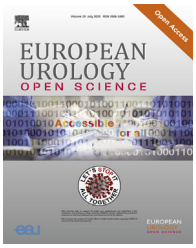
EUROPEAN UROLOGY OPEN SCIENCE 27 (2021) 88–93

available at www.sciencedirect.com
journal homepage: www.eu-openscience.europeanurology.com



European Association of Urology

Reconstructive Urology

Ectopic Germinal Centres with B and T Cells and Follicular Dendritic Cell Networks in Urethral Stricture Tissue: Possible Avenue for Immunological Treatments

Teresa Olsen Ekerhult [a,*], *Ola Grimsholm* [b], *Jenny Magnusson* [a], *Christina Kåbjörn Gustafsson* [c], *Ralph Peeker* [a]

[a] *Department of Urology, Institute of Clinical Sciences at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden;* [b] *Department of Rheumatology and Inflammation Research, University of Gothenburg, Gothenburg, Sweden;* [c] *Department of Pathology, Ryhov Hospital, Jönköping, Sweden*

Article info

Article history:
Accepted March 8, 2021

Associate Editor:
Silvia Proietti

Keywords:
Urethral stricture
Sclerosis
Inflammation
Histopathology
Germinal centre
B cell
T cell
Follicular dendritic cell

Abstract

Background: The underlying cause of a urethral stricture can sometimes be obscure. It is possible that an injury to the urethra induces an immunological cascade that generates scar tissue and fibrosis, eventually resulting in a stricture. If such immunological reactions could be better elucidated, immunological therapies could possibly emerge.

Objective: To evaluate if ectopic germinal centres exist in urethral stricture disease.

Design, setting, and participants: Resected stricture specimens from 45 patients undergoing open bulbar urethroplasty with excision and anastomosis were assessed. Histopathological characteristics, such as fibrosis (grade I–III), inflammation, and sclerosis, were evaluated using immunostaining for CD3 (T cells), CD20 (B cells), and CD21 (follicular dendritic cells).

Outcome measurements and statistical analysis: The primary outcome measure was the presence or absence of a germinal centre. The secondary outcome was evaluation of any correlation between the degree of fibrosis and germinal centres. Fisher's exact test was used for univariate analysis.

Results and limitations: In six patients, ectopic germinal centres were found. In ten patients, there was no inflammation at all. There was no correlation found between the degree of fibrosis and the abundance of immunohistochemically detected immune cells.

Conclusions: Ectopic germinal centres, with B and T cells as well as follicular dendritic cell networks, do exist in urethral stricture disease. This finding may open up for novel research avenues on the possibility of adopting immunological treatments for urethral stricture disease.

Patient summary: In patients with a narrowing of the urethra due to any kind of trauma, we looked for the presence of centres of immunological reaction in urethral tissue. We identified these immunological centres (also called germinal centres) in some patients. This intriguing finding suggests that immunological treatments may have potential for men with scar tissue in a narrowed urethra.

* Corresponding author. Department of Urology, Institute of Clinical Sciences at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. Tel. +46 31 3429019.
E-mail address: teresa.ekerhult@vgregion.se (T.O. Ekerhult).

http://dx.doi.org/10.1016/j.euros.2021.03.006

1. Introduction

Urethral stricture is a common condition almost exclusively affecting men, with an incidence of 0.6–1.4% [1–3], although there are considerable regional variations. The development of a urethral stricture is in many cases idiopathic. However, it is generally agreed that the cause of stricture development includes some sort of trauma. In many cases the trauma in question is obvious, such as pelvic trauma with urethral disruption, instrument damage, long-term catheterisation, or straddle injury. Besides trauma, certain infectious conditions and stone passage can also cause the development of urethral stricture disease, such as infections with gonococci or chlamydia.

After an insult to the urothelium, inflammatory reactions are induced in various mediators such as immune cells, including T cells, B cells, macrophages, and neutrophils. Moreover, signalling molecules such as cytokines, mTOR protein, and transcription factors may also lead to the formation of a circumscribed scar and fibrosis with narrowing of the urethral lumen [2,4–6].

Cumulative evidence indicates that the adaptive immune system consists of T and B cells, with the latter responsible for humoral immunity in all vertebrates [7]. In secondary lymphoid organs, naïve follicular B cells are located in B cell follicles, surrounded by T cell zones. When a mature naïve B cell encounters its relevant cognate antigen, it becomes activated and with the help of the cognate T cell it can form germinal centres (GCs) that are secondary structures within B cell follicles [8]. In the GC, B cells proliferate in the dark zone, after which they undergo affinity maturation through the acquisition of somatic mutations in their antigen receptor. Subsequently, GC B cells pick up antigen from the deposit provided by follicular dendritic cells (FDCs) in the light zone and present these to T follicular helper (TFH) cells. The TFH cells then give a signal (IL-21) to the B cells, which then either acquire even more mutations by going back to the dark zone or, if appropriate, leave the GC as a memory B cell or a plasma cell [9]. GCs are sources of memory B and plasma cells that are partly responsible for the formation of a urethral stricture, as explained above. Targeting of these structures with inhibitory agents, such as rituximab, for example, would stop the signalling involved in GC formation and could prevent the occurrence of urethral stricture.

Ectopic lymphoid structures are often formed during various pathologic conditions such as autoimmunity (eg, rheumatoid arthritis) and infection. It has recently been shown that tertiary lymphoid tissues such as ectopic GCs are found in patients with chronic kidney disease and that ectopic GC formation is associated with the severity of kidney inflammation [10,11].

To the best of our knowledge, there are no reports on ectopic GCs in the urethra or urethral stricture tissue. The primary aim of this study was to investigate whether GCs are present in urethral strictures. The secondary aim was to determine if there is any correlation between the grade of fibrosis and the presence of GCs.

2. Patients and methods

2.1. Patient selection

The population for this retrospective study was consecutive patients with bulbar urethral strictures treated with urethroplasty with resection and end-to-end anastomosis (EA) between December 2011 and December 2014 at Sahlgrenska University Hospital, Gothenburg, Sweden for whom a specimen amenable to histopathological analysis was available (Table 1). The urethral specimens were analysed by one uropathologist using light microscopy on sections stained with haematoxylin-eosin (H&E), during which the degree of fibrosis was also assessed as previously described [12]. All specimens were also subjected to immunohistochemical staining. The research protocol was approved by the University of Gothenburg regional Ethical Review Board (permit no. 663-11) and the patients provided informed consent allowing their

Table 1 – Characteristics of the study population

	Overall (n = 45)	GCs (n = 6)	No GCs (n = 39)
Median age, yr (interquartile range)	36 (28–54)	44 (33–62)	37 (28–46)
Sclerosis (n)	11	3	8
Inflammation (n)	14	3	11
Failure (n)	11	1	10
Stricture length (n)			
0.5 cm	3	1	2
1.0 cm	14	2	12
1.5 cm	17	2	15
2.0 cm	4	0	4
Data missing	7	1	6
Aetiology (n)			
Idiopathic	31	4	27
Iatrogenic	3	0	3
Traumatic	8	1	7
Infection	1	1	0
Congenital	2	0	2
Fibrosis grade (n)			
Grade I	19	1	18
Grade II	13	2	11
Grade III	13	3	10

GCs = germinal centres.

specimens to be analysed and used for studies in the future before they underwent urethroplasty surgery.

2.2. Surgical technique

Patients underwent preoperative urethroscopy; in cases for whom this was not possible, combined retrograde and antegrade urethrography was performed. Perioperative antibiotics (trimethoprim sulfamethoxide or a quinolone) were also given during the postoperative catheter period. Anticoagulant (dalteparin 5000 IU/d subcutaneously) was given perioperatively to patients older than 75 yr and in cases with a history of venous thrombosis. This series of urethroplasties was carried out by two experienced surgeons who have been performing urethroplasty surgery since 1998. Careful dissection, mainly dorsally, to the bulbar urethra was performed. After mobilisation, the bulbar urethra was cut at the level of the stricture. The fibrotic part of the strictured urethra was resected and the ends were spatulated. An EA was created with between six and eight interrupted 4.0 polyglyconate sutures. After the first three sutures located at the 10, 12, and 2 o'clock positions were placed from inside the urethral mucosa, a 16Fr silicone urethral catheter was introduced, and then the remaining sutures were inserted from the outside. After finishing the mucosal anastomosis, an outer running suture of the tunica albuginea was used to create a second layer. No drainage was used. A cross-form compression bandage was applied in the perineal region for 2 d. Free mobilisation was recommended for all patients. The catheter was routinely removed after 14 d without urethrography control and the patients were advised to abstain from sexual activities for 6 wk. The patients were followed at 3 mo, 1 yr, and 2 yr postoperatively, and always on request, with uroflowmetry and urethroscopy performed as previously described [13].

2.3. Tissue handling and analysis

Visible scar tissue was excised and an EA was performed as previously described [13]. Specimens were prepared by routine fixing in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin. Routine H&E and van Gieson staining was carried out and specimens were analysed. The degree of fibrosis was classified as mild, moderate, or severe, essentially according to a scheme previously described [12,14,15]. New sections of the same paraffin-embedded tissue were sliced for immunohistochemistry. The specimens were again evaluated by the same uropathologist and were categorised according to whether they contained GCs or not.

The degree of fibrosis was dichotomised between specimens with grade III fibrosis and those with grade I–II fibrosis (Table 2) in accordance with previous findings [6] showing that sclerosis only occurred in grade III fibrosis and a higher number of failures in grade III fibrosis.

2.4. Immunohistochemistry

The initial H&E-stained slides were re-evaluated under a microscope and areas of inflammation and GCs were marked. Adjacent sections were stained with rabbit antibody to human CD3 (ab5690; Abcam, Cambridge, UK; dilution 1:100), rabbit antibody to human CD20 (ab78237; Abcam; dilution 1:100), and rabbit antibody to human CD21 (ab75985; Abcam; dilution 1:250). After routine deparaffinisation, antigen retrieval was performed using a pressure cooker in Tris-EDTA buffer at pH 9.0 for 20 min. The sections were blocked using 4% bovine serum albumin in Tris buffer for 20 min and incubated with the primary antibody for 1 h at room temperature. Then the sections were incubated with MACH2 Universal HRP-Polymer Detection reagent (Biocare, Pacheco, CA, USA) for 30 min and finally developed with DAB solution (Biocare). The resulting positive cells in the marked areas were counted and compared (percentage) in each case.

Staining for CD3 and CD20 was performed in order to localise T- and B-cell areas inside lymphoid aggregates in the urethral strictures. Staining with anti-CD21 was used to detect FDCs inside GC-like structures. H&E and immunohistochemical stains were compared and the percentage inflammatory area in the premarked area was calculated.

Table 2 – Presence of germinal centres by fibrosis grade[a]

Fibrosis grade	No GCs (neg)	GCs (pos)
Grade I–II	28	3
Grade III	11	3

GCs = germinal centres.
[a] Fisher's exact test: $p = 0.36$; odds ratio 2.5, 95% confidence interval 0.4–14.6.

2.5. Statistical analysis

Fisher's exact test was used for univariate analysis. A p value of <0.05 was considered significant. Statistical calculations were performed with SPSS v20 for Mac software and R Statistical Software v3.3.1.

3. Results

Among 71 consecutive patients, 45 with a specimen amenable to histopathological analysis were included in the study (Table 1). Reasons for exclusion were as follows: the patient declined participation ($n = 13$); no specimen was available ($n = 5$); and only spatulation without resection was carried out or there was conversion to an onlay procedure or the specimen was too small for analysis ($n = 8$).

In 35 cases a mixed population of inflammatory cells containing diffuse B-cell (CD20) and T-cell (CD3) areas was observed. GCs were found subepithelially as well as deeper in the lamina propria. In the GCs, B- and T-cell areas, as well as FDC networks, were detected. The T cells were diffusely scattered in the stroma and at the periphery of the GCs, whereas there were only a few scattered B cells and they were more often observed in clusters with a tendency to form GCs. We only found fully formed GCs in six cases, despite preparation of extra sections from the tissue available. B cells were strongly positive for CD20 and more weakly positive for CD21, but were observed in the same area as the more strongly CD21-positive FDC networks (Fig. 1). GCs, including layers with B and T cells, were observed for six patients, and three of these six specimens displayed grade III fibrosis (Table 3). There was no significant difference in GCs between the most severe grade III fibrosis ($n = 14$) and grade I–II fibrosis (grade I, $n = 17$; grade II, $n = 14$) and hence there was no correlation between the degree of fibrosis and the abundance of immunohistochemically detected immune cells (Table 2). In the specimens with GCs, 30–50% of immune cells expressed CD3, 20–80% expressed CD20, and 25–90% expressed CD21 (Table 3).

4. Discussion

In this study, GCs were found in urethral resection specimens from 6/45 patients (8%) undergoing urethro-

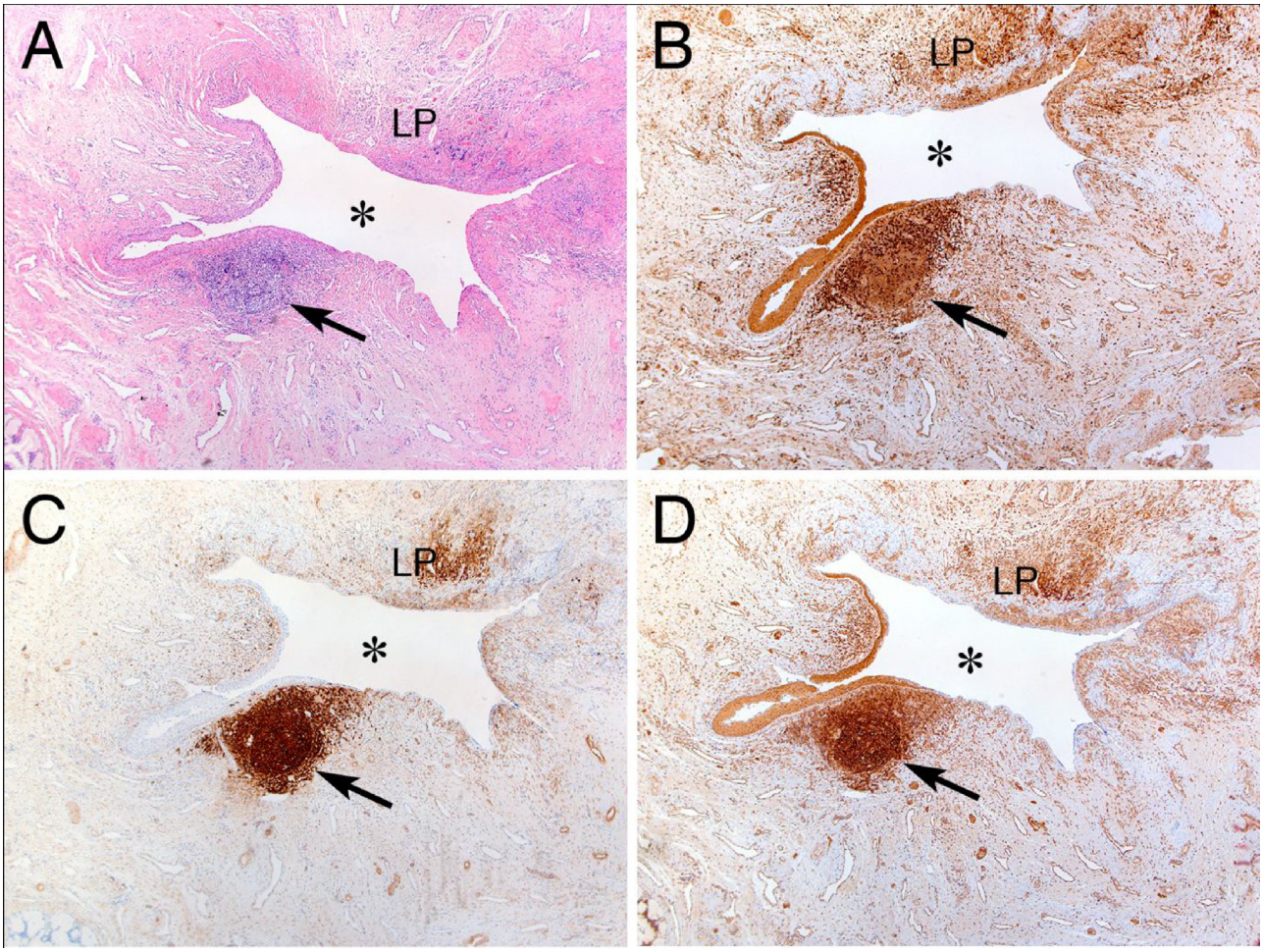


Fig. 1 – Cross-section of the urethral lumen (asterisk) with urothelium. There is diffuse chronic inflammation in the lamina propria (LP). The arrow denotes the formation of a lymphoid follicular centre (germinal centre). Magnification 100×. (A) Routine haematoxylin and eosin staining showing dark-stained chronic inflammatory cells in the LP. (B) CD3-positive cells are diffuse in the stroma and mostly at the periphery of the lymphoid follicle. (C) CD20-positive cells are present more focally in the stroma and in the centre of the lymphoid follicle. (D) CD21-positive cells are evident in the centre of the lymphoid follicle.

plasty for bulbar urethral stricture, an intriguing pathophysiological phenomenon never reported previously. These specimens displayed GC formation, which was sometimes subepithelial but also deeper in the lamina propria, with detectable clear CD21-positive FDC networks. There were also some sections with possible beginning of a GC, which may be explained by the cutting and preparation process for the sections. There was no significant difference in GC formation between grade III and grade I–II fibrosis.

In 35 cases in the present series, a mixed population of diffuse B (CD20) and T (CD3) inflammatory cells was seen in the histopathological specimen, with B cells being more abundant.

Table 3 – Fibrosis grade and T- and B-cell infiltration rate for the patients with germinal centres

Patient	Fibrosis grade	Stricture length (cm)	Infiltration (%)			Aetiology	Age at surgery (yr)	Follow-up (mo)
			T cell CD3	B cell CD20	FDC CD21			
Patient 1	III	0.5	30	70	90	Idiopathic	21	54
Patient 23	III	1.5	50	50	25	Trauma	54	24
Patient 24	III	1.0	40	60	60	Idiopathic	33	37
Patient 25	II	1.5	50	50	80	Infection	64	21
Patient 33	II	MD	50	80	80	Idiopathic	64	8
Patient 34	I	1.0	50	20	90	Idiopathic	34	12

FDC = follicular dendritic cell; MD = missing data.

Our findings of tertiary lymphoid structures and in some cases full-blown GCs indicate that inflammation in the urethra can induce the formation of tertiary lymphoid tissue. This was recently observed in chronic kidney disease [10]: patients with pyelonephritis more often had fully developed GCs. It is therefore possible that GC abundance could be more frequent among patients with intense sclerosis and stricture formation, although this hypothesis was not proven in our cohort.

We speculate that lymphoid aggregates may perhaps be formed, which in turn would start to produce antibodies, even in the absence of GCs. However, it is hard to predict whether antibody production in this case would be protective or harmful for the patient, but targeting of B cells with biological therapy could possibly be a new way to treat patients in order to avoid fibrosis and stricture formation.

In addition, it has been reported that interferon α and TGF-β1, which are secreted by macrophages and other cells, play a significant role in inflammatory and fibrotic conditions [16–18]. Injection of mesenchymal stem cells in an animal model (rats; $n = 12$) induced various cell phenotypes that played a role in immunosuppression, inhibition of TGF-β1, and reduction of oxidative stress to reduce urethral stricture formation and penile fibrosis [19,20]. Gul hypothesised that injection of platelet-rich plasma with TGF-β1 antibody during internal urethrotomy may provide superior healing [21].

Other treatments include oral steroids and injection of antifibrotic mitomycin C along the incision during internal urethrotomy as a safe option for patients with bulbar strictures unfit for open reconstruction [22,23]. The coated balloon technique, which combines balloon dilatation and concomitant drug delivery, was used in a nonrandomised clinical trial ($n = 53$) to deliver paclitaxel, which inhibits cell replication and limits fibrotic scar formation; the success rate at 1 yr was 70% [24].

The hypothesis that lichen sclerosus could be a cause of GCs in bulbar urethral strictures was challenged by a study that found that lichen is almost always situated in the penile urethra rather than in the bulbar region [25,26].

There are several limitations to this study. This was a small retrospective study and the consecutive patient approach meant that all aetiologies and idiopathic strictures were included. Maybe an additional blinded pathologist should have reviewed the specimens, which could have had enhanced the validation of the results. Twenty-six patients were excluded for various reasons, which is more than a third of all the consecutive patients in the study. This could possibly have affected the results. However, a review of the patients not included revealed similar demographics, including stricture aetiology, stricture length, and age, leading us to believe that there is no significant selection bias in our study.

Our study failed to demonstrate an association between the degree of fibrosis in urethral stricture specimens and the occurrence of GCs. It can be speculated that this is because the present series is fairly small, as previously mentioned. However, there was a trend towards such an association: one patient with grade I, two patients with grade II, and three patients grade III fibrosis had GCs (Table 3).

Of course, an alternative explanation would be that the development of fibrosis on one hand and the development of GCs on the other represent two separate pathophysiological events.

5. Conclusions

GCs do exist in bulbar urethral stricture disease. This finding might be of importance for future immunological studies in urethral stricture disease. From the results of the present study we cannot firmly conclude that there is an association between the degree of inflammation/fibrosis and the formation of GCs.

Author contributions: Teresa Olsen Ekerhult had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Olsen Ekerhult, Peeker, Grimsholm.
Acquisition of data: Olsen Ekerhult, Grimsholm, Kåbjörn Gustafsson.
Analysis and interpretation of data: Olsen Ekerhult, Grimsholm, Kåbjörn Gustafsson, Peeker.
Drafting of the manuscript: Olsen Ekerhult, JM, Grimsholm, Kåbjörn Gustafsson, Peeker.
Critical revision of the manuscript for important intellectual content: Olsen Ekerhult, Peeker, Grimsholm, Magnusson.
Statistical analysis: Olsen Ekerhult, Peeker.
Obtaining funding: Peeker, Olsen Ekerhult.
Administrative, technical, or material support: Kåbjörn Gustafsson.
Supervision: Peeker.
Other: None.

Financial disclosures: Teresa Olsen Ekerhult certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This study was supported by grants from the Anna-Lisa and Bror Björnssons Foundation, Gothenburg, Sweden, and the Märta and Gustaf Ågrens Research Foundation, Gothenburg, Sweden, and ALF/LUA research grants from Gothenburg University, Sweden. The sponsors played no role in analysis of the data.

Acknowledgments: The authors thank Ulric Pedersen of the Department of Pathology, Sahlgrenska University Hospital, Gothenburg, Sweden, for the excellent microphotographs.

References

[1] Anger JT, Buckley JC, Santucci RA, Elliott SP, Saigal CS. Trends in stricture management among male Medicare beneficiaries: underuse of urethroplasty? Urology 2011;77:481–5.
[2] Mundy AR, Andrich DE. Urethral strictures. BJU Int 2011;107:6–26.
[3] Santucci RA, Joyce GF, Wise M. Male urethral stricture disease. J Urol 2007;177:1667–74.
[4] Huang E, Peng N, Xiao F, Hu D, Wang X, Lu L. The roles of immune cells in the pathogenesis of fibrosis. Int J Mol Sci 2020;21:5203.

[5] Cavalcanti AG, Costa WS, Baskin LS, McAninch JA, Sampaio FJ. A morphometric analysis of bulbar urethral strictures. BJU Int 2007;100:397–402.
[6] Zhang P, Shi M, Wei Q, et al. Increased expression of connective tissue growth factor in patients with urethral stricture. Tohoku J Exp Med 2008;215:199–206.
[7] Cooper MD, Peterson RD, Good RA. Delineation of the thymic and bursal lymphoid systems in the chicken. Nature 1965;205:143–6.
[8] Schwickert TA, Victora GD, Fooksman DR, et al. A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center. J Exp Med 2011;208:1243–52.
[9] Zotos D, Tarlinton DM. Determining germinal centre B cell fate. Trends Immunol 2012;33:281–8.
[10] Sato Y, Boor P, Fukuma S, et al. Developmental stages of tertiary lymphoid tissue reflect local injury and inflammation in mouse and human kidneys. Kidney Int 2020;98:448–63.
[11] Gatto D, Brink R. The germinal center reaction. J Allergy Clin Immunol 2010;126:898–907.
[12] Ekerhult TO, Lindqvist K, Grenabo L, Kabjorn Gustafsson C, Peeker R. Sclerosis as a predictive factor for failure after bulbar urethroplasty: a prospective single-centre study. Scand J Urol 2018;52:302–8.
[13] Ekerhult TO, Lindqvist K, Peeker R, Grenabo L. Low risk of sexual dysfunction after transection and nontransection urethroplasty for bulbar urethral stricture. J Urol 2013;190:635–8.
[14] Sangkum P, Yafi FA, Kim H, et al. Collagenase Clostridium histolyticum (Xiaflex) for the treatment of urethral stricture disease in a rat model of urethral fibrosis. Urology 2015;86, 647.e1–6.
[15] Sangkum P, Gokce A, Tan RB, et al. Transforming growth factor-beta1 induced urethral fibrosis in a rat model. J Urol 2015;194:820–7.
[16] El-Sakka AI, Hassoba HM, Chui RM, Bhatnagar RS, Dahiya R, Lue TF. An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. J Urol 1997;158:2284–90.
[17] Border WA, Noble NA. Fibrosis linked to TGF-beta in yet another disease. J Clin Invest 1995;96:655–6.
[18] Delaney TA, Morehouse C, Brohawn PZ, et al. Type I IFNs regulate inflammation, vasculopathy, and fibrosis in chronic cutaneous graft-versus-host disease. J Immunol 2016;197:42–50.
[19] Castiglione F, Dewulf K, Hakim L, et al. Adipose-derived stem cells counteract urethral stricture formation in rats. Eur Urol 2016;70:1032–41.
[20] Milenkovic U, Albersen M, Castiglione F. The mechanisms and potential of stem cell therapy for penile fibrosis. Nat Rev Urol 2019;16:79–97.
[21] Gul M. Modified platelet-rich plasma with transforming growth factor β1 neutralization antibody injection may reduce recurrence rate of urethral stricture. Med Hypotheses 2016;97:1–3.
[22] Farrell MR, Lawrenz CW, Levine LA. Internal urethrotomy with intralesional mitomycin C: an effective option for endoscopic management of recurrent bulbar and bulbomembranous urethral strictures. Urology 2017;110:223–7.
[23] Gupta S, Roy S, Pal DK. Efficacy of oral steroids after optical internal urethrotomy in reducing recurrence of urethral strictures. Turk J Urol 2018;44:42–4.
[24] Mann RA, Virasoro R, DeLong JM, et al. A drug-coated balloon treatment for urethral stricture disease: two-year results from the ROBUST I study. Can Urol Assoc J 2020;14:187–91.
[25] Zhang X, Hu W. Re: Histological evidence of urethral involvement in male patients with genital lichen sclerosus: a preliminary report: G. Barbagli, F. Mirri, M. Gallucci, S. Sansalone, G. Romano and M. Lazzeri J Urol 2011;185:2171–6. J Urol 2012;187:1512.
[26] Grimes MD, Tesdahl BA, Schubbe M, et al. Histopathology of anterior urethral strictures: toward a better understanding of stricture pathophysiology. J Urol 2019;202:748–56.