Turk J Urol 2019; 45(2): 139-45 • DOI: 10.5152/tud.2018.36824

RECONSTRUCTIVE UROLOGY
Original Article

Outcome of patients with failed pelvic fracture-associated urethral injury repair: A single centre 10-year experience

Gaurav Garg, Manmeet Singh, Manoj Kumar, Ajay Aggarwal, Siddharth Pandey, Deepanshu Sharma, Satya Narayan Sankhwar

Cite this article as: Garg G, Singh M, Kumar M, Aggarwal A, Pandey S, Sharma D, et al. Outcome of patients with failed pelvic fracture-associated urethral injury repair: A single centre 10-year experience. Turk J Urol 2019; 45(2): 139-45.

ABSTRACT

Objective: The management of recurrent posterior urethral strictures developing after pelvic fracture urethral injury (PFUI) is a challenging task. Despite availability of many surgical approaches, there is no consensus regarding the optimal approach. The objective of this study was to present our 10-year experience in the management of recurrent urethral strictures due to PFUI.

Material and methods: We did a retrospective single-institution review of patients who underwent surgical management for recurrent posterior urethral strictures from January 2006 to December 2016 using descriptive statistics. We included only those patients with PFUI who underwent some definitive surgical procedure for their previous failed repair(s).

Results: The final analysis included data of 50 male patients (10 adolescents and 40 adults). Mean age of the patients was 29.92±10.62 years. The average length of stricture was 3.02±1.47 cm. Progressive perineal urethroplasty (PPU) was done in 40 cases. Two patients with concomitant rectourethral fistula/false passage underwent transpubic urethroplasty (TPU). Three patients with complete bulbar necrosis were managed with single stage/staged preputial tube reconstruction. One patient underwent microsurgical urethroplasty using radial free forearm flap while in two patients each Mitrofanoff appendicovesicostomy and perineal urethrostomy was done. Majority of complications were minor (Clavien Grade 1 and 2). Overall success rate of PPU was 75%. Mean follow-up period was 29.46±10.68 months (range: 13-60 months).

Conclusion: Most cases of recurrent posterior urethral strictures of <3 cm in length can be operated by PPU with reasonable success rates. Complex and long-segment (higher than 3 cm) strictures require use of ancillary procedures like TPU, substitution urethroplasty and Mitrofanoff appendicovescostomy.

Keywords: Outcome; pelvic fracture, urethral injury; stricture; urethroplasty.

ORCID IDs of the authors:
G.G. 0000-0001-5198-8934;
M.S. 0000-0002-4445-3111;
M.K. 0000-0001-6993-8548;
A.A. 0000-0003-3157-7067;
S.P. 0000-0003-3314-1347;
D.S. 0000-0002-1754-5918;
S.N.S. 0000-0001-9875-5505

King George's Medical University, Lucknow, India

Submitted:
01.06.2018

Accepted:
17.07.2018

Available Online Date:
21.11.2018

Corresponding Author:
Manmeet Singh
E-mail:
manudocuro@yahoo.co.in

Available online at
www.turkishjournalofurology.com

Introduction

Pelvic fracture associated urethral injury (PFUI) occurs as a result of road traffic accidents (RTA) and fall from height (FFH), and PFUI is associated with 10% of all pelvic fractures particularly with pubic bone diastasis, inferomedial pubic bone fracture, and displacement.[1] The first surgical management of PFUI comprises of excision of the fibrotic urethral segment between distracted urethral ends with primary anastomosis of the normal urethra (EPA).[2] The management of failed PFUI repairs is a challenging task for the urologists due to more proximal location of stricture behind the pubic bone, extensive fibrosis and increased concerns of patients regarding sexual and urological outcomes with redo urethroplasty.[3,4] Presence of concomitant false passages, recto-urethral fistulas, peri-urethral cavities, incompetent bladder neck etc with PFUI also increases the complexity of re-do repairs.[3] At present a variety of procedures have been described in the literature as progressive perineal urethroplasty (PPU), transpubic urethroplasty (TPU), laparoscopic omentoplasty etc for the management of complex and recurrent posterior urethral strictures.[2-5] These approaches are technically demanding and such cases are performed at very limited centres in developing countries like India. There is no consensus regarding the optimal approach for the management of recurrent posterior urethral strictures after PFUI. In the

Turk J Urol 2019; 45(2): 139-45
DOI:10.5152/tud.2018.36824

current study we reviewed our 10-year experience of redo urethroplasty performed for recurrent posterior urethral strictures.

Material and methods

We did an analysis of prospectively maintained urethroplasty database at a tertiary care center in North India. A total of 62 patients underwent redo urethroplasty for PFUI between January 2006 and December 2016. Approval from Institutional Ethics Committee was taken for the study. We included only those patients with PFUI who underwent some definitive surgical procedure for their previous failed repair(s). The hospital records were reviewed noting demographic details, duration of suprapubic catheterization (SPC) before surgery, cause of PFUI, defect site and length of the defect (calculated with retrograde, and voiding cystourethrography, RGU+VCU). Data concerning previous surgical interventions, surgical techniques used in redo procedures, average blood loss and postoperative hospital stay were analysed. Procedures such as progressive PPU, TPU, pedicled preputial tube urethroplasty, Mitrofanoff appendicovesicostomy, radial free forearm flap urethroplasty and perineal urethrostomy were performed based on operating surgeon's discretion, patient's wishes and taking into account characteristic of the defect. Postprocedural urethral stenting was done with 16 F silicon Foley catheters in adults and 12-14 F catheter in adolescents. A SPC was also kept for 4 to 6 weeks postoperatively. A pericatheter RGU was performed before removal of intraurethral catheter. If there was no contrast leak then intraurethral catheter (IUC) was removed. In case of leak, IUC was removed after an interval of additional 2 weeks. The eventual surgical outcome was considered as acceptable if patients were voiding with an average peak flow rate of >15 mL/min with minimal post void residual urine (PVRU) and in case of some obstruction 1-4 times minimally invasive endoscopic procedures were attempted.[6] Patients were followed up after 6-8 weeks, and then every 6 months for 2 years. We also recorded complications as per modified Clavien-Dindo classification system.[7] Erectile dysfunction (ED) if present was classified based on 5-item version of International Index of Erectile Function (IIEF-5) scale scores as mild (17-21), mild to moderate (12-16), moderate (8-11), severe (5-7), and no ED (22-25).[8] Patients lost to follow up and/or with incomplete records were excluded from final analysis.

Statistical analysis

All the analysis was done using Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) version 16. Qualitative data were analyzed using *chi*-square test and quantitative data was analyzed using Student t-test. A p-value of <0.05 was considered significant.

Results

More than 260 anastomotic urethroplasty for PFUI have been done till date at our institution since 2006. Twelve patients who either lost to follow up or had incomplete records were excluded from the study. The final analysis was performed on data of 50 male patients. The baseline characteristics are depicted in Table 1. There were 10 adolescents (range 9 to 18 years), and 40 adult patients (>18 to 45 years). Mean age was 29.92±10.62 years. Cause of PFUI was secondary to RTA in 45 cases and FFH in 5 cases. Mean time to failure after previous surgery was 15.04 months (range 2-24). Most patients had an indwelling SPC. Mean period on SPC before surgery was 20.24 months (range 15-25). The average number of prior urethroplasties performed was 1.22 (range 1-3). Most commonly affected site was bulbomembranous urethra in 40 (80%), prostatomembranous junction in 7 (14%) and proximal bulbar urethra in 3 (6%) cases. The average stricture length was 3.02±1.47 cm. The details of redo urethroplasty are depicted in Tables 2 and 3. Majority (19/21; 90.47%) of complications after redo urethroplasty were minor (Clavien Grades 1 and 2) (Table 4). Mean follow-up period was 29.46±10.68 months (range 13-60 months). The results of PPU were comparable between adolescents and adults (p>0.05) (Table 5).

Discussion

There is no consensus on the management of recurrent and complex urethral strictures after PFUI. Some authors have attempted endoscopic treatments like Optical Internal Urethrotomy (OIU) before urethroplasty while the others have performed redo urethroplasty directly.[6] The objectives of urethroplasty in PFUI should be complete excision of scarred urethra with tension-free anastomosis of the urethral ends.[3-5] The initial urethral surgery offers the best chance to achieve a successful outcome.[9] Redo urethroplasty is more challenging for the urologists as there is extensive fibrosis and loss of planes. The local blood supply to urethra is often precarious as the paired bulbar arteries are almost always sacrificed during initial surgery and the blood supply depends mainly on retrograde supply. As depicted in Table 2 most patients (34 adults and 6 adolescents) with single failed urethroplasty underwent PPU as described by Webster et al.[10] We encountered some unique findings during redo urethroplasty. The scar excision and urethral mobilization were not adequate in 24 (60%) cases during the primary surgery and we had to do extensive scar excision and urethral mobilization from penoscrotal junction to the prostatic apex. There was evidence of stitch granulomas in around 8 cases (20%). Additionally inferior pubectomy was either not performed or inadequately performed during primary surgery. Hence incomplete mobiliza-

Table 1. Baseline characteristics of patients

Parameter	Value
Mean age (Years)	29.92+10.62 (range 9–45)
Sex	Male
Number of patients	50
	10-adolescents (9-18 years)
	40 adults (>18-45 years)
Mechanism of trauma	RTA-45 (90%)
	FFH-05 (10%)
Mean time to failure after previous surgery	15.04±5.45 (range 6-24)
Patients with SPC in situ	46 (92%)
Previous interventions	
Patients referred from elsewhere (n=45)	Single urethroplasty-35
Patients operated at our centre (n=5)	Single urethroplasty-35
	Recurrent urethroplasties-10
	OIU/dilatation-13
	Single urethroplasty-5
	Recurrent urethroplasties-0
	OIU/dilatation-2
Defect site	BMU-40
	PMU-7
	BU-3

RTA: road traffic accident; FFH: fall from height; OIU: optical internal urethrotomy; BMU: bulbomembranous urethra; PMU: prostomemebranous urethra; BU: bulbar urethra; SPC: suprapubic catheterization

Table 2. Details of redo-urethroplasty. Patients with history of one failed urethroplasty

Surgery performed; No of patients	Location	Stricture length (cm) Mean±SD	Operative time (minutes) Mean±SD	Blood loss (mL) Mean±SD	Hospital stay (days) Mean±SD	Success rate	Follow up period (months) Mean±SD
PPU (n=40)	BMU-35 PMU-5	2.46±0.69	135±9.86	450±86.09	7.62±1.67	75%	30.97±10.72

PPU: progressive perineal urethroplasty; BMU: bulbomembranous urethra; PMU: prostomembranous urethra

tion of urethra, inadequately performed inferior pubectomy and stitch granulomas might be the cause of failure in primary surgery. Length of stricture, extent of injury, vascularity of urethra and number of previous attempts at repair have been proposed as determining factors for successful outcome after redo urethroplasty.[11] The mean operative time for PPU in the present study was 135±9.86 mins. Gupta et al.[6] reported that redo urethroplasty takes significantly more time to perform compared to primary urethroplasty (140 mins vs 90 mins; $p<0.05$). Majority of redo cases require use of inferior pubectomy, corporal separation and corporal rerouting to straighten the course of urethra leading to increase in operative time. Previous studies have reported variable success rates in patients with redo urethroplasty for PFUI ranging from 80 to 95%.[6,9,11-13] Bhagat et al.[12] reported overall success rate of 83.72% in 43 cases of redo urethroplasty for PFUI. In the author's study PPU was done in 28, TPU in 12, staged Buccal mucosal graft (BMG) with scrotal inlay in 2 cases, and single stage preputial tube reconstruction was done in one patient. Webster et al.[13] reported 95% success rate in a series of 20 patients, however redo anastomotic

Turk J Urol 2019; 45(2): 139-45
DOI:10.5152/tud.2018.36824

Table 3. Details of redo-urethroplasty. Patients with history of more than one failed urethroplasty

Surgery performed; No of patients	Location	Mean stricture length (cm)	Mean operative time (minutes)	Mean blood loss (mL)	Mean hospital stay (days)	Success rate	Mean follow up period (months)
TPU (n=2)	PMU-02	5	190	750	9	100%	25
Single stage preputial flap urethroplasty (n=1)	BMU-01	5.5	240	500	10	100%	25
Staged BMG+ preputial flap urethroplasty (n=2)	BMU-02	6	260	650	10	50%	17
Mitrofanoff appendicovesi-costomy (n=2)	PMU+ bladder neck-02	6.5	320	700	14	-	30
Radial free forearm flap urethroplasty (n=1)	BMU+BNP-1	7	420	1200	13	100%	15
Perineal urethrostomy (n=2)	PMU-02	5.5	120	100	5	-	18

PPU: progressive perineal urethroplasty; TPU: transpubic urethroplasty; BMU: bulbomembranous urethra; PMU: prostomembranous urethra; BNP: bladder neck prostate

urethroplasty was not performed in all patients. Kulkarni et al.[9] described success rate of 79.13% in a retrospective analysis of 541 cases of redo urethroplasty performed for PFUI. Gupta et al.[6] reported a success rate of 80.8% in a study of 52 patients of redo-urethroplasty. The overall success rate of PPU in the present study was 75% and there was no difference in outcomes between adolescents and adults ($p>0.05$). The patients who failed after PPU had significantly higher stricture length compared to patients with successful outcomes (3.45 cm vs 2.13 cm; $p<0.01$). Apparently low success rates of PPU in the present study might be due to the fact that we encountered patients who had undergone several attempts of endoscopic interventions/urethroplasties at other centers before they were referred to us. Previous interventions (endoscopic realignment/urethroplasty) in patients with post-traumatic posterior urethral strictures have been shown to have a significant adverse impact ($p<0.05$) on success rates of subsequent anastomotic urethroplasty.[14] Presence of concomitant perineal or rectourethral fistulas, false passages and bladder neck incompetence increase the complexity of PFUI repair.[3] Two adolescent patients with a mean age 12.5 years underwent TPU as described by Waterhouse et al.[15] These patients had narrow pelvis, long stricture segment (5 cm) and concomitant rectourethral fistula/false passage from bulb to bladder posterior to bladder neck. The patient with recto-urethral fistula underwent prior diversion colostomy before urethroplasty. The transpubic approach is preferred in pediatric PFUI cases owing to narrow and deep location of child pelvis with an intraabdominal bladder.[16] Cases with complete bulbar necrosis (n=3) were managed with either single stage preputial tube reconstruction (1 adult patient) or staged reconstruction with buccal mucosal graft and preputial flap (one adult and one adolescent patient). Microsurgical urethroplasty using radial free forearm flap was done in one patient who had dual narrowing localized at BMU and bladder neck-prostate (BNP) junction as described by Chauhan et al.[17] Two cases (one adult and one adolescent) having incompetent bladder neck underwent urinary diversion with Mitrofanoff appendicovesicostomy.[18] Two adult patients with long segment defects (5.5 cm) were not willing to undergo extensive surgeries like TPU etc and opted for permanent perineal urethrostomy. Hence patients with long (>3 cm)

Table 4. Complications after redo urethroplasty

Surgery performed; No of patients	No of patients developing complications	Clavien Grade I	Clavien Grade II	Clavien Grade III
PPU (n=40)	14	Wound sepsis-6	UTI-2 Urinary Incontinence-2 Erectile dysfunction-4	
TPU (n=2)	2	Wound sepsis-1	UTI-1	
Pedicled preputial tube urethroplasty (n=1)	1	Wound sepsis-1		Vesicocutaneous fistula-1
Staged BMG& ventral preputial flap urethroplasty (n=2)	1			
Mitrofanoff appendicovesicostomy n=2)	1		Hoarseness of voice due to endotracheal tube-1-	-
Radial free forearm lap urethroplasty (n=1)	1		-	Rectal injury-1
Perineal urethrostomy (n=2)	1	Wound sepsis-1	-	-

PPU: progressive perineal urethroplasty; TPU: transpubic urethroplasty; UTI: urinary tract infection; BMG: Buccal mucosal graft

Table 5. Comparison of results between adolescents and adults who underwent redo anastomotic urethroplasty by PPU

Variable	Adolescents (n=6)	Adults (n=34)	p
Stricture length (cm)	2.25±0.41	2.5±0.72	0.42
Operative time (minutes)	135.33±10.26	134.94±7.89	0.92
Blood loss (mL)	446.83±97.79	450.56±85.48	0.92
Hospital stay (days)	12.17±8.23	9.26±3.19	0.12
Follow up (months)	34.5±10.59	30.35±10.78	0.38
No of patients with successful outcomes	4	26	0.62
No of patients developing complications	2	12	1.00

PPU: progressive perineal urethroplasty

and complex recurrent posterior urethral strictures require use of ancillary procedures. In the present study, superficial wound sepsis (n= 9: 18%), and urinary tract infection (n=3: 6%) developed after redo urethroplasty. None of wound infections needed surgical intervention. Bascom et al.[19] described an overall wound complication rate of 21% in a retrospective analysis of 829 urethroplasties. The authors proposed that any long term sequelae of wound complications did not develop.[19] In the present study there was mild urinary incontinence in 2 patients which was successfully treated with pelvic floor exercises and anticholinergics. In patients with PFUI urinary continence mainly depends on the integrity of proximal continence mechanism especially the bladder neck.[20] Patients with concomitant bladder neck dysfunction should be counseled preoperatively as there is greater risk of urinary incontinence.[20] Mild to moderate erectile dysfunction was reported by 4 patients after PPU in the present study. The cause of ED in PFUI is attributable to primary injury itself rather than urethroplasty.[21,22] Pratap et

Turk J Urol 2019; 45(2): 139-45
DOI:10.5152/tud.2018.36824

al.[23] proposed that there was no change in the potency status of the patients even after combined abdomino-perineal urethroplasty for long segment (>3 cm) and complex posterior urethral strictures. Erectile dysfunction responded well to medical therapy with phosphodiestrase-5 (PDE-5) therapy. One patient of staged urethroplasty developed vesicocutaneous fistula after removal of the catheter. He was managed with pedicled flap closure from thigh. There was intraoperative rectal injury during microsurgical reconstruction with radial free forearm flap which was repaired later on. Almost 90% of the patients in the present study had undergone primary urethroplasty at primary care centres. In developing countries like India the disease dynamics is complicated, a very few centres and specialists are operating upon failed and complex cases. Anastomotic urethroplasty for complex and redo cases should preferably be done by experienced persons at high volume tertiary care centres.[9]Our study had some limitations. It was a retrospective study, and lacked data of pediatric patients. We also did not analyze factors associated with failure in redo cases. Further large population-based prospective studies are warranted to clearly outline the ideal approach for the management of failed and complex posterior urethral strictures.

In conclusion, most cases of recurrent posterior urethral strictures of ≤3 cm in length can be operated by PPU with reasonable (75%) success rates. Complex and long segment (>3 cm) strictures require use of ancillary procedures like TPU, substitution urethroplasty and Mitrofanoff appendicovescostomy.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of King George's Medical University Institutional Ethical Committee (2544/Ethics/R-cell-18).

Informed Consent: Written informed consent was obtained from all individual participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.G., M.K., A.A., S.P., D.S., S.N.S.; Design – G.G., M.K., A.A.; Supervision – G.G., M.K., A.A., M.S., S.P., D.S., S.N.S.; Data Collection and/or Processing – G.G., M.K., A.A., M.S.; Writing Manuscript – G.G., M.K., A.A., M.S., S.P., D.S., S.N.S.; Critical Review – G.G., M.K., A.A., M.S., S.P., D.S., S.N.S.

Acknowledgements: I acknowledge the cooperation of residents of Urology department of King George's medical university who participated in data collection and evaluation of the patient. We also appreciate the commitment and compliance of the patient who reported the required data.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Demetriades D, Karaiskakis M, Toutouzas K, Alo K, Velmahos G, Chan L. Pelvic fractures: epidemiology and predictors of associated abdominal injuries and outcomes. J Am Coll Surg 2002;195:1-10. [CrossRef]
2. Webster GD, Goldwasser B. Perineal transpubic repair: a technique for treating post-traumatic prostatomembranous urethral strictures. J Urol 1986;135:278-9. [CrossRef]
3. Koraitim MM. Posttraumatic posterior urethral strictures in children: a 20-year experience. J Urol 1997;157:641-5. [CrossRef]
4. Koraitim MM. On the art of anastomotic posterior urethroplasty: a 27-year experience. J Urol 2005;173:135-9. [CrossRef]
5. Kulkarni SB, Barbagli G, Joshi PM, Hunter C, Shahrour W, Kulkarni J, et al. Laparoscopic omentoplasty to support anastomotic urethroplasty in complex and redo pelvic fracture urethral defects. Urology 2015;85:1200-5. [CrossRef]
6. Gupta NP, Mishra S, Dogra PN, Hemal AK, Seth A, Kumar R. Does a previous end-to-end urethroplasty alter the results of redo end-to-end urethroplasty in patients with traumatic posterior urethral strictures? Int J Urol 2008;15:885-8.
7. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240:205-13. [CrossRef]
8. Rhoden EL, Teloken C, Sogari PR, Vargas Souto CA. The use of the simplified International Index of Erectile Function (IIEF-5) as a diagnostic tool to study the prevalence of erectile dysfunction. Int J Impot Res 2002;14:245-50. [CrossRef]
9. Kulkarni SB, Surana S, Desai DJ, Orabi H, Iyer S, Kulkarni J, et al. Management of complex and redo cases of pelvic fracture urethral injuries. Asian J Urol 2018;5:107-17. [CrossRef]
10. Webster GD, Ramon J. Repair of pelvic fracture posterior urethral defects using an elaborated perineal approach: experience with 74 cases. J Urol 1991;145:744-8. [CrossRef]
11. Mundy AR. Transperineal bulbo-prostatic anastomotic urethroplasty. World J Urol 1998;16:164-70. [CrossRef]
12. Bhagat SK, Gopalakrishnan G, Kumar S, Devasia A, Kekre NS. Redo-urethroplasty in pelvic fracture urethral distraction defect: an audit. World J Urol 2011;29:97-101. [CrossRef]
13. Webster GD, Ramon J, Kreder KJ. Salvage posterior urethroplasty after failed initial repair of pelvic fracture membranous urethral defects. J Urol 1990;144:1370-2. [CrossRef]
14. Singh BP, Andankar MG, Swain SK, Das K, Dassi V, Kaswan HK, et al. Impact of prior urethral manipulation on outcome of anastomotic urethroplasty for post-traumatic urethral stricture. Urology 2010;75:179-82. [CrossRef]
15. Waterhouse K, Abrahams JI, Gruber H, Hackett RE, Patil UB, Peng BK. The transpubic approach to the lower urinary tract. J Urol 1973;109:486-90. [CrossRef]
16. Senocak ME, Ciftci AO, Buyukpamukcu N, Hicsonmez A. Transpubic urethroplasty in children: report of 10 cases with review of the literature. J Pediatr Surg 1995;30:1319-24. [CrossRef]
17. Chauhan A, Sham E, Chee J. Microsurgical Urethroplasty for Complex Bulbar Urethral Strictures Using the Radial Forearm Free Flap Prelaminated with Buccal Mucosa. J Reconstr Microsurg 2016;32:378-85. [CrossRef]

18. Harris CF, Cooper CS, Hutcheson JC, Snyder HM, 3rd. Appendicovesicostomy: the mitrofanoff procedure-a 15-year perspective. J Urol 2000;163:1922-6. [CrossRef]
19. Bascom A, Ghosh S, Fairey AS, Rourke KF. Assessment of Wound Complications After Bulbar Urethroplasty: The Impact of a Lambda Perineal Incision. Urology 2016;90:184-8. [CrossRef]
20. Bagga HS, Angermeier KW. The mechanism of continence after posterior urethroplasty. Arab J Urol 2015;13:60-3. [CrossRef]
21. Anger JT, Sherman ND, Dielubanza E, Webster GD, Hegarty PK. Erectile function after posterior urethroplasty for pelvic fracture-urethral distraction defect injuries. BJU Int 2009;104:1126-9. [CrossRef]
22. Dogra PN, Singh P, Nayyar R, Yadav S. Sexual Dysfunction After Urethroplasty. Urol Clin North Am 2017;44:49-56. [CrossRef]
23. Pratap A, Agrawal CS, Pandit RK, Sapkota G, Anchal N. Factors contributing to a successful outcome of combined abdominal transpubic perineal urethroplasty for complex posterior urethral disruptions. J Urol 2006;176:2514-7. [CrossRef]