients can be easily managed with watchful waiting for UTI and antibiotic therapy “on demand”.~~ However, surgery is indicated in ~~high-risk~~ moderate symptomatic and high grade VUR and in symptomatic patients with moderate-risk VUR (grade III) [13,14].

Although open ureteral reimplantation is still considered the gold standard, the minimally invasive techniques ~~have reached more and more indications in the surgical approach of VUR, widening the technical armamentarium in the hands of the pediatric urologist~~ Open ureteral reimplantation is still considered the gold standard for achieving the maximum result in terms of recovery from reflux. From this point of view, the various minimally invasive procedures expand the technical equipment while maintaining a high percentage of resolution, an optimal aesthetic result, while minimizing postoperative hospitalization and the administration of analgesics.[1,13-17].

As mentioned above, the pneumesvesicoscopic cross trigonal intravesical reimplantation described by CK Yeung, the most recent robotic-assisted techniques as well as the extravesical laparoscopic reimplantation according to Lich-Gregoire are proof of this [3, 18-30].

~~In 2005 Yeung CK et al showed how CO2 bladder insufflation allows pneumovesicoscopic classic cross trigonal intravesical reimplantation by the same instruments used in pediatric~~

laparoscopy [18]. More recently, robotic-assisted surgery showed to be an important tool to overcome many technical limitations of laparoscopic surgery, with reliable experiences in intra- and extravesical reimplantation in children [19-22]. Moreover, extravesical laparoscopic reimplantation according to Lich-Gregoire has shown to be a valid option for pediatric urologists, offering promising results [3,23-30]. In this scenario we would highlight Nevertheless that laparoscopic and robotic-assisted techniques, especially intravesical laparoscopic reimplantation, need a long learning curve and remain technically demanding procedures. Robotic-assisted surgery is still too expensive, and is limited to a minority of centers.

The classical open intravesical or extravesical procedures are still an important therapeutic options, but are now reserved to selected patients, in which endoscopic procedures might result less effective: high grade secondary VUR, high grade bilateral VUR, megaureters requiring tapering before reimplantation and duplex systems [22-25].

In this scenario, the cystoscopic injection of dextranomer/hyaluronic acid has shown to be the best option in the management of grade III-IV VUR, compared to continuous antibiotic prophylaxis and watchful waiting, and The endoscopic procedure by injection of (Dx/HA)

is considered as the first minimally invasive surgical approach to manage grade III-IV VUR by many pediatric urologists, with an overall resolution rate between 71 and 83%, with some Authors reporting resolution rates higher than 90% [2, 25,26]; in a recent literature, moreover, recently indications for endoscopic treatment have been widened including the above mentioned patients with high grade secondary VUR, high grade bilateral VUR, megaureters requiring tapering before reimplantation and duplex systems [2, 22-25] with treat several cases with specific populations, historically not considered for endoscopic

treatment [2]. Nevertheless, the effectiveness of cystoscopic injection of dextranomer/hyaluronic acid Dx/HA seems to be strongly related to patients' selection, surgeon experience and VUR grade, suggesting a difficult standardization of the procedure [2]. To this point, we found a strong relation with the mound volume of the dextranomer/hyaluronic acid Dx/HA injected and VUR resolution. In a cohort of 32 patients and 53 ureters we observed that the mound height of (Dx/HA) injected resulted the major predictive parameter of procedure effectiveness (sensitivity 100%, specificity 65.9%), suggesting VUR resolution when a maximum diameter $\geq$ than 1 cm was found on postoperative ultrasound (US) examination [7]. Starting from these observations in a subsequent study we found that intraoperative ultrasound (IO-US) may represent a valid tool for pediatric urologists in determining the exact location, shape and volume of the Dx/HA mound, concurring to standardize the technique and to overcome the lack in surgeon experience, allowing to improve the effectiveness of the procedure [8-9]. In the presented study we observed how the IO US can make the endoscopic procedure more accurate in the VUR treatment.

Effectiveness of the procedures raised in all VUR grade subpopulations, with a significant increase when series are globally compared raising from 70.8% to 83.9% ($p=0.049$).

Although no significant difference has been shown when comparison of series is performed according to grade, a significant difference stands out when series are compared considering all aggregated ureters. We hypothesize that a larger study population could have shown significant differences in VUR grade subgroups as well.

In a classic paper by Elder *et al*, reported in the EAU guidelines in pediatric Urology, aggregate resolution rates after endoscopic treatment (up to three procedures) was 85%, involving all VUR grades [14,27].

In our series, the US-assisted procedure allowed a similar result for aggregate grade III-V VUR, excluding grades I-II, in which a higher resolution rate is expected. Moreover, only two procedures have been considered in the presented paper. Comparing our retrospective series A and B, the overall success rate after two cystoscopic procedures increased from 88.6% to 97% for grade III VUR, from 65% to 78,4% for grade IV and from 52.4% to 63.6% for grade V. One can argue that this improvement might be due to the increasing learning curve; nevertheless, in 2010, the surgeon performing all procedures already had a wide experience in endoscopic treatment of VUR, thus the impact of the increasing learning curve in effectiveness rates could be considered negligible [28].

Operative time was significantly lower in series B, suggesting that IO-US may allow a more rapid identification of the injection site, helping in locating the correct injection site with fewer attempts, and finally resulting in a reduction in overall operative time. Moreover, the mean volume of bulking agent injected was higher in series B, even if the difference was not significant. We hypothesize that the higher volume injected may be related to the attempts to reach the value of 10mm in sagittal diameter of the bulking agent, even in those cases in which the endoscopic evaluation alone would suggest concluding the procedure.

The choice of surgical treatment in patients with symptomatic grade III-V VUR is still a matter of debate. As mentioned above, open procedures are still considered as the gold standard by many pediatric urologists and laparoscopic and robotic approaches are not

recommended as routine procedures, due to the wider variation of results and unfavorable cost/effectiveness rates [14, 29-32], offering well known excellent resolution rates. Laparoscopic and robotic reimplantation techniques have been successfully performed with results comparable to the classical open procedures, (effectiveness ranging from 92 to 98%), but a wider variation of results has been highlighted when laparoscopic/robotic and open approaches are compared [14,28-31]. Moreover, laparoscopic and robotic procedures have shown a higher complications rate leading to reintervention, a longer learning curve and higher costs. Thus, laparoscopic and robotic approaches are not recommended as routine procedures [14]. In a recent retrospective study, Soulier *et al* reported a success rate of 98.3% after a mean follow-up of 59,3 months in 159 ureters and 117 patients treated with laparoscopic extravesical reimplantation (LEVUR), which is similar to Cohen results [3]. The Author reported intraoperative or early postoperative complications in 8 cases. In 5 cases a bladder mucosal perforation occurred during the procedure, immediately treated with endo-loop repair. One patient presented with acute urine retention treated with a supra pubic catheter left *in situ* for 10 days, while in two patients uretero-peritoneal fistulas occurred, requiring respectively a double-J stenting and an open Cohen procedure.

No intraoperative or postoperative complications have been observed in the presented series, but a minor, self-limiting hematuria in 6 patients.

Effectiveness of endoscopic procedures is known to be lower than other reimplantation techniques, especially in higher VUR grades.

Esposito *et al* compared the open Cohen, the endoscopic STING and the LEVUR procedures in terms of effectiveness, complications rates, costs and requirement of

analgesic drugs concluding that the LEVUR is comparable to the Cohen procedure and superior to the STING endoscopic procedure in terms of effectiveness, but it shows the same advantages of STING procedure in terms of postoperative pain and lower postoperative morbidity [29] .

In a recent prospective randomized study Salih *et al* compared the open extravesical reimplantation and the endoscopic procedure, reporting a higher success rate of the open Lich-Gregoir. Nevertheless, in the described series, the patients enrolled in the endoscopic arm of the study underwent a single endoscopic procedure [32].

The endoscopic procedure, however, should not be considered as a single stage therapy. It is well described how the reiteration of the procedure concurs to reach satisfying success rates, although each procedure appears to be less effective than the previous one.

This study has some limitations. First, it is a retrospective study; a prospective randomized study may offer more robust conclusions, demonstrating differences within single grades of VUR. Second, despite every attempt to obtain a standardization of combined endoscopic-ultrasonographic procedure, all procedures were performed by the same couple of surgeon-radiologists; furthermore, US is an operator-dependent procedure. It is difficult to evaluate the potential effect of introducing different surgeons and radiologists in the study. Third, in the presented paper, we observed a male-to-female ratio unusual for VUR distribution as females are usually more affected than males. The introduction of different surgeons and radiologists in the study would have strengthened, or weakened our conclusions.

Conclusions

In conclusion, endoscopic injection of DX/Ha plays an important role in the miniminvasive treatment of VUR. The intraoperative US-assistance can objectively refine the endoscopic procedure, in order to identify the exact location of the intramural ureter, monitoring at the same time the injection procedure and reducing operative times. This simple, non-invasive intraoperative US assistance could optimize results for experienced surgeons, and reduce learning-curve for inexperienced ones, and thus should be considered as a standard integration of endoscopic VUR treatment.

Table 1. Global distribution of patients

Series	A	B	Total
Males	32	27	59 (47,97%)
Females	35	29	64 (52,03%)
Patients Tot	67	56	123
Bilateral	29	31	60 (48%)
Ureteric units	96	81	177

Table 2. Series A

Endoscopic injection of Dx/HA		First procedure Success rate		Second procedure Success rate		Global success rate	
VUR Grade		Ureters	%	Ureters	%	Ureters	%
III		29/35	82,8%	2/6	33%	31/35	88,6%
IV		18/40	45%	8/22	36,4%	26/40	65%
V		7/21	33%	4/14	28,6%	11/21	52,4%%
Global Success		54/96	56,2%	14/42	33%	68/96	70,8%
Persistence		42/96	43.8%	28/42	67%	//	//

Table 3. Series B

US assisted Endoscopic injection of Dx/HA		First procedure Success rate		Second procedure Success rate		Global success rate	
VUR Grade		Ureters	%	Ureters	%	Ureters	%
III		30/33	90.9%	2/3	66.6%	32/33	97%
IV		17/37	45,9%	12/20	60%	29/37	78.4%
V		3/11	27.3%	4/8	50%	7/11	63.6%
Global Success		50/81	61,7%	18/31	58%	68/81	83.9%
Persistence		31/81	38.3%	13/31	42%	//	//

Table 4: differences and significance between series

Endoscopic injection of Dx/HA		US-assisted Endoscopic injection of Dx/HA		p-value
VUR -Grade		Global effectiveness	Global effectiveness	
	III	31/35 (88.6%)	32/33 (97%)	0.357
	IV	26/40 (65%)	29/37 (78.4%)	0.217
	V	11/21 (52.4%)	7/11 (63.6%)	0.712
VUR - Global	III-V	68/96 (70.8%)	68/81 (84%)	0.049
		Mean	Range	
Operative time	III-V	27.64 min	10 - 70 min	0.007
Volume injected	III-V	0.7923 ml	0.3 - 2 ml	0.273

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract: retrospective case-control study	2	<i>"we retrospectively compared two series.."</i>
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	<i>"Results of endoscopic injection of DX/Ha can be improved with IO-US. [...] allows a significant reduction of operative time...."</i>
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	<i>However, the best surgical option for primary intermediate and high risk VUR is still questionable</i>
Objectives	3	State specific objectives, including any prespecified hypotheses	3	<i>we aimed to compare two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA), respectively without and with intraoperative US-guidance</i>
Methods				
Study design	4	Present key elements of study design early in the paper	3	<i>The present retrospective study compares two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA) between January 2010 and December 2019</i>

Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3	<i>The first series (A) refers to the period January 2010 - December 2014 and includes patients treated with endoscopic procedures without the intraoperative US guidance.</i>
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	3,4	<i>For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable. In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US. In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned Patients with secondary VUR or with a follow-up shorter than 3 years were excluded</i>
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	3	<i>all patients with primary symptomatic intermediate risk vesicoureteral-reflux (VUR) and primary high risk VUR, even if preserved renal function, were treated</i>
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4	<i>For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable. In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US.</i>
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4	<i>In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned. In case of VUR persistence or recurrent UTI after the second procedure, it was considered ineffective, even in cases of VUR downgrading.</i>

Bias	9	Describe any efforts to address potential sources of bias	4	<i>All procedures were carried out by the same surgeon (MC) for both series. The intraoperative US was performed by a single pediatric radiologist (SS). For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable.</i>
Study size	10	Explain how the study size was arrived at	3	<i>The present retrospective study compares two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA) between January 2010 and December 2019 for primary VUR</i>
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	4	<i>Significant differences in effectiveness of techniques were tested with the Fisher exact test, while the Student test was used for comparison of operative time and bulking-agent volume injected. Significance was identified with $p < 0.05$.</i>
		(b) Describe any methods used to examine subgroups and interactions		
		(c) Explain how missing data were addressed		
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy		
		(e) Describe any sensitivity analyses		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4	<i>A total of 123 patients matching inclusion criteria have been treated with endoscopic procedures for primary VUR at our institution, 59 males and 54 females between January 2010 and December 2019</i>
		(b) Give reasons for non-participation at each stage		<i>Only patients with primary VUR were included</i>
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	<i>59 males and 54 females 59 presented with bilateral VUR Series A. Mean age at surgery was 20 months (range 14 - 44 months). Series B. the mean age was 23 months (range 18-55 months).</i>

		(b) Indicate number of participants with missing data for each variable of interest		
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)		<i>Series A.</i> mean follow-up was 82 months. <i>Series B.</i> Mean follow-up was 46 months.
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time		
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	4	<i>Series A.</i> Persistent VUR was present in 28 ureters, respectively, 4 ureters (11.4%) with grade III VUR, 14 ureters (21.6 %)with grade IV , 10 ureters (47,6%)with grade V VUR. Endoscopic procedures globally were effective in 68/96 ureters (70,8%). <i>Series B.</i> VUR persistence was detected in 13 refluxing ureters after the second procedure, namely 1 ureter with (3%) grade III VUR, 8 ureters (21,6%) with grade IV VUR, and 4 ureters (36,4%) with grade V VUR. Endoscopic procedures globally were effective in 68/81 ureters (83.9%). Mean operative time was 21,5 minutes (range 8-70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$). Mean Dx/HA volume injected per ureter was 0.834 ml, respectively 0.7923 (range 0.3 - 2ml) in series A and 0.8516 in series B (range 0.5 - 1.3 ml); the difference was not significant ($p= 0.2733$).
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4,5	<i>Series A.</i> Endoscopic procedures globally were effective in 68/96 ureters (70,8%). <i>Series B.</i> Endoscopic procedures globally were effective in 68/81 ureters (83.9%). [...]Mean operative time was 21,5 minutes (range 8-70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$)..
		(b) Report category boundaries when continuous variables were categorized		
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		
Discussion				

Key results	18	Summarise key results with reference to study objectives	6	<i>Comparing our retrospective series, the overall success rate after two cystoscopic procedures increased from 88.6% to 97% for grade III VUR, from 65% to 78,4% for grade IV and from 52.4% to 63.6% for grade V. Operative time was significantly lower in series B, suggesting that IO-US may allow a more rapid identification of the injection site, helping in locating the correct injection site with fewer attempts, and finally resulting in a reduction in overall operative time. Effectiveness of the procedures raised in all VUR grade subpopulations, with a significant increase when series are globally compared raising from 70.8% to 83.9%</i>
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	6	<i>First, it is a retrospective study; a prospective randomized study may offer more robust conclusions, demonstrating differences within single grades of VUR. Second, despite every attempt to obtain a standardization of combined endoscopic-ultrasonographic procedure, all procedures were performed by the same couple of surgeon-radiologists; furthermore, US is an operator-dependent procedure. The introduction of different surgeons and radiologists in the study would have strengthened, or weakened our conclusions.</i>
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6,7	
Generalisability	21	Discuss the generalisability (external validity) of the study results	6,7	
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1	

Summary

Background: Endoscopic treatment of vesicoureteral reflux (VUR) is ~~the first~~ **an important** minimally invasive surgical approach in patients undergoing surgical treatment of VUR. In our past experience, we observed that a bulking agent mound sagittal diameter of 10 mm is the main predictor of effectiveness of the procedure. Moreover we noticed that the use of intraoperative ultrasound, allows the surgeon to better identify the site, volume and shape of the bulking agent injected, finally reducing operative time.

Objective: We aimed to evaluate if the intraoperative ultrasound assistance could definitively improve effectiveness of the endoscopic procedure.

Methods: we retrospectively compared two series treated with endoscopic procedures for intermediate and high risk primary VUR, respectively without (series A) and with (series B) intraoperative ultrasound (IO-US). In all patients VCUG was performed to assess VUR grade and to verify resolution or VUR downgrading during the follow-up.

Results: A total of 177 ureteric units were treated. Endoscopic procedures globally were effective in 68/96 ureters (70,8%) in series A and in 68/81 ureters (83.9%) in series B. No significant differences in effectiveness were observed comparing the series with regard to VUR grades, but a significant difference is shown ($p < 0.05$) **when grouping grades III-V VUR** ~~when the series are globally compared~~. No significance in differences of volume injected were detected, but operative time was significantly lower in series B (**27.5 min vs 19.6 min**, $p < 0.05$). **Mean sagittal mound diameter measured during cystoscopy in series B was 10.45mm (range 8.5 - 14.2mm)**

Discussion: The intraoperative ultrasound assistance during endoscopic treatment of VUR could represent a valid tool for surgeons to better identify location, volume and shape of the bulking agent. Furthermore, the use of an objective parameter of evaluation of the implant can overcome the subjective intraoperative evaluation of the implant itself, improving results for experienced surgeons and reducing the learning-curve for inexperienced ones.

Conclusions: Results of endoscopic injection of bulking-agent can be improved with intraoperative ultrasound, allowing at the same time a significant reduction of operative time.

		Endoscopic injection of Dx/HA		US-assisted Endoscopic injection of Dx/HA		p-value
VUR -Grade		Global effectiveness		Global effectiveness		
	III	31/35 (88.6%)		32/33 (97%)		0.357
	IV	26/40 (65%)		29/37 (78.4%)		0.217
	V	11/21 (52.4%)		7/11 (63.6%)		0.712
VUR - Global	III-V	68/96 (70.8%)		68/81 (84%)		0.049
		Mean	Range	Mean	Range	
Operative time	III-V	27.64 min	10 - 70 min	19.6 min	8 - 40 min	0.007
Volume injected	III-V	0.7923 ml	0.3 - 2 ml	0.8516 ml	0.5 - 1.3 ml	0.273

Key words: Vesicoureteric reflux, Bulking agents, Endoscopy, Intraoperative Ultrasound, Children.

List of abbreviations

VUR: Vescicoureteral reflux

UTI: Urinary tract infections

Dx/HA: dextranomer/hyaluronic acid copolymer

US: Ultrasound

VCUG: voiding cystourethrography

HIT: Hydrodistention injection technique

CAP: continuous antibiotic prophylaxis

IO-US: intraoperative ultrasound

Introduction

Vesicoureteral reflux (VUR) is a well-known condition involving 1% to 3% of children, often presenting with febrile urinary tract infections (UTIs) [1]. VUR is considered the most important risk factor for acute pyelonephritis in children and, in untreated patients, it could determine a progressive loss of renal function, due to renal scarring, especially in intermediate and high grade VUR [2,3].

The surgical treatment has evolved in the last 20 years, an increasing number of patients being treated conservatively but recommendations regarding VUR treatment are still different and controversial. There are several unresolved questions about what is the best management in patients with VUR; this could be related to many variables that can increase the risk of UTI and persistence of VUR, such as patient's age, sex, circumcision, presence of LUTS and acquired toilet training. Most Some Authors recommend continuous antibiotic prophylaxis as the initial management, as a spontaneous resolution or downgrading of reflux is expected within the first year of life; other Authors, conversely, do not use antibiotic prophylaxis according to low evidence of effectiveness in preventing renal scarring and its undefined impact on antimicrobial resistance. A persistent low-risk low-grade VUR should could only be managed with *on-demand* antibiotics if UTI occurs, while some Authors prefer to offer surgical treatment also in low-grade VUR in order to prevent UTI [1,4,5]. Nevertheless, surgical treatment is recommended in patients with symptomatic intermediate and persistent high-grade reflux risk reflux (grade III) and in those with persistent high-risk reflux (grade IV and V VUR) [6].

However, the best surgical option for primary intermediate and high-risk high-grade VUR is still questionable, and results are often controversial. The endoscopic procedures have

gained more and more acceptance, proving to be quite effective as other open or miniminvasive procedures, with the well-known benefits of minimally invasive surgery in terms of cost/effectiveness, patient's tolerance, hospital stay and pain killer's administration [1]. However ~~the~~ only **few** intraoperative tools are available to predict the effectiveness of the procedure. **For example, the choice of injection technique on the basis of ureteral orifice appearance and hydrodistention can improve the effectiveness of the procedure, as the "double HIT" technique has showed better results if compared to single HIT or STING in higher VUR grades;** **secondly, the effectiveness of the procedure can be deduced on the basis of the endoscopic appearance of the bulking-agent mound and the ureteral orifice modifications in shape and hydrodistensibility.** Thus effectiveness rates have been strongly related to the surgeon-experience, VUR grades and patient's selection. In a recent paper we suggested that the effectiveness of endoscopic injection of bulking agents can benefit from intraoperative ultrasound, allowing the surgeon to optimize site, shape and volume of the bulking-agent mound introducing moreover a more objective parameter for evaluation of procedure's effectiveness[7,8] . Moreover, we observed that an adequate size of the bulking-agent mound can predict effectiveness of the procedure and VUR resolution, potentially avoiding postoperative cystourethrogram introducing a standardization in the treatment and allowing a greater reliability of the procedure[9]. In order to confirm our previous observations the aim of this paper is to compare two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA), respectively without and with intraoperative US-guidance in terms of general aspects of the implant, intra-operative time, and DX/Ha amount.

Materials and Methods.

The present retrospective study compares two series of patients treated with endoscopic injection of ~~dextranomer/hyaluronic acid copolymer~~ (Dx/HA) between January 2010 and December 2019 for primary VUR. The first series (A) refers to the period January 2010 - December 2014 and includes patients treated with endoscopic procedures without the intraoperative US guidance. The second series (B) refers to the period January 2015- December 2019, and all endoscopic procedures were integrated with intraoperative US, as previously described [7-8] . Patients presenting with VUR under 1 years of age were initially managed conservatively with continuous antibiotic prophylaxis, and thereafter re-evaluated with VCUG. According to the EAU/ESPU guidelines [10], all patients with primary symptomatic ~~intermediate risk~~ **III grade** vesicoureteral-reflux (VUR) and primary ~~high-risk~~ **IV-V grade** VUR, even if preserved renal function, were treated. In all patients VUR was detected by voiding cystourethrography (VCUG), and assessed according to the International Reflux Study Committee parameters [11-13]. Low/moderate grade reflux was treated only if a contralateral concomitant higher grade reflux was treated, but ureteric units with second grade VUR were not included in the study. Patients with secondary VUR or with a follow-up shorter than 3 years were excluded.

Ureteric units were divided into 3 main groups according to the VUR grades. All procedures were carried out by the same surgeon (MC) for both series. In the series B, the intraoperative ultrasound was performed contemporary to the endoscopic procedure, in order to visualize the bladder trigone, the ureteral dilatation, the meatus hydrodistention before and after injection, the distribution of material along the course of ureter, allowing

an optimal location and size of the implant [7-9]. The intraoperative US was performed by a single pediatric radiologist (SS).

For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable .

In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US.

In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned. In case of VUR persistence or recurrent UTI after the second procedure, it was considered ineffective, even in cases of VUR downgrading, and patients were managed with a classical open cross-trigonal Cohen ureteral reimplantation.

Patients had an indwelling catheter for an average 24 hours (18-36) in order to reduce intravesical pressure, allowing the early bulking-agent stabilization in the first postoperative period, and were discharged the day after the procedure.

All patients have been followed-up with clinical and US examination on postoperative months 1,3, 6, 9, then every year.

Significant differences in effectiveness of techniques were tested with the Fisher exact test, while the Student test was used for comparison of operative time and bulking-agent volume injected. Significance was identified with $p < 0.05$.

Results.

A total of 123 patients matching inclusion criteria have been treated with endoscopic procedures for primary VUR at our institution, 59 males and 54 females between January 2010 and December 2019; of them, 59 presented with bilateral VUR, and a total of 177 ureteric units were treated (Table 1).

Series A. It includes 67 patients treated without intraoperative US-assessment of endoscopic injection of Dx/HA, respectively 32 males and 35 females. Mean age at surgery was 20 months (range 14 - 44 months), and mean follow-up was 82 months (range 75 – 114 months). Twenty-nine patients, 19 females and 10 males, presented with a bilateral VUR. Thirty-five ureters presented with III grade VUR, 40 with grade IV, and 21 with grade V . A total of 96 ureteric units were finally included.

Persistent VUR was present in 28 ureters, respectively, 4 ureters (11.4%) with grade III VUR, 14 ureters (21.6 %)with grade IV , 10 ureters (47,6%)with grade V VUR.

Endoscopic procedures resulted in radiographic resolution of VUR globally were effective in 68/96 ureters (70,8%). Results are resumed in Table 2.

Series B. 56 patients were included, 27 males and 29 females; the mean age was 23 months (range 18-55 months). Mean follow-up was 46 months (range 35 – 76 months). In 30 patients, 12 males and 19 females, a bilateral VUR was observed. Thirty-three ureters showed a grade III VUR, 37 a grade IV, and the remaining 11 a grade V. VUR persistence was detected in 13 refluxing ureters after the second procedure, namely 1 ureter with (3%) grade III VUR, 8 ureters (21,6%) with grade IV VUR, and 4 ureters (36,4%) with grade V VUR.

Endoscopic procedures globally were effective in 68/81 ureters (83.9%), and Mean sagittal mound diameter measured during cystoscopy in series B was 10.45mm (range 8.5 - 14.2mm). Results are resumed in table 3.

Mean operative time was 21,5 minutes (range 8-70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$). Mean Dx/HA volume injected per ureter was 0.834 ml, respectively 0.7923 (range 0.3 - 2ml) in series A and 0.8516 in series B (range 0.5 - 1.3 ml); the difference was not significant ($p= 0.2733$). No intraoperative or postoperative complications were reported. ~~Patients had an indwelling catheter for an average 24 hours (18-36) in order to reduce intravesical pressure, allowing the early bulking agent stabilization in the first postoperative period, and were discharged the day after the procedure.~~ In 6 patients a minor self-limiting hematuria was observed. In Series A 22/67 (33%) patients underwent open ureteral reimplantation. Six of them (29%) presented recurrent UTI under CA. In Series B 11/56 (19%) patients underwent open ureteral reimplantation. Four of them (36%) presented recurrent UTI under CAP.

Globally, a bilateral condition was observed in 15/33 patients with persistent VUR, respectively, 6/22 in series A, and in 9/11 patients (Series B). Endoscopic procedures were effective in 136/177 ureters, respectively 68/96 (70.8%) ureters in series A, and in 68/81 ureters (84%) in series B. ~~Results are resumed in tables 2 and 3.~~

As for Unilateral or Bilateral condition, no statistically significant difference was found between the two series, as well as for the severity of VUR (categorized in Grade III, IV and V) and the clinical outcome when results are compared according to the VUR grade.

Nevertheless, a significant difference is shown when grouping grades III-V VUR if series are globally compared (p= 0.049, Table 4).

Discussion

Indications for surgical treatment of VUR have progressively reduced in the last 20 years, but, at the same time, technical options have widened. Only in selected cases low-grade VUR usually does not undergo surgical treatment. Furthermore, and continuous antibiotic prophylaxis (CAP) becomes less used is no more indicated, as on-demand antibiotic therapy could be administrated when UTI occurs. since these patients can be easily managed with watchful waiting for UTI and antibiotic therapy “on demand”. However, surgery is indicated in high risk—moderate symptomatic and high grade VUR and in symptomatic patients with moderate risk VUR (grade III) [13,14].

Although open ureteral reimplantation is still considered the gold standard, the minimally invasive techniques have reached more and more indications in the surgical approach of VUR, widening the technical armamentarium in the hands of the pediatric urologist. Open ureteral reimplantation is still considered the gold standard for achieving the maximum result in terms of recovery from reflux. From this point of view, the various minimally invasive procedures expand the technical equipment while maintaining a high percentage of resolution, an optimal aesthetic result, while minimizing postoperative hospitalization and the administration of analgesics.[1,13-17].

As mentioned above, the pneumevesicoscopic cross trigonal intravesical reimplantation described by CK Yeung, the most recent robotic-assisted techniques as well as the

extravesical laparoscopic reimplantation according to Lich-Gregoire are proof of this [3, 18-30].

In 2005 Yeung CK *et al* showed how CO₂ bladder insufflation allows pneumovesicoscopic classic cross trigonal intravesical reimplantation by the same instruments used in pediatric laparoscopy [18]. More recently, robotic-assisted surgery showed to be an important tool to overcome many technical limitations of laparoscopic surgery, with reliable experiences in intra and extravesical reimplantation in children [19-22]. Moreover, extravesical laparoscopic reimplantation according to Lich-Gregoire has shown to be a valid option for pediatric urologists, offering promising results [3,23-30]. In this scenario we would

highlight Nevertheless that laparoscopic and robotic-assisted techniques, especially intravesical laparoscopic reimplantation, need a long learning curve and remain technically demanding procedures. Robotic-assisted surgery is still too expensive, and is limited to a minority of centers.

The classical open intravesical or extravesical procedures are still an important therapeutic options, but are now reserved to selected patients, in which endoscopic procedures might result less effective: high grade secondary VUR, high grade bilateral VUR, megaureters requiring tapering before reimplantation and duplex systems [22-25].

In this scenario, the cystoscopic injection of dextranomer/hyaluronic acid has shown to be the best option in the management of grade III-IV VUR, compared to continuous antibiotic prophylaxis and watchful waiting, and The endoscopic procedure by injection of (Dx/HA) is considered as the first minimally invasive surgical approach to manage grade III-IV VUR by many pediatric urologists, with an overall resolution rate between 71 and 83%, with some Authors reporting resolution rates higher than 90% [2, 25,26]; in a recent literature,

moreover, recently indications for endoscopic treatment have been widened including the above-mentioned patients with high grade secondary VUR, high grade bilateral VUR, megaureters requiring tapering before reimplantation and duplex systems [2, 22-25] with treat several cases with specific populations, historically not considered for endoscopic treatment [2]. Nevertheless, the effectiveness of cystoscopic injection of dextranomer/hyaluronic acid Dx/HA seems to be strongly related to patients' selection, surgeon experience and VUR grade, suggesting a difficult standardization of the procedure [2]. To this point, we found a strong relation with the mound volume of the dextranomer/hyaluronic acid Dx/HA injected and VUR resolution. In a cohort of 32 patients and 53 ureters we observed that the mound height of (Dx/HA) injected resulted the major predictive parameter of procedure effectiveness (sensitivity 100%, specificity 65.9%), suggesting VUR resolution when a maximum diameter $\geq$ than 1 cm was found on postoperative ultrasound (US) examination [7]. Starting from these observations in a subsequent study we found that intraoperative ultrasound (IO-US) may represent a valid tool for pediatric urologists in determining the exact location, shape and volume of the Dx/HA mound, concurring to standardize the technique and to overcome the lack in surgeon experience, allowing to improve the effectiveness of the procedure [8-9]. In the presented study we observed how the IO US can make the endoscopic procedure more accurate in the VUR treatment.

Effectiveness of the procedures raised in all VUR grade subpopulations, with a significant increase when series are globally compared raising from 70.8% to 83.9% ($p = 0.049$).

Although no significant difference has been shown when comparison of series is performed according to grade, a significant difference stands out when series are

compared considering all aggregated ureters. We hypothesize that significant differences in VUR grade subgroups would have been showed with a study population at least three times larger. We hypothesize that a larger study population could have shown significant differences in VUR grade subgroups as well.

In a classic paper by Elder *et al*, reported in the EAU guidelines in pediatric Urology, aggregate resolution rates after endoscopic treatment (up to three procedures) was 85%, involving all VUR grades [14,27].

In our series, the US-assisted procedure allowed a similar result for aggregate grade III-V VUR, excluding grades I-II, in which a higher resolution rate is expected. Moreover, only two procedures have been considered in the presented paper. Comparing our retrospective series A and B, the overall success rate after two cystoscopic procedures increased from 88.6% to 97% for grade III VUR, from 65% to 78,4% for grade IV and from 52.4% to 63.6% for grade V. One can argue that this improvement might be due to the increasing learning curve; nevertheless, in 2010, the surgeon performing all procedures already had a wide experience in endoscopic treatment of VUR, thus the impact of the increasing learning curve in effectiveness rates could be considered negligible [28].

Operative time was significantly lower in series B, suggesting that IO-US may allow a more rapid identification of the injection site, helping in locating the correct injection site with fewer attempts, and finally resulting in a reduction in overall operative time. Moreover, the mean volume of bulking agent injected was higher in series B, even if the difference was not significant. We hypothesize that the higher volume injected may be related to the attempts to reach the value of 10mm in sagittal diameter of the bulking agent, even in

those cases in which the endoscopic evaluation alone would suggest concluding the procedure.

The choice of surgical treatment in patients with symptomatic grade III-V VUR is still a matter of debate. As mentioned above, open procedures are still considered as the gold standard by many pediatric urologists and laparoscopic and robotic approaches are not recommended as routine procedures, due to the wider variation of results and unfavorable cost/effectiveness rates [14, 29-32], offering well known excellent resolution rates. Laparoscopic and robotic reimplantation techniques have been successfully performed with results comparable to the classical open procedures, (effectiveness ranging from 92 to 98%), but a wider variation of results has been highlighted when laparoscopic/robotic and open approaches are compared [14,28-31]. Moreover, laparoscopic and robotic procedures have shown a higher complications rate leading to reintervention, a longer learning curve and higher costs. Thus, laparoscopic and robotic approaches are not recommended as routine procedures [14]. In a recent retrospective study, Soulier *et al* reported a success rate of 98.3% after a mean follow-up of 59,3 months in 159 ureters and 117 patients treated with laparoscopic extravesical reimplantation (LEVUR), which is similar to Cohen results [3]. The Author reported intraoperative or early postoperative complications in 8 cases. In 5 cases a bladder mucosal perforation occurred during the procedure, immediately treated with endo-loop repair. One patient presented with acute urine retention treated with a supra pubic catheter left *in situ* for 10 days, while in two patients uretero-peritoneal fistulas occurred, requiring respectively a double-J stenting and an open Cohen procedure.

No intraoperative or postoperative complications have been observed in the presented series, but a minor, self-limiting hematuria in 6 patients.

Effectiveness of endoscopic procedures is known to be lower than other reimplantation techniques, especially in higher VUR grades.

Esposito *et al* compared the open Cohen, the endoscopic STING and the LEVUR procedures in terms of effectiveness, complications rates, costs and requirement of analgesic drugs concluding that the LEVUR is comparable to the Cohen procedure and superior to the STING endoscopic procedure in terms of effectiveness, but it shows the same advantages of STING procedure in terms of postoperative pain and lower postoperative morbidity [29] .

In a recent prospective randomized study Salih *et al* compared the open extravesical reimplantation and the endoscopic procedure, reporting a higher success rate of the open Lich-Gregoir. Nevertheless, in the described series, the patients enrolled in the endoscopic arm of the study underwent a single endoscopic procedure [32].

The endoscopic procedure, however, should not be considered as a single stage therapy. It is well described how the reiteration of the procedure concurs to reach satisfying success rates, although each procedure appears to be less effective than the previous one.

This study has some limitations. First, it is a retrospective study; a prospective randomized study may offer more robust conclusions, demonstrating differences within single grades of VUR. Second, despite every attempt to obtain a standardization of combined endoscopic-ultrasonographic procedure, all procedures were performed by the same couple of surgeon-radiologists; furthermore, US is an operator-dependent procedure. It is difficult to evaluate the potential effect of introducing different surgeons and radiologists

in the study. Third, in the presented paper, we observed a male-to-female ratio unusual for VUR distribution as females are usually more affected than males. Forth, the use of hydrodistension grading system would have allowed us a better standardization of procedures [33]. The introduction of different surgeons and radiologists in the study would have strengthened, or weakened our conclusions.

Conclusions

In conclusion, endoscopic injection of DX/Ha plays an important role in the mininvasive treatment of VUR. The intraoperative US-assistance can objectively refine the endoscopic procedure, in order to identify the exact location of the intramural ureter, monitoring at the same time the injection procedure and reducing operative times. This simple, non-invasive intraoperative US assistance could optimize results for experienced surgeons, and reduce learning-curve for inexperienced ones, and thus should be considered as a standard integration of endoscopic VUR treatment.

Table 1. Global distribution of patients

Series	A	B	Total
Males	32	27	59 (47,97%)
Females	35	29	64 (52,03%)
Patients Tot	67	56	123
Bilateral	29	31	60 (48%)
Ureteric units	96	81	177

Table 2. Series A

Endoscopic injection of Dx/HA		First procedure Success rate		Second procedure Success rate		Global success rate	
VUR Grade		Ureters	%	Ureters	%	Ureters	%
III		29/35	82,8%	2/6	33%	31/35	88,6%
IV		18/40	45%	8/22	36,4%	26/40	65%
V		7/21	33%	4/14	28,6%	11/21	52,4%%
Global Success		54/96	56,2%	14/42	33%	68/96	70,8%
Persistence		42/96	43.8%	28/42	67%	//	//

Table 3. Series B

US assisted Endoscopic injection of Dx/HA		First procedure Success rate		Second procedure Success rate		Global success rate	
VUR Grade		Ureters	%	Ureters	%	Ureters	%
III		30/33	90.9%	2/3	66.6%	32/33	97%
IV		17/37	45,9%	12/20	60%	29/37	78.4%
V		3/11	27.3%	4/8	50%	7/11	63.6%
Global Success		50/81	61,7%	18/31	58%	68/81	83.9%
Persistence		31/81	38.3%	13/31	42%	//	//

Table 4: differences and significance between series

		Endoscopic injection of Dx/HA		US-assisted Endoscopic injection of Dx/HA		p-value
VUR -Grade		Global effectiveness		Global effectiveness		
	III	31/35 (88.6%)		32/33 (97%)		0.357
	IV	26/40 (65%)		29/37 (78.4%)		0.217
	V	11/21 (52.4%)		7/11 (63.6%)		0.712
VUR - Global	III-V	68/96 (70.8%)		68/81 (84%)		0.049
		Mean	Range	Mean	Range	
Operative time	III-V	27.64 min	10 - 70 min	19.6 min	8 - 40 min	0.007
Volume injected	III-V	0.7923 ml	0.3 - 2 ml	0.8516 ml	0.5 - 1.3 ml	0.273

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract: retrospective case-control study	2	<i>"we retrospectively compared two series.."</i>
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	<i>"Results of endoscopic injection of DX/Ha can be improved with IO-US. [...] allows a significant reduction of operative time...."</i>
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	<i>However, the best surgical option for primary intermediate and high risk VUR is still questionable</i>
Objectives	3	State specific objectives, including any prespecified hypotheses	3	<i>we aimed to compare two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA), respectively without and with intraoperative US-guidance</i>
Methods				
Study design	4	Present key elements of study design early in the paper	3	<i>The present retrospective study compares two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid</i>

				<i>copolymer (Dx/HA) between January 2010 and December 2019</i>
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3	<i>The first series (A) refers to the period January 2010 - December 2014 and includes patients treated with endoscopic procedures without the intraoperative US guidance.</i>
Participants	6	<i>(a) Cohort study</i>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i>—Give the eligibility criteria, and the sources and methods of selection of participants <i>(b) Cohort study</i>—For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i>—For matched studies, give matching criteria and the number of controls per case	3,4	<i>For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable.</i> <i>In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US.</i> <i>In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned</i> <i>Patients with secondary VUR or with a follow-up shorter than 3 years were excluded</i>
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4	<i>For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable.</i> <i>In series A, the procedure was empirically concluded if a satisfying “volcano-like” mound of Dx/HA was obtained on endoscopic appearance and if the implant was able to avoid hydrodistention of the ureteral orifice; in series B, in addition to the already mentioned empiric evaluation parameters, the goal of the procedure was to obtain a mound of Dx/HA with a minimum of 10 mm in sagittal diameter, measured by intraoperative US.</i>
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4	<i>In both series patients were studied with a postoperative VCUG three months after the procedure. If persistent VUR was shown, a second endoscopic procedure was planned. In case of VUR persistence or recurrent UTI after the second procedure, it was considered ineffective, even in cases of VUR downgrading.</i>

Bias	9	Describe any efforts to address potential sources of bias	4	<i>All procedures were carried out by the same surgeon (MC) for both series. The intraoperative US was performed by a single pediatric radiologist (SS). For both series, endoscopic procedure consisted of a submucosal injection of Dx/HA within the ureteral orifice (multi-HIT) and/or at the basis of the ureteral orifice only if hydrodistention was not achievable.</i>
Study size	10	Explain how the study size was arrived at	3	<i>The present retrospective study compares two series of patients treated with endoscopic injection of dextranomer/hyaluronic acid copolymer (Dx/HA) between January 2010 and December 2019 for primary VUR</i>
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	4	<i>Significant differences in effectiveness of techniques were tested with the Fisher exact test, while the Student test was used for comparison of operative time and bulking-agent volume injected. Significance was identified with $p < 0.05$.</i>
		(b) Describe any methods used to examine subgroups and interactions		
		(c) Explain how missing data were addressed		
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy		
		(e) Describe any sensitivity analyses		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4	<i>A total of 123 patients matching inclusion criteria have been treated with endoscopic procedures for primary VUR at our institution, 59 males and 54 females between January 2010 and December 2019</i>
		(b) Give reasons for non-participation at each stage		<i>Only patients with primary VUR were included</i>
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	<i>59 males and 54 females 59 presented with bilateral VUR Series A. Mean age at surgery was 20 months (range 14 - 44 months). Series B. the mean age was 23 months (range 18-55 months).</i>

		(b) Indicate number of participants with missing data for each variable of interest		
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)		<i>Series A.</i> mean follow-up was 82 months. <i>Series B.</i> Mean follow-up was 46 months.
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time		
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	4	<i>Series A.</i> Persistent VUR was present in 28 ureters, respectively, 4 ureters (11.4%) with grade III VUR, 14 ureters (21.6 %)with grade IV , 10 ureters (47,6%)with grade V VUR. Endoscopic procedures globally were effective in 68/96 ureters (70,8%). <i>Series B.</i> VUR persistence was detected in 13 refluxing ureters after the second procedure, namely 1 ureter with (3%) grade III VUR, 8 ureters (21,6%) with grade IV VUR, and 4 ureters (36,4%) with grade V VUR. Endoscopic procedures globally were effective in 68/81 ureters (83.9%). Mean operative time was 21,5 minutes (range 8- 70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$). Mean Dx/HA volume injected per ureter was 0.834 ml, respectively 0.7923 (range 0.3 - 2ml) in series A and 0.8516 in series B (range 0.5 - 1.3 ml); the difference was not significant ($p= 0.2733$).
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4,5	<i>Series A.</i> Endoscopic procedures globally were effective in 68/96 ureters (70,8%). <i>Series B.</i> Endoscopic procedures globally were effective in 68/81 ureters (83.9%). [...]Mean operative time was 21,5 minutes (range 8-70 min), respectively 27,5 min in series A (range 10 - 70 min), and 19.6 min in series B (range 8 - 40 min); operative time was significantly lower in series B ($p=0,007894$)..
		(b) Report category boundaries when continuous variables were categorized		
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		
Discussion				
Key results	18	Summarise key results with	6	<i>Comparing our retrospective series, the overall</i>

		reference to study objectives		success rate after two cystoscopic procedures increased from 88.6% to 97% for grade III VUR, from 65% to 78,4% for grade IV and from 52.4% to 63.6% for grade V. Operative time was significantly lower in series B, suggesting that IO-US may allow a more rapid identification of the injection site, helping in locating the correct injection site with fewer attempts, and finally resulting in a reduction in overall operative time. Effectiveness of the procedures raised in all VUR grade subpopulations, with a significant increase when series are globally compared raising from 70.8% to 83.9%
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	6	First, it is a retrospective study; a prospective randomized study may offer more robust conclusions, demonstrating differences within single grades of VUR. Second, despite every attempt to obtain a standardization of combined endoscopic-ultrasonographic procedure, all procedures were performed by the same couple of surgeon-radiologists; furthermore, US is an operator-dependent procedure. The introduction of different surgeons and radiologists in the study would have strengthened, or weakened our conclusions.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6,7	
Generalisability	21	Discuss the generalisability (external validity) of the study results	6,7	
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1	