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Kh.S. Ibishev, E.A. Mamedov, Z.R. Gusova, A.I. Palenyy, Y.O. Prokop

SERUM TESTOSTERONE AND TESTICULAR HEMODYNAMICS BEFORE AND AFTER INFECTION WITH SARS-COV-2 (PILOT STUDY)

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Introduction. The current epidemiological situation dictates a detailed study of the effect of a new coronavirus infection (SARS-CoV-2) on various anatomical, histological, physiological and biochemical parameters of the human body, which has become an integral part of many medical research, including urology, andrology, endocrinology.
Objective. To study serum testosterone levels and testicular hemodynamics before and after infection with SARS-CoV-2.
Materials and methods. 20 patients were examined, before and after suffering SARS-CoV-2 infection (who fell ill at the beginning of 2020). Average age 37.8 years (min=27, max=50). The level of total testosterone (Ts) in the blood serum and hemodynamic parameters of the testicles (maximum and minimum blood flow velocity and resistance index) were studied.
Results: When studying the level of Ts 3 months after the transferred SARS-CoV-2, its decrease from the initial value was noted ($r=0.47$; $p=0.35$), and after 6 months ($r=0.98$; $p<0.007$) there was no recovery of the target level of Ts before infection with SARS-CoV-2. Regarding the hemodynamics of the testicles, there was also a decrease in Vmax and Vmin, and an increase in RI, which indicates a deterioration in the blood flow of the testicles ($p<0.001$).
Conclusion: SARS-CoV-2 affects the level of Ts in the blood serum and the parameters of the hemodynamics of the testicles. The extent to which SARS-CoV-2 affects patient performance depends largely on the severity of the disease and to a lesser extent on baseline performance.

K e y w o r d s: *coronavirus; COVID-19; SARS-CoV-2; testosterone; testicles; hemodynamics of testicles*

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Introduction. Diagnostics, treatment and follow-up of men with testosterone deficiency have become a very important issue for many medical specialties, including urology, andrology, endocrinology and others [1, 2].

Endothelial dysfunction is one of the causes for a decrease in serum testosterone level (Ts). It can be due to metabolic syndrome (MS), which is associated with visceral obesity, cardiovascular diseases, type 2 diabetes mellitus, chronic obstructive pulmonary disease, etc. However, it is generally accepted that MS ranks first among all factors contributing to the development of endothelial dysfunction, and thereby causes a decrease in the level of Ts. The main pathogenetic factors include deterioration of metabolic processes occurring in visceral adipose tissue, as well as their multifactorial effect on the endothelium as an endocrine organ. In recent years, dysfunction of the immune system has been studied as another very important pathogenetic mechanism of MS, significantly affecting the development of endothelial dysfunction. This MS-mediated etiological component of testosterone deficiency is based on the initiation of a number of pathochemical and pathophysical processes, among which the activation and an imbalance of the cytokines, especially proinflammatory ones (IL-6, TNFα, CRP) play an important role. That is why all these mechanisms often form a vicious circle in the pathogenesis of metabolic disorders, hypogonadism, and sometimes male infertility, which is very difficult to break [3].

In addition, it is known that chronic obstructive pulmonary disease is also one of the risk factors for endothelial dysfunction and Ts deficiency. The pulmonary ischemia seems to be the primary triggering mechanism, which subsequently contributes to the development of systemic endothelial dysfunction [2].

Thereby, the pathogenetic effect of all these disturbances is based on the development of endothelial and immune system dysfunction, which provokes cascading inflammatory reactions in the vascular wall, causing both local ischemia in the organs of the reproductive system and systemic hemodynamic failure [4]. In addition, it has been proven that whatever the cause, endothelial dysfunction and Ts deficiency have mutually reinforcing negative effect.

It is now generally known that SARS-CoV-2 is also one of the powerful triggering factors that cause dysfunction of the immune and endothelial part of the respiratory system [5].

The main target cells for SARS-CoV-2 are alveolar epithelial cells, in the cytoplasm of which viral replication occurs. After assembly, virions pass into cytoplasmic vacuoles and migrate to the cell membrane, where they are released into the extracellular space by exocytosis. Expression of virus antigens on the cell surface before the release of virions does not occur, therefore, antibody formation and synthesis of interferons at this moment are also not observed and start relatively late. Syncytium formation under the influence of SARS-CoV-2 facilitates rapid penetration of the virus into unaffected areas

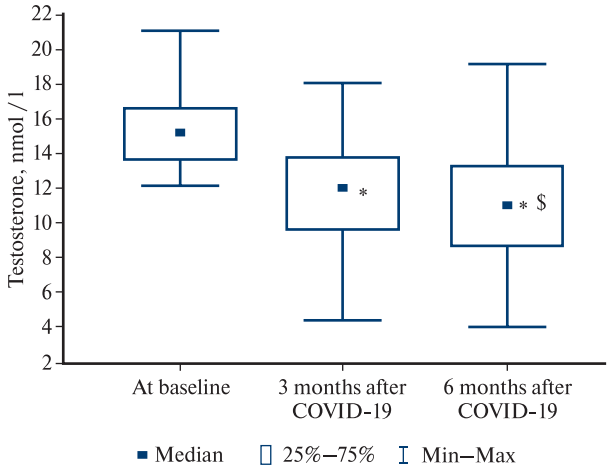

Fig. 1. Dynamics in changes of serum total testosterone after COVID-19 vs. baseline

of the lungs. The virus causes an increase in the permeability of cell membranes and transport of protein-rich filtrate into the lung interstitial tissue and alveolar lumen. It leads to the destruction of surfactant with subsequent alveolar collapse; as a result, acute respiratory distress syndrome (ARDS) may develop due to violation of gas exchange. The immunosuppressive state is associated with increased risk of opportunistic bacterial and mycotic infections of the respiratory tract. There are currently no data on the duration and intensity of immunity for SARS-CoV-2. The effect of the virus on endothelial and epithelial cells of other organs and systems has not been studied, as there is no more detailed analysis of the pathogenesis of the new type of coronavirus infection. Certainly, by causing dysfunction of endothelium of respiratory and immune systems, SARS-CoV-2 can also lead to endothelial dysfunctions in other organs, in particular the cardiovascular and reproductive systems. In this regard, it is very important to study more thoroughly the effect of the virus and its consequences on other organs and systems, including the male reproductive system [5].

Aim. To study serum total testosterone level and testicular hemodynamics before and after COVID-19.

Materials and methods. A total of 20 patients were examined at the beginning of 2020 before and after the COVID-19. The average age of patients was 37.8 years (min = 27, max = 50). The level of Ts and hemodynamic parameters of testicles (maximum [V_{max}] and minimum [V_{min}] blood flow velocity and resistive index [RI]) were evaluated. Hemodynamic parameters were determined in the parenchymal arteries of the testicles by scrotal Doppler examination on the machine PhilipsEnVisor CHD with a linear transducer in a pulsed mode. The analysis of Ts was carried out on a Rochecobas 6000 analyzer (Switzerland) by immunoassay method (Rochediagnostics-Germany). Before the COVID-19, all patients underwent a routine examination by a urologist for infertility, including a study of the hormonal status and scrotal Doppler.

The examination was performed 3 and 6 months after COVID-19. All patients with mild and moderate course of COVID-19 received similar treatment. Hormonal and immunosuppressive drugs were stopped, while recombinant interferon alfa 3 drops per side of nose (3000 IU) 5 times a day for 5 days, Umifenovir 200 mg T.I.D. for 5-7 days, Vitamin C 500 mg QD, IV Ceftriaxone 2000 mg B.I.D. for 14 days, Thrombo ASS 50 mg B.I.D. for 30 days, paracetamol 200 mg for high fever were prescribed.

Statistical analysis was carried out using the statistical package STATISTICA 6.1 (StatSoft Inc., USA). Normality was checked using the Shapiro-Wilk test. Due to the deviation from the normal distribution of most of the parameters, the median (Me) was used. In addition, variability was assessed using interquartile range (Q1; Q3). To analyze the significance of differences between time points Wilcoxon test for dependent samples was done. Bonferroni correction was used to counteract the problem of multiple comparisons between values at three stages of the study; when evaluating the results, an adjusted level of significance was used: $p<0.05/3 = 0.017$. The interrelation of the values at different stages was assessed using Spearman's rank correlation coefficient. Legend: * - significant differences in values vs. baseline ($p<0.017$), $ - significant differences in indicators vs. values at 3 months after SARS-COV-2 ($p<0.017$). The study was not sponsored. The authors declare that they have no conflicts of interest. The study was approved as part of the Ph.D. «Assessment of the effect of SARS-COV-2 on the reproductive potential of men with idiopathic infertility» (approved by the NLEC of FGBOU VO RostGMU, Ministry of Health of the Russian Federation, October 22, 2021).

Results and discussion. The analysis of total testosterone level and hemodynamic features of the intratesticular arteries revealed a significant decrease in Ts and a deterioration in the testicular blood flow compared to baseline (*Tables 1 and 2*).

When evaluating Ts level after COVID-19, a decrease from the baseline after 3 months was noted (r=0.47; p=0.35). In addition, 6 months after disease no normalization of Ts level occurs (r=0.98; $p<0.007$) compared to baseline. The analysis of the testicular hemodynamics also revealed a decrease in V_{max} and V_{min}, as well as an increase in RI, which indicates a deterioration in the testicular blood flow ($p<0.001$). Our data indicate a long-term persistent effect of coronavirus infection on the reproductive system even after 6 months; moreover, in some patients, there is a progressive decrease in the parameters. For example, we observe a continuing decrease in the serum Ts level by 6 months after COVID-19 (*Fig. 1*).

Patients with coronavirus infection prove to have a decline in the number of Leydig and Sertoli cells [6], which are also responsible for the synthesis of Ts. Consequently, observed decrease in total Ts after 3 and 6 months is caused precisely by

Table 1

Serum testosterone level

Characteristics	Me (Q1 Q3)	Mean (M)	Min	Max
Ts (nmol/l)		Baseline		
	15,3 (13,7 16,7)	15,5	12,2	21,1
		After 3 months		
	12,1 (9,7 13,9)	11,7	4,5	18,1
		After 6 months		
	11,1 (8,8 13,4)	11,1	4,1	19,2

death of endocrine cells. Thereby, our work complements other researches on this issue.

It should be noted that there is no persistence of the virus in the testicular tissue, which is explained by the absence of the ACE2 receptor and TMPRSS2 serine protease in the membrane of testicular cells (including spermatozoa), through which the coronavirus invades a cell [6, 7]. Alveolar epithelium is the main target for coronaviruses. When the surfactant is destroyed, alveoli collapse, and violation of gas exchange results in ARDS, which in turn leads to oxidative stress and an increase in the level of reactive oxygen species. Therefore, the effect of the SARS-CoV-2 on the testes may be indirect. It is also known that with a mild course of COVID-19, sperm parameters remain normal, while in case of moderate course, pronounced changes develop: the average sperm concentration is 16.2 versus 95.9 million/ml in patients with mild course of the disease [8]. However, it is not yet clear how persistent deteriorations are.

The vascular endothelium is a target for Ts, which has antiatherogenic and vasoprotective effects. Low and low-normal Ts levels have a negative impact on numerous factors of cardiovascular risks, such as insulin resistance, diabetes mellitus, obesity, etc., which in turn serve as one of the causes for the decrease in Ts; this creates a vicious circle [2].

Ts enhances the production and release of endothelial NO, which is a powerful vasodilator [2]. A low level of Ts may cause a decrease in the vasodilating effect, therefore, the vascular resistance increases, which was observed in our study. At the same time, by the 6th month after COVID-19, an even greater increase in RI was observed (*Fig. 2*). It is also known that physiological NO antagonists have endothelial origin, including endothelin-1 and angiotensin II (ATII), which are both powerful vasoconstrictors. Angiotensin-converting enzyme (ACE) is expressed in the vascular endothelium. In case of endothelial injury, ACE expression increases, which in turn leads to an increase in ATII synthesis. In addition, vascular tone,

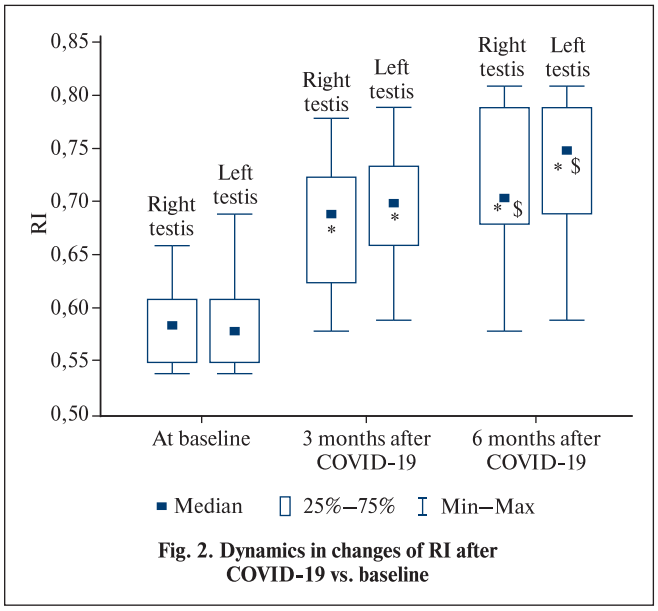

Fig. 2. Dynamics in changes of RI after COVID-19 vs. baseline

proliferation of vascular wall components, thrombus formation and oxidative stress enhance [4]. Endothelin-1, in turn, triggers a cascade of events that can lead to the development of such serious complications as myocardial infarction, heart failure, etc.

As discussed earlier, SARS-CoV-2 has a high affinity for ACE2 receptors, which results in an imbalance in the renin-angiotensin system, leading to vasoconstriction and the development of pro-inflammatory state. The endothelium is one of the primary targets for SARS-CoV-2. Some authors even use the term «endotheliitis» [9]. Expression of ACE2 on the endothelium and vascular smooth muscle cells after SARS-CoV-2 invasion contributes to the involvement of

Table 2

Hemodynamic parameters in parenchymatous organs

Characteristics		Me (Q1 Q3)	Mean (M)	Min	Max
		Baseline			
Right testis	Volume (cc)	19,0 (16,8 21,0)	19,3	14,8	26,4
	V_{max} (cm/s)	12,6 (11,0 13,2)	12,3	8,0	18,0
	V_{min} (cm/s)	5,6 (4,0 6,3)	5,5	3,0	8,0
	RI	0,58 (0,55 0,61)	0,59	0,54	0,66
Left testis	Volume (cc)	17,7 (15,9 20,1)	17,8	13,0	23,1
	V_{max} (cm/s)	11,8 (9,5 13,4)	11,6	5,0	16,0
	V_{min} (cm/s)	5,8 (4,5 6,2)	5,3	1,0	8,0
	лев, RI	0,58 (0,55 0,61)	0,59	0,54	0,69
		After 3 months			
Right testis	Volume (cc)	19,0 (16,5 20,5)	19,1	14,8	26,4
	V_{max} (cm/s)	9,3 (8,0 11,0)	9,9	7,0	18,0
	V_{min} (cm/s)	4,0 (3,2 5,0)	4,1	2,0	7,0
	RI	0,69 (0,63 0,72)	0,68	0,58	0,78
Left testis	Объем ($см^3$)	17,7 (15,9 20,1)	17,8	13,0	23,1
	V_{max} (cm/s)	9,1 (7,5 11,5)	9,5	5,0	16,0
	V_{min} (cm/s)	4,2 (2,5 5,0)	3,9	1,0	6,5
	RI	0,70 (0,55 0,73)	0,70	0,59	0,79
		After 6 months			
Right testis	Volume (cc)	19,0 (16,2 20,3)	18,8	14,8	26,1
	V_{max} (cm/s)	9,0 (7,0 10,0)	9,1	6,0	18,0
	V_{min} (cm/s)	4,0 (3,0 5,0)	3,8	2,0	6,0
	RI	0,72 (0,68 0,79)	0,72	0,58	0,81
Left testis	Volume (cc)	17,5 (15,5 19,6)	17,6	12,9	23,0
	V_{max} (cm/s)	9,0 (7,0 10,0)	9,0	5,0	16,0
	V_{min} (cm/s)	4,0 (2,5 4,9)	3,6	1,0	6,0
	RI	0,75 (0,69 0,79)	0,73	0,59	0,81

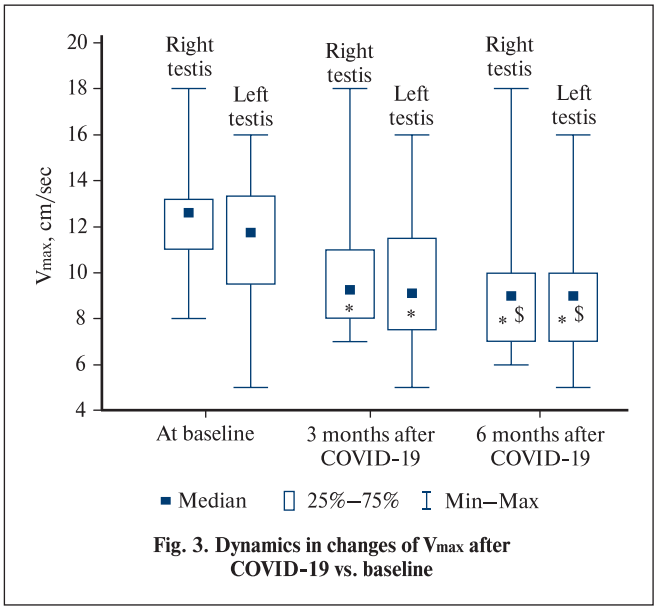

Fig. 3. Dynamics in changes of V_{max} after COVID-19 vs. baseline

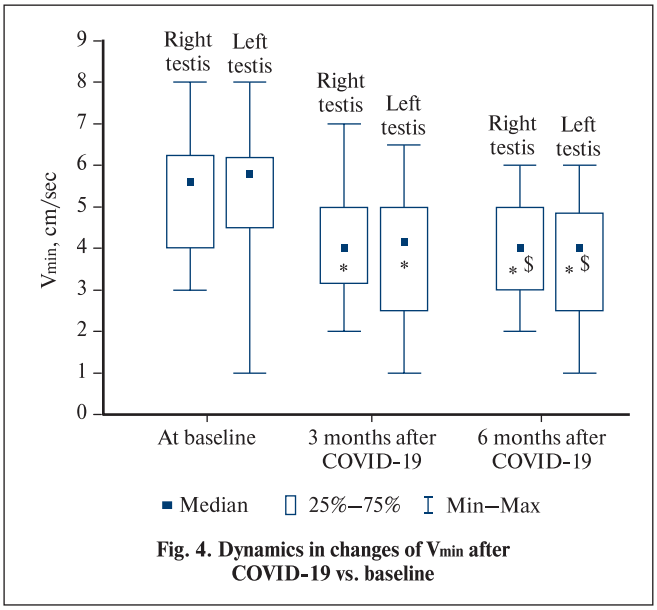

Fig. 4. Dynamics in changes of V_{min} after COVID-19 vs. baseline

the cardiovascular system in systemic disorder, and is also accompanied by endothelial damage, endothelial dysfunction and perivascular inflammation, which further enhances endothelial damage and leads to microcirculation dysfunction in various organs [10]. These data are confirmed in our research. A decrease in V_{max}, V_{min} and an increase in RI were noted, which indicates a decline in testicular blood flow.

The higher the vascular resistance, the more negative its influence on the endothelium, which in turn has an effect on its function (*Fig. 2*). The increased vascular resistance also results in decreased blood flow, which predisposes for the development of various complications such as testicular hypotrophy, varicocele and infertility (*Fig. 3, 4*).

Our results and published data prove the role of viral infection in the pathogenesis of many disorders of the urinary tract and reproductive organs, which requires the development of standards for the diagnosis and treatment of viral genitourinary tract infection [11, 12].

The SARS-CoV-2 pandemic has become a serious challenge for the global healthcare system and even today, more than a year after the first cases of the COVID-19 were detected, it remains one of the most serious problems in the world, since, owing its pathogenic properties, the virus has negative impact on many organs and systems [12, 13].

Conclusions. COVID-19 has a long-term, and in some cases a progressive negative effect on the level of Ts and hemodynamic parameters of the testes, which serve as a risk factor for the development of serious, including life-threatening, diseases, and dictates the need for long-term monitoring of patients after recovery. SARS-CoV-2 certainly affects various parts of the reproductive system, which requires further researches and search for possible ways to prevent and treat complications.

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PERINEAL AND PENILE URETHROSTOMY: SURGICAL OUTCOME AND RISK ASSESSMENT OF COMPLICATIONS

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Introduction. Urethral reconstruction in the complex urethral strictures is associated with a high risk of failure. In some cases, urethrostomy is justified when choosing a method of treatment for this category of patients.
Aim. To assess the results of perineal and penile urethrostomy and identify factors associated with the development of early surgical complications and urethrostomy stenosis.
Materials and methods. 85 patients aged 53.9 years underwent urethrostomy from 2010 to 2019: permanent – 48 (56.5%), due to refusal of urethroplasty – 37 (43.5%). Penile urethrostomy was formed in 41 (48.2%) patients, perineal urethrostomy – in 44 (51.8%), respectively. The urethral stricture etiology was as follows: inflammatory – 32.9%, iatrogenic – 29.4%, idiopathic – 28.2%, traumatic – 9.4%. The urethral strictures length was 6 cm or more in 58.8% patients, multifocal stricture occurred in 22.4% cases, subtotal – in 28.2%. The criterion for successful treatment was the absence of complications requiring repeated surgery on the urethra and/or systematic urethral dilation (median follow-up – 58 mo). The contribution of various factors to the increased risk of developing urethrostomy stenosis was assessed using univariate analysis by calculating the odds-ratio (OR, 95% CI). Statistical significance was tested using the χ^2 test, Fisher's test. Multivariate analysis was performed using logistic regression.
Results. Early postoperative complications were detected in 7 (8.2%) patients [urethritis (2), wound purulent infection (2), scrotal hematomas (1), unsuccessful trial of void (1), acute urinary retention (1)]. Of these, only 2 (2.4%) cases required additional surgical intervention. Urethrostomy stenosis was detected in 14 (16.5%) patients during the follow-up period from 3 to 200 mo (median - 8 mo). Surgical correction was required in 12 (14.1%) cases, systematic urethral dilation – in 2 (2.4%) cases. Independent risk factors for all complications were urinary tract infection (OR=3.3; 95% CI – confidence interval (CI)=1,17 – 9,1; p=0.013), arterial hypertension (OR=2.3; 95% CI=1.02 – 5.05; p=0.044), prior urethral dilation (OR=2.4; 95% CI=1.08 – 5.33; p=0.031), the multifocal stricture (OR=2.8; 95% CI=1.28 – 6.05; p=0.011), and for stenoses, in particular, urinary tract infection (OR=6.1; 95% CI=1.45 – 25.22; p=0.003), arterial hypertension (OR=2.6; 95% CI=1.05 – 6.40; p=0.035), failed hypospadias repair (OR=3.3; 95% CI=1.27 – 8.55; p=0.031) and early postoperative complications (OR=4.1; 95% CI=1.74 – 9.41; p=0.004). The combination of unfavorable factors identified in multivariate analysis determines the risk of early and late complications in 32.1-49.9% of cases.
Conclusion. Urethrostomy may be the ultimate treatment for complex urethral stricture with an 82.4% primary success rate. The main factors negatively affecting the surgery outcomes are arterial hypertension, chronic kidney disease, multifocal stricture, prior urethral dilation, failed hypospadias repair, urethrocutaneous fistulas and early postoperative complications.

K e y w o r d s: *urethral stricture, urethrostomy, early complications, urethrostomy stenosis, risks of complications*

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Introduction. Urethrostomy is traditionally considered as a procedure, which is indicated, if there are no other options for urethral reconstruction [1-3]. It is also considered in elderly men and/or patients with comorbidities precluding prolonged anesthesia [3]. In addition, urethrostomy is necessary in case of traumatic amputation of the penis and malignant tumors of the urethra and penis [4, 5].

However, in the past decade, a role of urethrostomy as a rational choice of treatment for men with extended (panurethral), recurrent anterior urethral strictures, as well as patients with advanced lichen sclerosus and strictures developed after failed hypospadias surgeries has been increasingly discussed [6–9].

This point of view is largely explained by the results of treatment and the degree of patient satisfaction. For example, a study conducted at the Lahey Hospital (USA) with an average follow-up period of 32.4 months showed that among patients with urethral stricture associated with lichen sclerosus the highest success rate was observed after perineal urethrostomy (93%), while after one- and two-stage buccal urethroplasty success rates were 75 and 76%, respectively [10]. Similar data were presented by A.C. Peterson et al. [11], who argued that perineal urethrostomy may be a better choice for patients with long urethral strictures associated with lichen sclerosus than staged reconstructive surgery. At the same time, the authors noted that all patients had satisfactory quality of life. A study

by G. P. Murphy et al. [8] confirmed that urethrostomy, relieving obstructive symptoms without adversely affecting erectile function, provided comparable satisfaction with similar functional outcomes with patients who underwent anterior urethroplasty. At the same time, the frequency of recurrence of the disease after 2 years of observation in the urethroplasty group was 30.2% in comparison with 14.5% after urethrostomy group.

After studying the trend of using various surgical techniques for complex strictures of the anterior urethra in a specialized center in Utah (USA), J. S. Fuchs et al. [12] showed a continuous growth of the proportion of urethrostomies from 2008 to 2017 from 4.3 to 38.7%, increasing almost 10-fold. The authors also emphasize that, at a median follow-up of 50.7 months, success rates were higher among patients with urethrostomies (94.8%) compared with those who underwent urethroplasty using buccal graft and local tissue flaps (78.5 and 78.2% respectively), even though patients undergoing urethrostomy were older and had more extensive strictures.

Another indirect reason for reassessment of the role of urethrostomy in the treatment of urethral strictures was the development and widespread use of staged procedures [13-15].

In this situation, urethrostomy, performed as the first stage, often becomes the ultimate form of urinary diversion due to patient satisfaction with urination and quality of life. In fact, only 24–58% of respondents undergo the second stage [2, 11, 16, 17].

Finally, many patients, in particular those with recurrent strictures, who have undergone repeated procedures, not willing to undergo a long-term urethral reconstruction and choose a simpler solution, namely urethrostomy [18].

Nevertheless, despite all the positive aspects of the urethrostomy, there are risks of developing both early and late postoperative complications, including one of the most common, urethrostomy stenosis.

Aim. To evaluate the results of perineal and penile urethrostomy, as well as to identify factors associated with the development of early surgical complications and urethrostomy stenosis.

Materials and methods. In 2010–2019 a total of 754 patients underwent surgical treatment of urethral strictures, 85 (11.3%) of them underwent urethrostomy. In 48 (56.5%) cases, urethrostomy was used as the definitive treatment for stricture disease (permanent), in 37 (43.5%) cases, urethroplasty was subsequently planned, but it was not performed due to the patient's refusal (94.6%) or the development of life-threatening complications of concomitant diseases (5.4%). The clinical characteristics of patients are presented in table 1.

In almost half of cases (51.8%), perineal urethrostomy was performed, while in remaining patients neomeatus was created on the penile shaft.

Surgical technique. When performing perineal urethrostomy, dorsal urethral wall was not transected. The patient lied in the lithotomy position. A vertical median perineal incision from the posterior semicircle of the scrotum to the upper edge of the anal sphincter was done. If subsequent urethroplasty was planned, the ventral urethral wall was transected along the entire length of the stricture with extending incision in the proximal direction for 1.5–2.0 cm. After that, to exclude the proximal urethral structure or bladder neck sclerosis, the urethra was calibrated with a 24–28 Ch metal sound and urethrocystoscopy was performed. Then the edges of the longitudinally transected urethra in the area of stricture and proximal part of the incision were sutured to the perineal skin with interrupted sutures (marsupialization). The urethral catheter was inserted through the neomeatus into the proximal part of the urethra. If perineal skin had limited elasticity, a Z-plasty technique was used in the lower part of the perineal incision to create a tension-free urethrostomy. In the case of the permanent urethrostomy, area of the stricture was not transected longitudinally, and the incision along the ventral surface of the urethra was made only in proximal part. When performing penile urethrostomy, Johanson technique with urethral marsupialization was also used.

Postoperative period and follow-up. The urethral catheter was removed on days 4–7 after surgery. Follow-up examinations were carried out after 3, 6 and 12 months during the first year and then annually. Examinations included urinalysis and uroflowmetry with measurement of residual urine. In patients with urinary disturbances, a decrease in Qmax (< 12 ml/s), or the amount of residual urine > 100 ml, retrograde urethrography was performed, as well as urethrocystoscopy, if indicated. In case of worsened urination, patients underwent standard examination in order to identify stricture recurrence.

Statistical analysis. The analysis was carried out using the SPSS Statistics v. 17.0. To calculate descriptive statistics, frequency for qualitative data and mean (M), standard deviation (SD), minimum (min), and maximum (max) values for quantitative data were used. For the univariate model of complications/urethrostomy stenosis, relative risk was calculated for qualitative variables. In addition, analysis of variance was performed for quantitative variables. Chi-square test and Fisher's test were used for checking significance of differences. Multivariate logistic regression analysis was also done.

Results. Early surgical complications developed in 7 (8.2%) patients (*Table 2*). In men with infectious and inflammatory complications, antibiotic therapy was augmented, and in two of these cases urethrostomy stenosis subsequently developed. One patient had acute urinary retention after removal of the urethral catheter, which required reconstruction of the neomeatus. Another patient with reduced bladder volume after restoring urination through a perineal urethrostomy refused spontaneous urination due to increased frequency and retained the cystostomy.

During follow-up of 3 to 200 months (median 8 months) urethrostomy stenosis was diagnosed in 14 (16.5%) cases. In 6 (42.9%) patients penile urethrostomy stenosis was seen, while in 8 (57.1%) men the stenosis of perineal urethrostomy developed. Surgical reconstruction was performed in 12 (14.1%) patients, and in three of them restenosis occurred over a period of 4–48 months after the first revision. Two (2.4%) patients received recommendations to perform systematic dilation of urethrostomy.

Thus, early and/or late urethrostomy stenosis occurred in 18 (21.2%) patients. At the same time, early postoperative complications and urethrostomy stenosis were observed in 3 (3.5%) men. The risk of surgical failure was studied among patients who experienced either early postoperative complications, or urethrostomy stenosis, or both.

Univariate analysis (*Table 3*) revealed an increased risk of complications (early and late) in patients with UTI (odds ratio [OR 3.3], p=0.013), arterial hypertension (OR 2.3, p=0.044), prior urethral dilation (OR 2.4, p=0.031) and multifocal stricture (OR 2.8, p=0.011). Other factors did not have a significant influence on the development of complications.

Multivariate analysis using a logistic regression showed that a combination of factors such as the patient age (every year reduces the risk by 0.924 (p<0.01)), urethra-cutaneous fistulas, hypertension, a history of urethral dilation, chronic kidney disease, multifocal strictures and failed hypospadias repair, determines the risk of early and late complications in 32.1-49.9% of cases (*Table 4*). The significance of the model was confirmed by chi-square test (p<0.0001).

Independent risk factors for the development of urethrostomy stenosis were also studied. Univariate analysis revealed that the

Table 1

Clinical features of the patients

Characteristics		Value	
Quantitative, $M \pm S$ (min–max)			
Age, years		53,86±14,30 (18,00 – 88,00)	
Body mass index, kg/m^2		26,36 ±4,24 (18,70 – 37,20)	
Stricture length, cm		7,04±3,68 (1,00 – 18,00)	
Duration of the disease, years		8,02±8,99 (0,30 – 40,00)	
Number of prior operations on the urethra		1,20±1,64 (0,00 – 7, 00)	
Number of comorbidities		3,74±2,94 (0,00 – 14,00)	
Qualitative, *n* (%)			
	UTI	44	(51,8%)
	Urethrocutaneous fistulas	4	(4,7%)
	Balanitis xerotica obliterans	4	(4,7%)
	Chronic kidney disease	9	(10,6%)
	BPH and/or bladder neck stenosis	19	(22,4%)
	Hypertension	26	(30,6%)
	Coronary artery disease	15	(17,6%)
	Diabetes	11	(12,9%)
	Obesity	12	(14,1%)
	Chronic hepatitis (B, C)	6	(7,1%)
Etiology of the stricture	inflammatory	28	(32,9%)
	iatrogenic	25	(29,4%)
	posttraumatic	8	(9,4%)
	idiopathic	24	(28,2 %)
	Primary stricture	44	(51,8%)
	Recurrent stricture	41	(48,2%)
	Prior endoscopic procedures	11	(12,9%)
	Prior open surgeries	22	(25,9%)
	A history of both types of procedures	7	(8,2%)
	Prior reconstruction of hypospadias	6	(7,1%)
	Prior urethral dilation	25	(29,4%)
Location of the stricture	penile	30	(35,3%)
	bulbar	14	(16,5%)
	penobulbar	22	(25,9%)
	multifocal	19	(22,4%)

Note: BPH – benign prostatic hyperplasia, UTI – urinary tract infection.

risk of stenosis was increased in case of UTI by 6 (p=0.003), hypertension – 2.6 (p=0.035) and stricture after failed hypospadias repair – 3.3 (p=0.031). Also, development of early postoperative complications was associated with an increased risk of urethrostomy stenosis by 4 (p=0.004; Table 5).

In table 6 the results of the multivariate analysis of the risk of developing urethrostomy stenosis using a logistic regression model with the dependent variable "presence of stenosis" are shown. A combination of factors, such as urethrocutaneous fistulas, hypertension, failed hypospadias repair and early postoperative complications contributes to 21.8-36.0% of cases of urethrostomy stenosis. The significance of the model was confirmed by chi-square test (p<0.0001).

Discussion. In the present study, we defined primary treatment success as the absence of complications requiring repeated urethral surgery and/or systematic urethral dilation. Thus, with an average follow-up period of 58 months success rate was 81.2% (70/85), while urethrostomy stenosis was diagnosed in 16.5% (14/85) of cases, which corresponds to the literature data, according to which the incidence of stenosis varies from 0 to 30 % [2, 3, 7, 8, 10, 11, 12, 16–21]. Such a wide range may be due to the heterogeneity of patients'

Table 2

Early postoperative complications

Complications		Treatment tactics	Number of patients (%)
Clavien-Dindo I	Unsuccessful trial of void	Continuous diversion by cystostomy	1 (1.2)
Clavien-Dindo II	Urethritis	Antibacterial therapy	2 (2.4)
	Wound purulent infection	Antibacterial therapy	2 (2.4)
Clavien-Dindo IIIa	Scrotal hematoma	Surgical drainage	1 (1.2)
Clavien-Dindo IIIb	Acute urinary retention	Neomeatoplasty	1 (1.2)

Table 3

Univariate risk assessment of early and late complications

Qualitative variables	Relative risk	95% confidence interval	*p**
UTI	3.26	1.17–9.1	0.013
Urethrocutaneous fistulas	2.53	0.87–7.4	0.148
Balanitis obliterans ■	–	–	–
Chronic kidney disease	1.69	0.6–4.7	0.345
BPH and/or bladder neck stenosis	1.34	0.55–3.3	0.534
Hypertension	2.27	1.02–5.05	0.044
Coronary artery disease	1.33	0.51–3.49	0.566
Diabetes	0.84	0.22–3.17	0.794
Obesity	1.74	0.69–4.4	0.266
Chronic hepatitis (B, C) ■	–	–	–
Primary stricture	0.9	0.7–1.1	0.146
Recurrent stricture	1.4	0.6–3.2	0.416
Prior endoscopic procedures	1.35	0.46–3.9	0.596
Prior open surgeries	0.82	0.3–0.22	0.69
A history of both types of procedures	2.23	0.85–5.87	0.143
Prior reconstruction of hypospadias	2.63	1.05–6.6	0.073
Prior urethral dilation	2.4	1.08–5.33	0.031
Inflammatory stricture	1.02	0.43–2.43	0.968
Iatrogenic stricture	1.23	0.47–3.25	0.678
Posttraumatic stricture	1.07	0.45–2.56	0.87
Idiopathic stricture	0.51	0.16–1.6	0.219
Penile stricture	1.7	0.7–4.1	0.229
Bulbar stricture	0.87	0.35–2.2	0.771
Penobulbar stricture	1.07	0.45–2.6	0.827
Multifocal stricture	2.78	1.28–6.05	0.011
Penile urethrostomy	1.47	0.61–3.54	0.383
Perineal urethrostomy	0.68	0.28–1.64	0.383
Quantitative variables	Class mean (patients with complications)	Class mean (patients without complications)	*p*
Age, years	49.33	55.07	0.131
Body mass index, kg/m^2	27.17	26.14	0.366
Stricture length, cm	7.86	6.81	0.286
Duration of the disease, years	7.16	8.25	0.649
Number of previous urethral procedures	1.61	1.09	0.233
Number of comorbidities	4.5	3.54	0.219

Note. Here and in the table 5 * significance was checked using the chi-square test and Fisher's test.
■ – absence of complications despite a presence of the risk factor.

groups, as well as different follow-up periods. More than 10 years of experience at Duke University (USA) has shown favorable outcomes of urethrostomy in all cases during 38.5 months of follow-up. None of the patients required repeated surgery or urethral dilation [11]. However, in this study, only 18% of patients had a history of failed urethral surgery, which differs significantly from our group and studies carried out by J. B. Myers et al. [18], G. Barbagli et al. [2] and S. Kulkarni et al. [19]. The proportion of recurrent strictures in those publications was 48.2, 48, 52.6 and 96.3%, respectively, while urethrostomy stenosis developed in 16.5, 18, 30 and 28% of cases, respectively. In addition, with the exception of the work of J. B. Myers et al. [18], in other studies follow-up period was longer, ranging from 58 to 62 months.

Table 4

Multivariate risk assessment of early and late complications

Variable	Coefficient	Standard error	*p*	Odds ratio
Age, years	-0.079	0.031	0.01	0.924
Urethrocutaneous fistulas (no)	3.192	1.526	0.036	24.343
Hypertension (no)	2.15*	0.855	0.012	8.586
Urethral dilation (no)	2.463	0.895	0.006	11.745
Multifocal strictures (no)	1.895	0.791	0.017	6.652
Stricture after failed hypospadias repair (no)	3.036	1.230	0.014	20.826
Chronic kidney disease (no)	3.267	1.225	0.008	26.240
Constant	-0.567	1.433	0.693	0.567
Chi-square for the model		32.96	*p*	<0.0001
Cox and Snell R2				0.321
Nagelkerke's R2				0.499
n				85

Note. Here and in *Table 6* reference categories are indicated in brackets.

Table 5

Univariate risk assessment of urethrostomy stenosis

Qualitative variables	Relative risk	95% confidence interval	*p**
UTI	6.057	1.454–25.224	0.003
Urethrocutaneous fistulas	3.115	1.038–9.352	0.082
Balanitis obliterans ■	–	–	–
Chronic kidney disease	2.111	0.732–6.092	0.192
BPH and/or bladder neck sclerosis	1.737	0.675–4.466	0.261
Hypertension	2.593	1.051–6.400	0.035
Coronary artery disease	1.967	0.625–4.610	0.313
Diabetes	1.035	0.269–3.981	0.960
Obesity	2.212	0.840–5.823	0.124
Chronic hepatitis ■	–	–	–
Primary stricture	0.947	0.776–1.156	0.592
Recurrent stricture	1.286	0.512–3.228	0.592
Prior endoscopic procedures	1.682	0.563–5.028	0.369
Prior open surgeries	0.441	0.108–1.800	0.221
A history of both types of procedures	2.786	1.023–7.582	0.068
Prior reconstruction of hypospadias	3.292	1.267–8.553	0.031
Prior urethral dilation	2.100	0.853–5.169	0.106
Inflammatory stricture	0.740	0.259–2.118	0.569
Iatrogenic stricture	1.568	0.573–4.292	0.392
Posttraumatic stricture ■	–	–	–
Idiopathic stricture	0.635	0.196–2.055	0.435
Penile stricture	1.278	0.496–3.258	0.614
Bulbar stricture	0.567	0.175–1.843	0.327
Penobulbar stricture	1.432	0.567–3.617	0.450
Multifocal stricture	2.316	0.943–5.685	0.071
Penile urethrostomy	1.469	0.550–3.929	0.436
Perineal urethrostomy	0.681	0.255–1.820	0.436
Early postoperative complications	4.052	1.743–9.419	0.004
Quantitative variables	Class mean (patients with urethrostomy stenosis)	Class mean(patients without urethrostomy stenosis)	*p*
Age, years	51.2	54.4	0.431
Body mass index, kg/m^2	27.6	26.1	0.212
Stricture length, cm	8.4	6.74	0.114
Duration of the disease, years	6.65	8.31	0.520
Number of previous urethral procedures	1.60	1.11	0.300
Number of comorbidities	5.0	3.47	0.067

Previous failed procedures are a well-known risk factor for an unfavorable outcome of any urethral reconstruction [22–25]. Urethrostomy is not an exception, which is confirmed by our data and the results of other studies [3, 26]. Although we observed a higher risk of complications in patients with a history of open and/or endoscopic surgery, this trend was not significant. At the same time, it was found that previous systematic urethral dilation, being an independent predictor of the development of early and late complications, increases the likelihood of their occurrence by 2.4 times and by 11.7 times, if it is combined with other unfavorable factors identified in the multivariate analysis (see Tables 3, 4).

The second most frequently studied risk factor for the development of urethrostomy stenosis is the etiology of strictures. Inflammatory etiology is considered unfavorable. G. Barbagli et al. [2] found the highest rate of complications among patients with infectious strictures (66.7%). Traumatic strictures may have a poor prognosis if the perineum is damaged. The negative impact

Table 6

Multivariate risk assessment of urethrostomy stenosis

Variable	Coefficient	Standard error	*p*	Odds ratio
Urethrocutaneous fistulas (no)	2.744*	1.195	0.022	15.550
Hypertension (no)	1.695*	0.746	0.023	5.449
Stricture after failed hypospadias repair (no)	2.883*	1.034	0.005	17.866
Early postoperative complications (no)	2.759**	0.962	0.004	15.779
Constant	-3.132**	0.647	0.000	0.044
Chi-square for the model		*20.94***	*p*	*<0.0001*
Cox and Snell R2				*0.218*
Nagelkerke's R2				*0.360*
n				*85*

of radiation therapy is unambiguous, since it is associated with the development of scar tissue and predisposes to poor wound healing. J. B. Myers et al. [18] reported a 12-fold increased risk of urethrostomy stenosis in patients with previous pelvic radiotherapy. Our study did not include patients with post-radiation strictures, and the number of patients with posttraumatic strictures was only 9.4%. we didn't see any urethrostomy stenosis in this subgroup. Among patients with inflammatory strictures, urethrostomy stenosis developed in 10.7% (3/28) of cases, and the infectious etiology did not significantly affect the risk. On the contrary, the presence of UTI significantly increasing the risk of development both early and late complications, as well as independent risk of late complications (see Tables 3 and 5).

With regard to scleroatrophic lichen and long-term results of urethrostomy, controversial data have been presented in the literature. On the one hand, there are a number of studies, in which urethrostomy in patients with lichen sclerosus demonstrates the highest success rates (93–100%) compared to various types of urethroplasty [10, 11, 18, 26]. In other studies, scleroatrophic lichen is considered as an unfavorable risk factor. According to G. Barbagli et al. [2], 36.7% of unsuccessful surgical outcomes were associated with lichen sclerosus, while J. C. Lopez et al. [27] state that patients with lichen sclerosis are 3 times more likely to undergo reoperation compared to patients with idiopathic strictures or strictures after failed hypospadias repair. This is probably due to progression of lichen to the area of creating the urethrostomy. It is possible that favorable outcomes are associated with the involvement of glandular or penile urethra, while the urethrostomy is performed on a healthy bulbar urethra. One of the possible causes of stenosis is progression of the disease to the area of urethrostomy. In our study, the proportion of patients with lichen was extremely low (4.7%), with all patients having a satisfactory outcome.

It is believed that patients after prior failed hypospadias surgery have the most favorable prognosis for perineal urethrostomy. In a study by G. Barbagli et al. [2], these patients had the best (87.5%) results compared to those with strictures of other etiology (33.3–73.6%). In our study, it was not confirmed; on the contrary, the previous hypospadias procedures were a significant risk factor for the development of urethrostomy stenosis (see Tables 5, 6), and among the patients of this group, the highest incidence of stenosis was found (50%, 3/6). At the same time, it is important to consider other risk factors, for example, patients with failed urethrostomy had multiple unsuccessful hypospadias procedures (from 3 to 7 times), long strictures (from 4 to 8 cm) with severely scarred paraurethral tissues, UTI, chronic comorbidities, and two of them were overweight.

Another risk factor for the development of urethrostomy stenosis is directly related to the procedure. The success of treatment is determined by the radical excision of the urethral scars, non-tension anastomosis between the urethral edges and skin, the preservation of the dorsal semicircle of the urethra and other surgical maneuvers. Studies comparing different urethrostomy techniques have not found any clear advantage of any one of them [3, 26, 28]. However, Johanson and Blandy non-transecting techniques, which preserve the dorsal urethral wall and retrograde blood supply from the dorsal penile artery, are expected to be associated with lower rate of urethrostomy stenosis [8, 18, 27, 29]. We used these techniques in our study, however, the predominance of the Johanson procedure (78/85) did not allow to assess the risks of complications with various techniques, which, on the one hand, is a limitation, but, on the other hand, ensures the homogeneity of our group. In addition, we were unable to separately calculate a risk of complications for perineal and penile urethrostomies, which can also be attributed to limitations of the study. Nevertheless, we determined that the localization of the urethrostomy does not have a significant effect on the development of stenosis (see Tables 3, 5).

Surgery outcomes may be also associated with comorbidities. Their negative impact was shown in a number of publications devoted to the stricture disease and outcomes of urethral surgery in patients with diabetes mellitus, coronary heart disease, arterial hypertension, hypogonadism, metabolic syndrome, etc. [24, 30–33]. However, according to the review, different comorbidities and their influence on the development of complications after urethrostomy remain understudied. In our group, we have analyzed concomitant diseases. A significant role of arterial hypertension in the development of overall complications and urethrostomy stenosis was confirmed (see Tables 3–6). The effect of arterial hypertension on the results of surgery can be explained by severe microcirculation dysfunction associated with changes in vascular tone, as well as blood rheological properties. These disorders lead to a decrease in capillary blood flow, deterioration of perfusion of organs and tissues, and the formation of chronic ischemia. The role of ischemia in the development of stricture disease has long been known from studies evaluating the incidence of urethral strictures in patients in intensive care units. For example, in the study published in the journal of the Tehran Heart Center the relationship between the severity of coronary heart disease and the risk of developing catheter-associated urethral strictures was shown. The authors found that a threshold value of the SYNTAX Score > 22.5 points predicts the likelihood of developing catheter-associated urethral strictures with a sensitivity of 76.7% and a specificity of 85.1% [34]. J. C. Lopez et al. [27] reported that coronary artery disease, indirectly affecting the wound healing process, increases the likelihood of urethrostomy stenosis by 2.3 times. This fact must be taken into account when planning urethral surgery in patients with arterial hypertension, and in addition to antihypertensive therapy, drugs that improves microcirculation should be prescribed.

There are no controversies in the literature that urethrostomy is associated with a low incidence of early postoperative complications. Anecdotal cases of bleeding, hematoma formation, wound dehiscence and other complications, which usually do not require additional surgical correction, has been described [2, 8, 17, 26, 27]. Our study was not an exception, also showing relatively low rate of postoperative complications (8.2%). This did not allow separately evaluating the risk factors of their development, which can be considered another limitation. However, when analyzing causes of urethrostomy stenosis, early postoperative complications was a significant risk factor, increasing the likelihood of stenosis by 4.1 times, while in combination with other adverse factors identified in the multivariate analysis, elevated risk was 15.8 (see Table 5 and 6).

Conclusions

1. Urethrostomy provides the restoration of satisfactory urination in 97.6% of cases and is associated with a low rate of early postoperative complications (8.2%). However, during long-term follow-up urethrostomy stenosis developed in 16.5% of cases. Primary success of urethrostomy at a median follow-up of 58 months is 81.2%.
2. The independent risk factors for the development of early and late complications are UTI, arterial hypertension, prior urethral dilation and multifocal stricture. The combination of unfavorable factors is associated with a probability of developing early and late complications of 32.1–49.9%.
3. The independent risk factors for the development of urethrostomy stenosis are UTI, arterial hypertension, previous

hypospadias repair and early postoperative complications. The combination of unfavorable factors is associated with a probability of developing urethrostomy stenosis of 21.8–36%.

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INVOLVEMENT OF P2 RECEPTORS IN THE CONTRACTILE ACTIVITY OF THE BLADDER IN PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA

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Aim: to study the role of P2 receptors in impaired bladder contractility in patients with lower urinary tract obstruction.

Materials and methods: in pharmacological studies, tissue samples from the bladder wall of 30 patients were used, obtained during planned surgical interventions for benign prostatic hyperplasia (transvesical simple prostatectomy without placement of cystostomy tube). Based on these tissue, isolated smooth muscle specimens were prepared. Their mechanical activity and the efficiency of ligands of purine P2 and other receptors were evaluated. With this aim, the following P2-receptor agonists were used: adenosine triphosphoric acid (ATP), adenosine diphosphoric acid (ADP), uridine-5'-triphosphoric acid (UTP), alpha, beta-methylene-ATP, 2-methylthio-ADP, as well as antagonists of P2-disulfonate receptors acid (PPADS), suramin, NF023, MRS2500. In addition, the efficiency of ligands of other receptors, including carbacholine, epinephrine, histamine, serotonin, atropine was evaluated.

Results: alpha-beta-methylene-ATP was the most effective agonist, while ATP and 2-methylthio-ADP were significantly less active. In our experiments, ADP and UTP did not show an effect on human bladder. The influence of P2 receptor agonists was inhibited by P2 receptor antagonists PPADS and suramin, as well as MRS2500, although to a lesser extent. Carbacholine caused a strong concentration-dependent contractile response of the bladder, which was inhibited by atropine. Histamine resulted in mild bladder contractions only at high concentrations. Epinephrine and serotonin did not cause significant changes in the contractile activity of the bladder.

Conclusion: The main subtype of P2 receptors involved in the contractile activity of the human bladder is P2X1. P2Y1 receptors also have some influence on the contraction, while other subtypes of P2 receptors are not detected by pharmacological methods.

K e y w o r d s: *P2 receptors, agonists, antagonists, bladder, benign prostatic hyperplasia*

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Introduction. Purinergic P2 receptors, the basic endogenous agonist of which is adenosine triphosphoric acid (ATP), are widely spread in the organs and tissues of human and animals [1, 2], including the genitourinary system [3, 4]. Under physiological conditions, the role of P2 receptors in the peripheral nervous system is not mostly the main; they only complement or modulate the action of the main neurotransmitters, such as acetylcholine and norepinephrine. However, in pathological states, the role of P2 receptors significantly increases and often become the leading in the pathogenesis of some disorders. In particular, the purinergic component of the human bladder contraction increases from 2% under normal conditions to 40% in some pathological processes (interstitial cystitis, neurogenic bladder, urinary tract obstruction) [5–8]. Voiding dysfunction due to bladder outlet obstruction associated with the prostate diseases remain an important urological issue in middle-aged and older men. Although there are a lot of options of drug therapy, their efficiency is mostly very low. Surgical treatment can radically resolve the obstruction, but it can lead to complications that often require additional procedures. As a result, the development of new mechanisms of action for potential drugs in the treatment of such conditions is an important task.

Aim. To study the role of P2 receptors in impaired bladder contractions in patients with obstructive disorders of lower urinary tract.

Materials and methods. The study involved 38 patients with a diagnosis of BPH, mainly with obstructive symptoms. The mean age of the patients was 70.6 ± 5.9 years, and average duration of the disease was 5.2 ± 2.6 years. During this period, patients received drug therapy (α1A-blockers; 5α-reductase inhibitors). Prior to surgery, all patients underwent a standard examination using mandatory and optional diagnostic methods. The mean IPSS score was 19.3 ± 5.9 points; PSA level was 2.8 ± 1.1 ng/ml and residual volume was 69.6 ± 36.8 ml. The adenomatous nodes were seen on transrectal ultrasound. The prostate volume was 93.97 ± 17.26 cc and the average Qmax according to uroflowmetry was 5.1 ± 1.7 ml/s.

Based on the results of the examination, a clinical diagnosis of BPH was confirmed. In 33 patients, second stage of BPH was diagnosed, while 5 mens had a cystostomy for acute urinary retention. In addition, there were 2 patients with concomitant bladder stones.

Indications for transvesical simple prostatectomy with a blind suture of bladder or a secondarily delayed blind suture were inefficiency of drug therapy, a very large prostate, the presence of cystostomy, severe disturbances in the lower urinary tract urodynamics with a predominance of obstructive symptoms and patients' refusal of transurethral procedure.

Material for experimental pharmacological studies was obtained during surgery. Before the study, all patients received all necessary documents and signed an informed consent to participate in the study. In addition, for each eligible patient, the clinical supervisor of the project filled out a patient card, in which the urological status and a diagnosis were mentioned.

The study was approved by the local ethics committee of the Kazan State Medical University.

During the simple prostatectomy, before performing the main stage (enucleation of the prostatic adenoma) a tissue sample from the upper edge of the incision, the anterior wall of the bladder was taken through all layers. From this sample, a piece of 0.5 × 1.5 cm in size was isolated and immediately placed in cooled (4°C) physiological Krebs-Henseleit solution and delivered to the pharmacological laboratory for research. The rest of the tissue was sent for morphological examination.

A total of 38 bladder tissue samples were obtained from 38 patients. For various reasons (small size, lack of detrusor muscle, improper storage, etc.) tissue samples from 8 patients were not suitable for evaluation, therefore, specimens from 30 patients were used for pharmacological experiments.

Pharmacological experiments. In the pharmacological laboratory, detrusor samples with a size of approximately 2 × 10 mm were prepared from the obtained tissue specimens. They were placed in a glass thermostat with 10 ml Krebs-Henseleit solution (37±0.5°C) to assess mechanical activity. Krebs – Henseleit buffer had the following composition: 118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO3, 1.2 mM MgSO4, 1.2 mM KH2PO4, 7.8 mM glucose and 1M 2.5 CaCl2 • 2H2O. For the entire experiment the solution was aerated with a gas mixture of 95% oxygen and 5% carbon dioxide (pH 7.4). One end of the smooth muscle sample was tightly fixed, the other was attached to an FSG-01 isometric sensor of mechanical activity (Linton, UK) using a silk thread. The recording was carried out on a computer using the MP100WSW Data Acquisition System program (UK). The program interface was developed by Biopack, UK. An initial load of 1 g was applied to the sample. Then it was left for 1 h for stabilization, while washing solution in a glass was changed every 15 min. Electric field stimulation was performed by Digitimer Multistim D330 stimulator (GB) using two platinum rings 2.5 mm in diameter, through which a smooth muscle sample was passed; the distance between the rings was 15 mm. Stimulation was performed until the contraction decreased by one third after reaching its maximum. The settings for stimulation were as follows: the frequency of contractions of 0.5–32 pulses/s, the width of 0.5 ms, the voltage of 100 V. The pulse had a rectangular shape. An interval between each subsequent stimulation was 60 s. In experiments with electrical stimulation, atropine (1 μM) and phentolamine (10 μM) were added to the Krebs-Henseleit solution to block the effect of M-choline and α-adrenergic receptors on the tissue response. The contractile agents were introduced directly into the glass and the specimen was washed several times with the Krebs-Henseleit solution after reaching the maximum contraction. Pharmacological methods were used to determine the subtypes of P2 receptors in the bladder. For this aim, the following agonists and antagonists were chosen: ATP (a universal agonist of all P2 receptors), alpha-beta-methylene-ATP (an agonist of P2X receptors, has more affinity for P2X1 and P2X3 receptors),

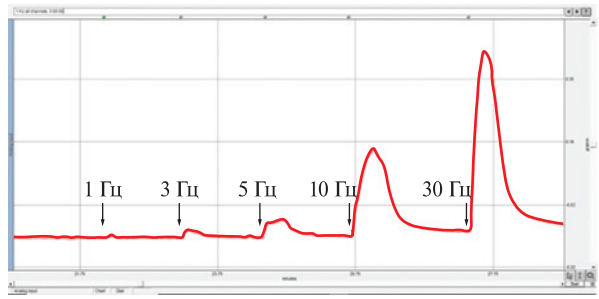

Fig. 1. Contractions of isolated human bladder smooth muscles sample, provoked by stimulation with an electric field of various frequencies and registered in the program AcqKnowlwdge (Biopack, UK)

2-methylthio-ADP (agonist P2Y1 -receptors), ADP (agonist of P2Y12-receptors), UTP (agonist of some subtypes of P2Y-receptors), PPADS (universal antagonist of P2-receptors, in low concentrations it has more affinity for P2X-receptors), suramin (universal antagonist of P2-receptors), NF023 (P2X1 receptor antagonist), MRS2500 (P2Y1 receptor antagonist). In addition, we evaluated the efficiency of ligands of other receptors, such as carbacholine (M- and H-cholinergic receptors agonist), epinephrine (alpha- and beta-adrenergic receptors agonist), histamine (agonist of histamine receptors), serotonin (agonist of 5-HT receptors), atropine (antagonist of M-cholinergic receptors). The relationships «frequency-contraction» and «concentration-contraction» depending on the agent and the experimental conditions were evaluated. Mathematical and statistical analysis of the results and presentation of the data was carried out using Microsoft Excel software. Comparison of groups was performed using Student's t-test.

Results. *Effect of electric field stimulation.* Stimulation with an electric field of 1-20 Hz produced frequency-dependent contractions of isolated human bladder smooth muscles sample (*Fig. 1*). After 20 minutes of tissue incubation with the M-anticholinergic atropine (1 μM), contractions caused by low frequencies (1–5 Hz) were not recorded, and at frequencies of 10–30 Hz, their amplitude decreased by 80% or more. The addition of PPADS at a concentration of 10 μM after tissue incubation with atropine did not lead to any additional changes in the bladder contractions. The addition of a nonselective P2 receptor antagonist PPADS (10 μM) did not significantly affect the contractions of the smooth muscles caused by electric field stimulation. Incubation of tissue with another nonselective P2 receptor antagonist, suramin (100 μM), resulted in a decrease in the contractions caused by electric field stimulation by about 30% from the baseline. The combined use of suramin (100 μM) and atropine (1 μM) completely inhibited the contractions under electric field stimulation, whatever frequency was used (*Table 1*).

Effect of P2 receptor agonists and antagonists. After addition of P2 receptor agonists to the incubation medium, various changes in the contractile activity of isolated human bladder smooth muscles sample were recorded. Alpha-beta-methylene-ATP (0.1-10 μM) produced the greatest effect on contractile activity, which depended on the concentration. ATP (1-100 μM) caused low-amplitude contractions only at the highest concentration, as well as 2-methylthio-ADP (0.1-10 μM). ADP and UTP, both at concentrations (1-100 μM), did not induce contractions of isolated human bladder smooth muscles sample (*Table 2*). Incubation of specimen with PPADS (10 μM) or suramin (100 μM) completely inhibited contractile responses induced

Table 1

Contractions of isolated human bladder smooth muscles sample, provoked by electric field stimulation in the control group vs. antagonists

Electric field stimulation frequency, Hz	Control (*n*=14)	Atropine, 1 μM (*n*=6)	PPADS, 10 μM *n*=5	Atropine, 1 μM + PPADS, 10 μM (*n*=6)	Suramin, 100 μM (*n*=5)	Atropine, 1 μM + suramin, 100 μM (*n*=5)
1	2,3±0,9	0	3,5±0,8	0	1,3±0,7	0
3	8,6±1,6	0	9,0±2,2	0	5,8±1,1	0
5	18,3±3,6	1,1±0,1*	23,7±3,5	1,9±0,4*	15,6±3,5	0
10	37,0±6,3	8,3±0,9*	33,4±5,6	12,3±2,4*	25,7±4,8	0
30	66,5±8,4	12,4±3,3*	56,8±7,9	16,6±4,1*	45,9±6,9*	0

Note. Here and in *tables 2, 3*, the results are presented as *M*±*m* in a percentage of the contraction caused by KCl at a concentration of 240 mM.
* $p<0.05$ vs. controls.

by alpha-beta-methylene-ATP, ATP, and 2-methylthio-ADP. However, when MRS2500 (1 μM), a selective P2Y1 receptor antagonist was added, the contractions caused by all agonists decreased by about 10–30%. It was found that a selective P2X1 receptor antagonist, NF023 (3 μM), completely inhibited contractions caused by P2 receptor agonists.

Influence of other agonists. Carbacholine (0.01-10 μM) caused strong, concentration-dependent contractions of isolated human bladder smooth muscles sample, which were much more pronounced compared to the most active agonist of P2 receptors (alpha-beta-methylene-ATP). *Fig. 2* shows the amplitudes of contractions of the isolated human bladder

Table 2

Contractions of isolated human bladder smooth muscles sample, caused by P2 receptor agonists

Substance	*n*	Agonist concentration, μM			
		0,1	1	10	100
Alpha-beta-methylene-ATP	8	4,8±2,2	28,9±7,5	75,7±12,6	–
Suramin (100 μM) + a, b-meATP	6	0*	0*	3,5±0,9*	–
PPADS (10 μM) + a,b-meATP	6	0*	0*	5,8±2,4*	–
MRS2500 (1 μM) + a,b-meATP	5	0*	15,8±2,8*	55,9±5,3*	–
NF023 (3 μM) + a,b-meATP	5	0*	0*	0+	–
ATP	6	0	0	0	15,2±2,4
Suramin (100 μM) + ATP	4	–	–	–	0*
PPADS (10 μM) + ATP	4	–	–	–	0*
MRS2500 (1 μM) + ATP	3	–	–	–	0*
NF023 (3 μM) + ATP	3	–	–	–	0*
2-methylthio-ADP	4	0	0	0	12,4±8,6
Suramin (100 μM) + 2-methylthio-ADP	3	–	–	–	0*
PPADS (10 μM) + 2-methylthio-ADP	3	–	–	–	0*
MRS2500 (1 μM) + a,b-meATP	3	–	–	–	0*
NF023 (3 μM) + a,b-meATP	3	–	–	–	0*
ADP	3	0	0	0	0
UTP	3	0	0	0	0

Note. "-", not evaluated, 0, no effect.
*$p<0.05$ compared to respective effects of agonists in the absence of antagonists.

Table 3

Contractions of isolated human bladder smooth muscles sample, caused by different receptor agonists

Substance	*n*	Agonist concentration, μM				
		0,01	0,1	1	10	100
Carbacholine	8	3,9±0,5	27,4±3,4	138,7±21,2	253,2±33,6	–
Atropine (1 μM) + Carbacholine	8	0*	0*	22,5±6,7*	39,8±11,3*	–
Histamine	3	–	0	0	17,5±5,3	–
Epinephrine	3	–	–	0	0	0
Serotonin	3	–	–	0	0	–

Note. "-", not evaluated, 0, no effect.
*$p<0.05$ compared to carbacholine in the control group.

smooth muscles sample caused by alpha-beta-methylene-ATP at a concentration of 10 μM and carbacholine at a concentration of 1 μM. Incubation of tissue with atropine (1 μM) completely prevented the contractions caused by carbacholine at concentrations up to 1 μM, and significantly inhibited the contractions caused by high concentrations of the agonist. Histamine (0.1-10 μM) produced mild bladder contractions only at the highest concentration. Epinephrine (1–100 μM) and serotonin (0.1–10 μM) did not cause significant changes in the bladder contractions (*Table 3*).

Discussion. It is well known that under physiological conditions the contractile activity of the bladder is regulated mainly by cholinergic nerves. According to our results, the same is true for patients with lower urinary tract obstruction. However, like under physiological conditions, patients with BPH have a small "non-cholinergic component". The effect of two nonselective P2 receptor antagonists, PPADS and suramin, does not allow one to determine the role of purinergic nerves, since PPADS did not affect contractions caused by electric filed stimulation, while suramin inhibited them. The trypanocidal drug suramin has a variety of effects, including non-selective inhibition of P2 receptor activity [9, 10]. Probably, its influence in our experiments is associated with other, not only purinergic, effects of suramin. The receptors P2X1, P2X3, P2Y1, P2Y2, which are involved in contractile activity, were previously found in the bladder of laboratory animals [3, 4]. Pharmacological methods are difficult to accurately detect the subtypes of P2 receptors, however, our studies suggest the presence of P2X1, P2X3, P2Y1 receptors in the human urinary bladder. In addition, we have shown a high efficacy of the P2X1 and P2X3 agonist alpha-beta-methylene-ATP and the efficacy of the P2X1 antagonist NF023. Moreover, we hypothesize the presence of P2Y1 receptors based on the effects of 2-methylthio-ADP (their agonist) and MRS2500 (their antagonist).

During last decades the possibility of using P2 receptor agonists and antagonists in clinical practice have been confirmed in different studies. The introduction of platelet P2Y12 receptor antagonists (ticlopidine, clopidogrel) into clinical practice as effective antiplatelet agents became one of the significant recent advances in pharmacology [11]. The wide variety and representation of P2 receptors in the human body make them very plausible as potential targets for new drugs; therefore, studies in this area should be continued.

Definitely, there are some limitations for the correct interpretation of our results. Since the material for the study was taken from patients with long-standing bladder outlet obstruction, this may affect the activity of receptors in the smooth muscles. In further studies, we are going to modify the design with taking this into account, as well as focusing on the duration of the disease and the previous drug therapy. We believe that the results described in this study and our subsequent studies will provide the basis for the search for new drugs that act on P2 receptors for the complex pathogenetic treatment of urogenital diseases.

Conclusion. Our studies have shown the contractile response of isolated human bladder smooth muscles sample responds to electric field stimulation, as well as agonists of cholinergic and purinergic receptors. The main subtype of P2 receptors involved in the contractile activity of the human bladder, including patients with BPH, is P2X1. Experiments have shown that P2Y1 receptors also have a certain effect on the contractile activity of the bladder smooth muscles, while the other subtypes of P2 receptors are not detected by our pharmacological methods.

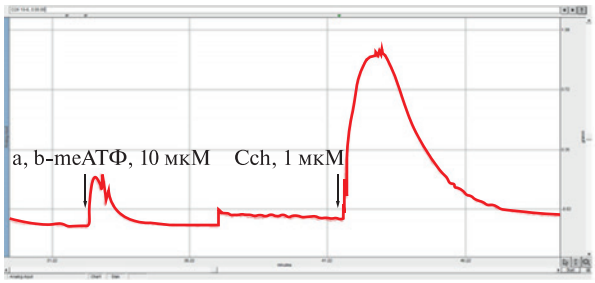

Fig. 2. Contractions of isolated human bladder smooth muscles sample, provoked by various agonists and registered in the program AcqKnowlwdge (Biopack, UK).
a, b-meATP, 10 μM - alpha, beta-methylene ATP;
Cch - carbacholine.

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STUDY OF THE STRUCTURE AND MICROFLORA OF URETHRAL TISSUES IN URETHRAL PAIN SYNDROME

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Urethral pain syndrome (UPS) is characterized by the occurrence of persistent or recurrent pain in the urethra in the absence of a confirmed infection and other obvious local pathological changes. The study of its pathogenetic aspects is important first of all for understanding the causes of the disease, to prescribe effective treatment, specific recommendations for the prevention and treatment of this disease are also absent. This paper presents the advanced experience of our research group on the study of the urethral state by the in vivo cross-polarization optical coherence tomography (CP OCT) method, and also the results of the microbiota analysis in the urethral tissues.

The purpose of the study is to search for the risk factors for UPS and the character of changes in the urethral tissues, using the data of: 1) concomitant pathology, 2) structural changes in the urethral wall in UPS in comparison with chronic cystitis of bacterial etiology 3) studying the microbiota of urethral tissues.

Materials and methods: The condition of the urethra was studied in 109 patients: 55 of them with UPS (group «UPS»), without clinical manifestations of inflammation; 41 – with chronic inflammation of the lower urinary tract of various origins (group «Inf»); in 14 patients with stones of the upper urinary tract without pyelonephritis, the urethra was taken as the norm (group «N»). All performed a clinical minimum of studies, also cystoscopy with the study of the bladder triangle, the neck of the bladder and the urethra by the method of in vivo tissue imaging – CP OCT. The device «OCT–1300U» with wavelength of 1300 nm is used. To determine the possible role of UPS disease background, the analysis of concomitant pathology preceding the development of UPS was performed. To analyze the relationship of changes in the urethral tissues with the composition of its microbiota, a PCR study of biopsies from the proximal segment of the urethra was performed in 13 patients with UPS.

Results: Qualitative comparison of the thickness and character of the OCT signal of the urethral wall layers observed using CP OCT in the studied groups of patients allowed us to establish that the state of the epithelium and connective tissue structures of the mucous membrane in patients with UPS is not the norm, changes are similar to those in chronic inflammation.

Changes in the character of the OCT signal were recorded in all parts of the urethra, but in the middle third they are most pronounced and most critical. In UPS, there is a brightly pronounced reorganization of the connective tissue stroma components. Pronounced fibrosis of subepithelial structures (increased signal brightness in the cross-channel compared to the norm) with their thickening was recorded in 48.2% of cases, and thinning/lack of visualization of the epithelial layer was detected in 20.5%, and in chronic inflammation 55.5% and 40.6% of cases, respectively. According to the results of PCR, only one patient had significant total bacterial contamination of the biopsy (TBC=104.7). In all other cases, the total bacterial mass of the biopsies was at the level of negative control.

Conclusions. In patients with UPS, the presence of several concomitant, often chronic, diseases was revealed, which may be a premorbid background and one of the risk factors for the occurrence and maintenance of UPS. Pilot PCR studies of biopsies from the proximal segment of the urethra indicate that low values of bacterial contamination in the majority of patients with UPS do not exclude the possible role of bacteria in the development of the disease in some patients.

The CP OCT method used in this study is currently the only one in vivo method of visualization of the urethral mucosa, which provides real-time images of structural changes in the epithelial (atrophy or hyperplasia) and connective tissue (active or latent inflammation with cellular infiltration or fibrosis) layers of the urethra, allowing better understanding of the pathogenesis of the disease and monitoring of therapy.

Key words: *urethral pain syndrome (UPS), cross-polarization optical coherence tomography (CP OCT), diagnosis of UPS, connective tissue matrix of the urethra, analysis of the microbiota of urethral tissues.*

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Introduction. Urethral pain syndrome (UPS) is a condition, which is characterized by persistent or recurrent pain in the urethra in the absence of confirmed infection and other obvious local pathological changes [1]. It is believed that up to a third of women of all ages complain of pain in the urethra, true pelvis, dysuria, dyspareunia [2], while in 40% of cases these symptoms are caused by urethritis and UPS [3]. As a rule, the UPS is considered synonymous with clinical diagnosis and is made as diagnosis of «exclusion». The study of pathogenetic aspects is of importance for understanding the causes of UPS and choosing effective therapy, since there are no clear recommendations for the prevention and treatment for this disorder.

UPS is a component of chronic pelvic pain syndrome, the main feature of which is the absence of proven infection or other obvious local pathology that could explain the pain [1]. If in EAU guidelines published in 2010 it was noted that there were no studies that could address the question of how often dysuria could occur in case of normal bladder and urethral examinations [4], currently the stereotypes for the diagnosis of genitourinary disorders are changing.

In 2012, the fact of urine sterility in humans was rejected. Genomic approaches to diagnostics have shown that traditional microbiological methods do not allow to identify the entire variety of microbial species that are present in urine [5, 6]. According to the results of bacteriological studies, even in otherwise healthy men and women, multicomponent aerobic-anaerobic associations of microorganisms was detected in 100% of cases. In addition to microbiota, the presence of virobiota in a small percentage has been shown [5]. Microflora may have a role in the development of overactive bladder [7]. The differences in urine microbiota of healthy volunteers and patients with chronic pelvic pain syndrome were revealed [8]. According to the study of G.A. Osipov (2016), dense mucopeptides of the mucosa in the intestines, respiratory tract, and also the urogenital tract can harbor anaerobes [9]. The localization of viral-bacterial associations in the genitourinary system is still unknown, the likelihood of their detecting in the bladder and urethral tissues is not excluded, which in unfavorable conditions, can provoke a chronic inflammation and, accordingly, serve as an underlying background for the development of functional disorders. There are some rationales to consider that the cause of the inflammatory process in patient with UPS is located in the urethral tissues (shown by our studies earlier [10]) and it serves as an additional stimulus for impaired microcirculation, innervation, and functioning of the urethra, indirectly affecting the development of pain.

Since UPS as a component of chronic pelvic pain syndrome has multifactorial etiology, examination of the lower urinary tract and the search for an optimal treatment approach requires a multimodal approach [11, 12]. The diagnostic armamentarium for patients with suspected UPS includes physical examination, bimanual palpation of the urethra and vaginal walls, and ultrasound examination of the pelvic organs, including the urethra. It should be noted that during widespread procedure for examining the lower urinary tract (cystoscopy), the urethra often remains overlooked.

Optical coherence tomography (OCT) is a promising method for studying changes in the urethral wall [13]. As in-vivo imaging method, OCT has a spatial resolution of 10–15 μm with a probing depth of 1–1.5 mm.

OCT refers to the so-called mesoscopic imaging and allows to characterize changes in the tissues associated with the pathology at the level of general architectonics (by the properties of the backscattering of probing radiation). Polarization sensitive OCT (PS OCT) and cross-polarization OCT (CP OCT) are used to register changes in the matrix components of connective tissues (for example, collagen fibers) by the properties of birefringence and cross-scattering, in order to selectively analyze these structures [14, 15].

Despite the significant experience in OCT in many fields of medicine, including urology (for differentiating inflammatory lesions and bladder tumors [16], detecting prostate cancer and establishing tumor boundaries during resection [17], diagnosing ureteral tumors [18]), this method has not been previously used for studying urethral walls in women with UPS.

The advanced experience of our research group on the study of urethral pathology by an in-vivo imaging method, CP OCT, and the results of the analysis of the urethral microflora are presented in the article.

Aim. To search for triggering factors of the development of UPS and analyze the changes in urethral tissues using data on: 1) concomitant diseases, 2) structural changes in urethral tissues in UPS compared to chronic inflammatory diseases of the lower urinary tract, 3) the composition of microflora of urethral tissues.

Materials and methods. The changes of urethral tissues in 109 females were studied. Among them, 55 patients had UPS (group of UPS) without clinical manifestations of inflammation; 41 females had lower urinary tract infections (group B), and in 13 patients with upper urinary tract stones without pyelonephritis, the urethra was considered as normal (control group). All patients underwent standard clinical studies, including blood and urine tests, urine culture, ultrasound of the urinary tract; gynecological examination with evaluation of the urethral meatus, palpation of the urethra through the anterior wall of the vagina, palpation of the vaginal walls, O'Donnell-Hirschhorn test and cystoscopy combined with CP OCT examination of the bladder neck and the urethra. To determine the possible role of underlying diseases in the development of UPS, the analysis of pre-existing concomitant pathology was done.

For in-vivo comparative analysis of changes in urethral tissues in female with inflammatory diseases and UPS, imaging with CP OCT was performed using the device «OCT-1300U». It has a wavelength of 1300 nm and provided images with a size of 1.7 (width) x 2.4 (height) mm. Each image consists of two parts: the lower image (co-polarization) shows layers of tissue, while the signal on the upper image (cross-polarization) appears only if there are anisotropic components (collagen and elastic fibers) in the tissue. The lower image allows to evaluate changes in the epithelium (to differentiate normal tissue, atrophy, dysplasia and malignancy). The condition of the subepithelial layers (mucous and submucous membrane) is easier to assess by the features and intensity of the signal in the upper image. Analysis of both images can reveal signs of inflammation, fibrosis, tumor transformation, and other tissue conditions [16, 19]. CP OCT of urethral tissues was performed during cystoscopy. A flexible endoscope with an outer diameter of 2.7 mm was introduced through the working channel of a cystoscope (Karl Storz, Germany) with a size 8 Ch. On average, 3-4 CP OCT images of the urethra and bladder neck was done in every patient; a total of 303 images were analyzed. On CP OCT images of the proximal, middle and distal parts of the urethra, as well as the bladder neck, the thickness of the epithelial and connective tissue layers was determined. All measurements were done manually by an independent reviewer using standard ImageJ function. If duration of UPS was up to a year, manifestations of the disease were considered as early, while in patients with more pronged course, tissue changes were regarded as late. Statistical analysis of changes in the thickness of urethral tissue, depending on the duration of the disease and age of the patients was carried

Table 1

Concomitant diseases and their source in patients with UPS

№	Affected system	N (%)	Cause of concomitant diseases	N (%)
	Gynecologic disorders	39 (70,9)	Hormonal	37 (94,8)
			Inflammatory	30 (76,9)
			Surgical interventions on the pelvic organs	12 (30,7)
	Respiratory diseases	37 (67,2)	Upper (nasal cavity, pharynx, larynx)	32 (86,4)
			Lower (trachea, bronchi, lungs)	5 (13,5)
			Psycho-emotional disorders	23 (41,8)
	Neurological diseases	35 (63,6)	Central nervous system	10 (18,2)
			Peripheral nervous system	42 (76,4)
			Psycho-emotional sphere	23 (41,8)
	Urological diseases	24 (43,6)	Inflammatory	10 (41,6)
			Non-inflammatory	17 (70,8)
	Gastrointestinal diseases	18 (32,7)	Inflammatory diseases of the stomach, duodenum, biliary tract	38 (69)
			Bowel disease	21 (38,2)
	Cardiovascular diseases	9 (16,3)	Arterial hypertension	5 (55,5)
			Other	4 (44,5)
	In total	162		

out using the «Statistica-12» package. Analysis of the linear trend of age-related changes in the thickness of epithelium and connective tissue stroma in three segments of the urethra and in the bladder neck was performed. Graphical approximation of the linear group trend was performed in MS Excel.

To analyze the relationship between changes in the urethral tissues and the composition of microflora of urethral tissues, PCR of samples from the proximal urethra, the largest part located above the pelvic floor, was performed. Such approach allows to minimize functional disorders due to trauma during biopsy, since along with the pinch biopsy during cystoscopy, some patients underwent a loop biopsy with a resectoscope. Tissue fragments from 13 patients with UPS were placed in a transport medium for bioassays «Stor-F» (DNA-Technology, Russia). All samples were stored in a refrigerator at a freezing temperature of -70°C. A set of reagents FEMOFLOR®-16 was used in order to study the urogenital biocenosis in women by real time PCR (NPO DNA-Technology, Russia).

Results.

1. The role of underlying diseases in the development of UPS

The results of the analysis of comorbidities in patients with UPS are presented in *table 1.*

As shown, each patient suffering from UPS had 2.94 (162/55) comorbidities. Gynecological diseases were predominant (70.9%): hormonal abnormalities were detected in 37 (94.8%) women, including 24 sexually active female of the premenopausal period and 13 women of the menopausal period. Bacterial and viral genital tract infections were recorded in 30 (76.9%) patients.

Past diseases of the upper respiratory tract, more often in adolescence, were recorded in 67.2% of women, of which the most of patients (64.9%) had recurrent viral respiratory infections and herpes infection. Concomitant pathology in patients with UPS included neurological diseases (63.6%), which were associated with the involvement of the peripheral nervous system and, importantly, with the psychoemotional disorders.

Thus, the role of foci of chronic infection and a decrease in immune defense factors as an underlying condition for the development of UPS cannot be excluded, since a history of respiratory, gastrointestinal, urological and gynecological inflammatory diseases was revealed in 55 patients with 146 of 162 episodes.

2. Changes in the urethral tissues in patients with UPS and chronic inflammation on CP OCT.

A qualitative comparison of the thickness of the layers of the urethral wall and the features of the CP OCT signal showed impairment of epithelium and connective tissue structures of the mucous membrane in patients with UPS, and the changes were similar to those in chronic inflammation (*Fig. 1*). The features of the abnormal OCT signal were recorded in all parts of the urethra, however, in the middle third they were most pronounced (where muscle structures of the sphincter mechanism are localized). Figure 1 shows CP OCT images of this particular area for three groups of patients to demonstrate typical changes in the structural organization of the layers of the urethral wall. The bottom image is related to co-polarized OCT, while the top one represents cross-polarized method.

Normally (*Fig. 1a*) in co-polarization, the upper epithelial layer is clearly distinguishable (indicated by an asterisk). The underlying bright connective tissue layer in co- and cross-polarizations has the same thickness, an organized pattern and an even lower border (the signal amplitude steadily decays with depth). On the images (*Fig. B, C*) thinning of the epithelial layer is observed in co-polarization vs. fibrosis of subepithelial structures in cross-polarization. On *Fig. D* partial atrophy of the epithelium is found in co-polarization vs. exudative edema of connective tissue in cross-polarization (significant decrease in signal); *Fig. e, f* demonstrates marked atrophy of the epithelial layer in co-polarization vs. significant thickening and compaction of connective tissue structures in cross-polarization, as well as the effect of birefringence (indicated by arrows), which is evidence of pronounced fibrosis.

In cases of pathological changes in the urethral wall (*Fig. 1 b – f*), the connective tissue layer has different thicknesses in co-

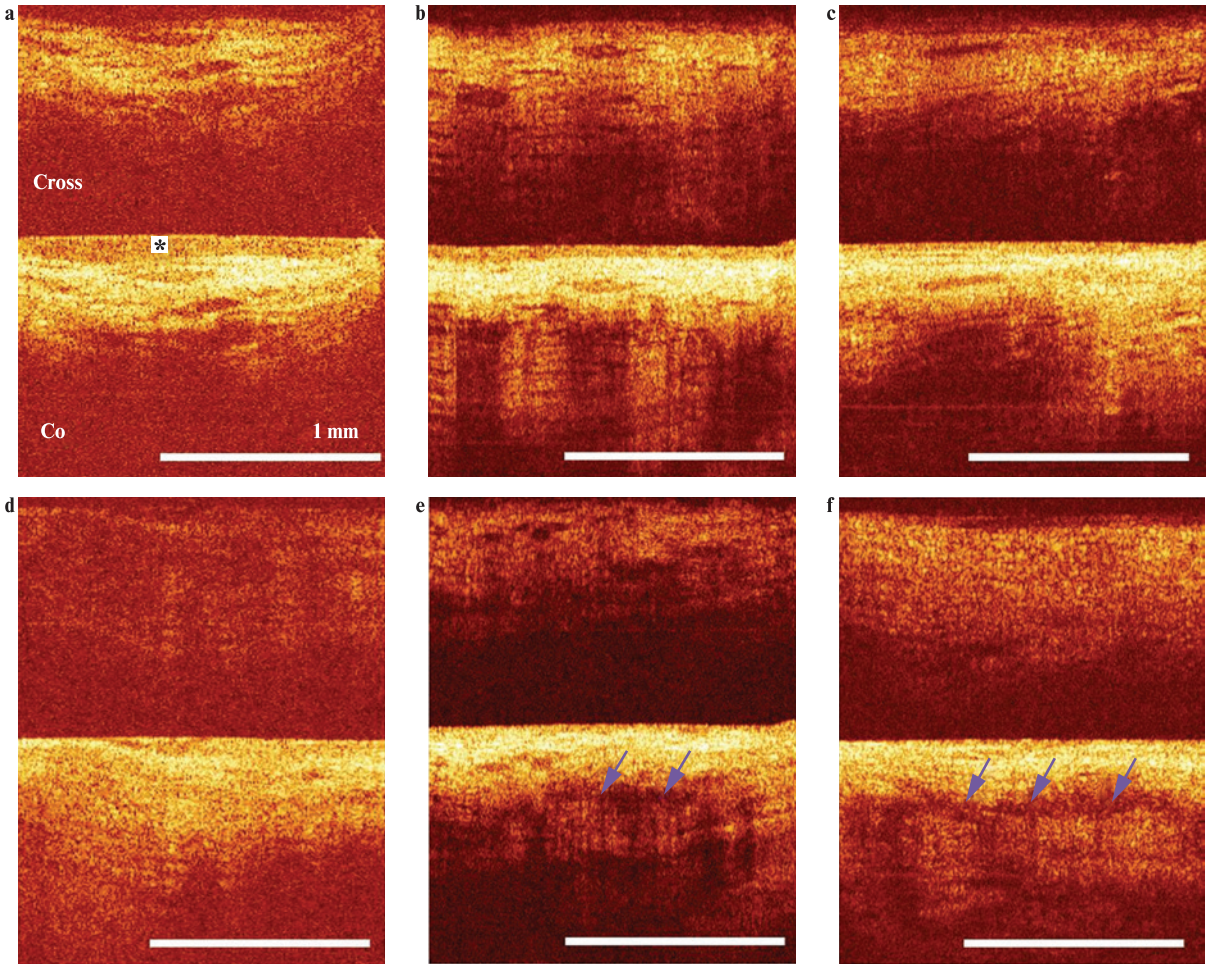

Fig. 1. CT OCT images of the middle part of the urethra: in group of otherwise healthy women (a) and patients with inflammatory etiology of the disease (b, c) and UPS (d, e, f)

The asterisk on (a) indicates the epithelial layer, the arrows on (d, e) indicate the effect of birefringence in the connective tissue stroma

and cross-polarization (*Fig. 1 c – e*), a non-matching pattern of structures (*Fig. 1 e, f*) and an uneven lower border (*Fig. 1 b-f*). Thus, with UPS, the reorganization of the connective tissue stroma is pronounced. Severe fibrosis of subepithelial structures (an increase in signal brightness in the cross-polarization compared to normal) with their thickening was recorded in 48.2% of cases, and thinning/absence of the epithelial layer was detected in 20.5% (*Fig. 1 d, e*). In patients with chronic inflammatory diseases, fibrosis in the urethral tissues was detected in 55.5% of cases, and hypo- or atrophy of the epithelium developed in 40.6% (*Fig. 1b, c*). When comparing images in co- and cross-polarization (bottom and top images) with pathological changes in the urethral wall (*Fig. 1 b-e*), it is obvious that the connective tissue in all cases has an uneven lower border (the signal amplitude doesn't steadily decay with depth) (*Fig. 1 b-e*), as well as either different thicknesses (*Fig. 1 c, d*), or a mismatched pattern of structures (*Fig. 1 d, e*).

According to the results of CP OCT, in women of reproductive age (up to 49 years old) with UPS, the differences between proximal 1/2 and distal 2/3 of the urethra were found. In the proximal part, CP OCT usually revealed the same changes as in the bladder neck, up to epithelial hyperplasia, while epithelial hypo- and atrophy was more often observed in the distal 2/3 of the urethra.

Analysis of age-related changes in the thickness of urethral tissues in three categories of patients showed that each of them had unique features. For example, for the UPS group, these changes correspond to an intermediate position between the controls and women with inflammation. In such cases, the epithelium was thinner than in normal conditions, and connective tissue stroma was significantly thickened. *Fig. 2* shows an example of the linear approximation of age-related changes in the thickness of the middle part of the urethra in patients with UPS, inflammation and women in the control group for the epithelium and connective tissue stroma, depending on the duration of the disease. There is a general tendency towards thinning of the epithelium and an increase in the thickness of connective tissue structures in both inflammation and UPS, and these changes depend on age in all groups, which is certainly associated with hormonal processes (*Fig. 2 a, b*).

3. Study of microflora of the proximal part of the urethra

The results of PCR of samples from the proximal part of the urethra are presented in *table 2*.

Only one patient (No. 9) had significant bacterial contamination of the biopsy specimen (total bacterial concentration = $10^{4.7}$). The main species in that case were Lactobacillus spp. ($10^{4.6}$) and Enterobacteriaceae ($10^{4.4}$). Moreover, patient No. 9 was found to have obligate anaerobes from the group of Clostridium spp./Lachnobacterium spp. ($10^{3.7}$), which may be involved in the development of bacterial vaginosis, while in the remaining 12 patients, bacteria of this

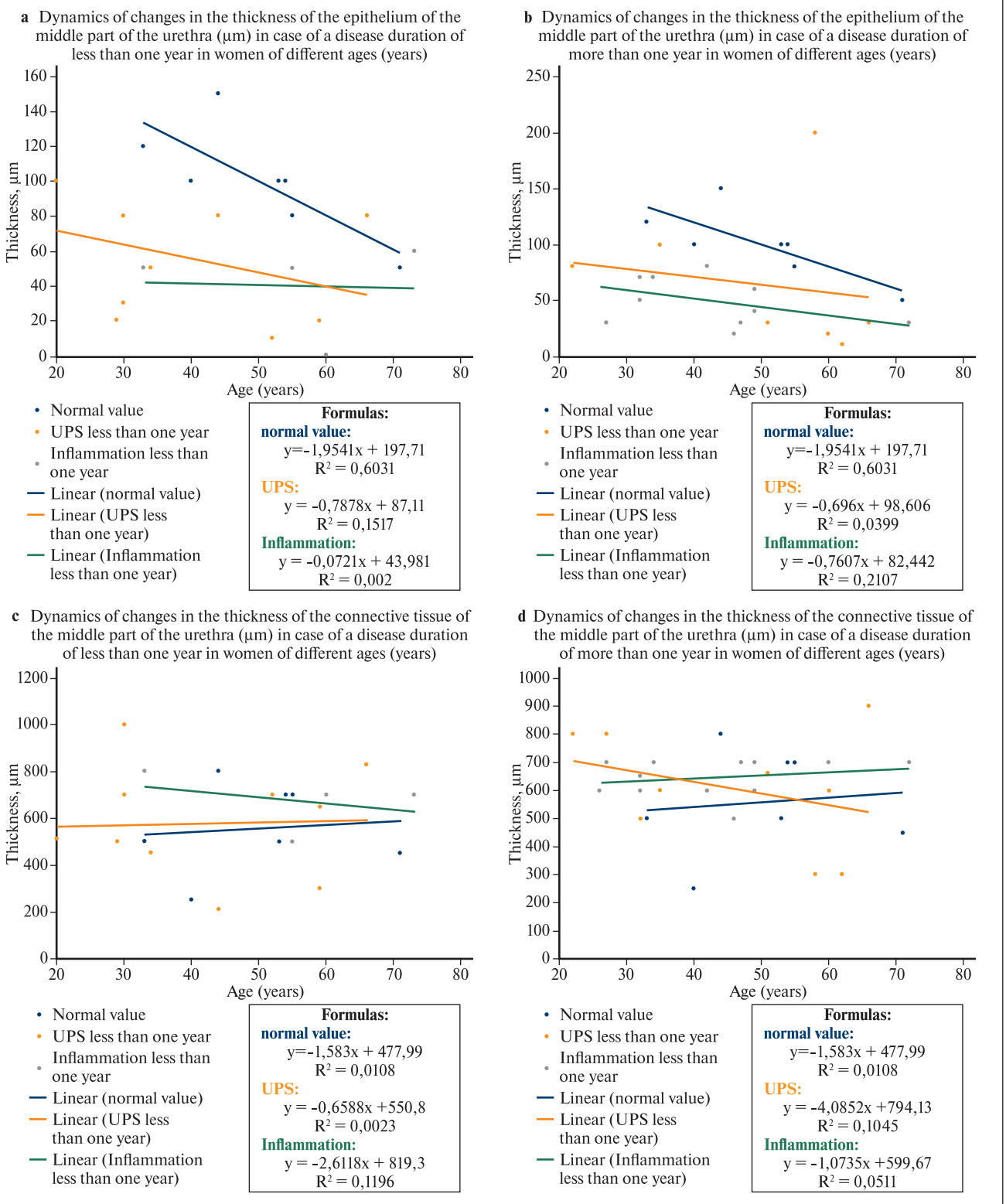

Fig. 2. Graphs of the linear approximation of age-related changes in the thickness of epithelium (a, b) and connective tissue stroma of the urethra (c, d) in otherwise healthy women (blue line), patients with UPS (orange line) and urethritis (green line) with a disease duration of less than one year (a, c) and more than one year (b, d)

group were not found. In another patient (No. 1), bacteria in a smaller but significant amount were detected in the sample from the urethral wall (total bacterial concentration = $10^{3.8}$). The predominant bacteria were Streptococcus spp. ($10^{3.9}$) and Enterobacteriaceae ($10^{3.7}$). In all other cases, the total bacterial concentration in biopsies was at the level of the negative group (a sample to which no DNA from patients was added). Low individual values of FEMOFLOR®-16 test, presented in *table 2* also cannot be considered as true positive.

Discussion. OCT is the unique modern non-invasive method for evaluating tissue structure, which has a resolution close to that of a cell (about 10–15 μm). It allows to assess the state

of tissues to a depth of 2 mm. Owing to use of probing light polarization in CP OCT it is possible to assess the connective tissue structures of the bladder and urethra [20, 21].

Age-related changes in the thickness of urethral tissues in UPS indicate their intermediate position between the normal state and inflammation. We showed earlier a significant rate of changes in the thickness of the epithelium in chronic inflammation in comparison with insignificant changes in UPS in the early stages. In addition, predominant changes in the thickness of the connective tissue stroma in the late stages of UPS in comparison with changes in chronic inflammation [10] became a rationale for our study. It was revealed that at the structural level, the pathogenesis of UPS is associated with the changes in the connective tissue stroma. This is reflected in an increase in the depth and level of cross-scattering on CP OCT (pronounced fibrosis), which occur in 48.2% of cases, up to birefringence effect [22, 23]. At the same time, in 32.5% of cases, a decrease in the signal on CP OCT was revealed, which is probably associated with a latent inflammatory process.

In turn, the analysis of concomitant diseases in patients with UPS indicates that women had a history of inflammatory processes of various localizations, which could serve as an underlying condition. It is known that inflammatory process becomes chronic due to transient dysfunction of the immune system, including functional insufficiency of the phagocytic cells [24]. Moreover, the infectious agent is able to persist inside the macrophage. On the other hand, macrophages serve as an effector of chronic inflammation with a predominance of cell proliferation and sclerosis [25]. Currently, a significant number of articles is dedicated to microsymbiocenosis in the female reproductive system. The combined influence of predominant microorganisms in the female reproductive system and endogenous host defense factors is to be studied [26]. Some authors obtained data on significant correlation coefficients between the types of microorganisms isolated from urine and large intestine of the same patient, which indirectly confirms the presence of a translocation mechanism [27]. In addition, studies by Yu. L. Nabok et al. (2019) of patients with urinary tract infection showed microbial interactions in the urinary tract with nearby biotopes (intestines and vagina). The significant correlation coefficients obtained by the authors between different taxa of the microbiota in the three studied samples prove the relationship between these loci [28].

In the work [6] there is a review of modern literature, dedicated to studying microbiota of the genitourinary system using chromatography-mass spectrometry and sequencing of urine specimens, which reflects cases of infection with potentially pathogenic microorganisms, despite negative routine urine tests. Data on the microbiocenosis of the urethral tissues (in particular, of the proximal part) have not been found in the available literature.

In a study of the microbial composition of a biopsy sample from the urethra, significant bacterial contamination was found in 2 (15%) out of 13 patients ($10^{3.8}$ and $10^{4.7}$). In patient No. 1 with total bacterial concentration of 103.8, the predominant species were Streptococcus spp. and Enterobacteriaceae. This 59-year-old postmenopausal woman had recurrent bacterial vaginosis as a concomitant disease. Another patient (No. 9) at the age of 31, with the highest bacterial contamination in the urethral sample (total bacterial concentration = 10^4), associated with Lactobacillus spp. (104.6), Enterobacteriaceae ($10^{4.4}$) and Clostridium spp./Lachnobacterium spp. ($10^{3.7}$) had a history of chlamydia and complained of a burning sensation in the urethra for many years. As a concomitant disease, vulvar condylomatosis was diagnosed. Thus, both women had symptoms and comorbidities related to the genitalia.

A pilot study (PCR of proximal urethral tissues) indicates the possible involvement of bacteria in the development of inflammatory processes in the urogenital tract, at least in some patients. In two cases, significant concentration of bacteria was detected (> $10^{3.5}$). Moreover, these women have concomitant gynecological infectious and inflammatory diseases. In 85% of cases, insignificant concentration of bacterial flora was detected, which unlike may contribute to the pathogenesis of UPS. Establishing the role of bacteria in initiating the pathogenesis of various diseases requires additional research.

Conclusions. In patients with UPS, several concomitant and often chronic diseases were revealed, which can serve as underlying condition and one of the triggering factors for the onset, and most importantly, maintenance of UPS.

CP OCT used in this work is currently the only in-vivo method for visualization of the urethral mucosa, which provides real-time evaluation of structural changes in the epithelium (atrophy or hyperplasia) and connective tissue (active or latent inflammation with cellular infiltration or fibrosis) of the urethra, allowing a better understanding of pathogenesis of the condition and monitoring of the therapy.

Pilot PCR studies of samples from the proximal urethra indicate that bacteria are present in the urethral tissues, but in 85% of cases their concentration is regarded as extremely insignificant and insufficient for affecting pathogenetic processes. However, for some patients, the significance of the

Table 2

Positive PCR test (FEMOFLOR®-16)

Samples: №/ isolated bacteria*	1	2	3	4	5	6	7	8	9	10	11	12	13
Total bacterial concentration	$10^{3,8}$	$10^{3,3}$	$10^{3,2}$	$10^{3,1}$	$10^{2,6}$	$10^{2,8}$	$10^{3,1}$	10^{3}	$10^{4,7}$	$10^{2,8}$	$10^{3,2}$	10^{3}	$10^{2,6}$
Lactobacillus spp.	0	0	0	0	0	0	0	0	$10^{4,6}$	0	0	0	0
Enterobacteriaceae	$10^{3,7}$	0	$10^{2,8}$	$10^{2,8}$	$10^{2,9}$	$10^{2,8}$	$10^{2,4}$	$10^{2,4}$	$10^{4,4}$	$10^{2,9}$	10^{3}	$10^{2,9}$	$10^{2,6}$
Streptococcus spp.	$10^{3,9}$	0	0	0	$10^{2,2}$	$10^{2,4}$	0	0	$10^{2,8}$	0	0	0	0
Staphylococcus spp.	$10^{2,3}$	$10^{1,7}$	$10^{2,6}$	0	$10^{2,4}$	102	$10^{2,6}$	0	$10^{2,9}$	0	0	$10^{2,6}$	0
Megasphaera spp./*Veilonella* spp/ *Dialister* spp.	$10^{2,3}$	$10^{1,9}$	0	0	0	0	$10^{2,4}$	0	0	0	0	0	0
Clostridium spp./ *Lachnobacterium* spp.	0	0	0	0	0	0	0	0	$10^{3,7}$	0	0	0	0

*Absolute analysis.
The total bacterial concentration, exceeding the value of $10^{3,5}$, are highlighted by the color

bacterial factor is not excluded. Undoubtedly, further research is needed to understand the pathogenesis of this socially significant group of diseases.

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A NOVEL ATRAUMATIC PUNCTURE NEEDLE «MG». RESULTS OF A COMPARATIVE MORPHOLOGICAL STUDY

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Introduction. Despite the low morbidity of percutaneous nephrolithotomy, this procedure has its inherent complications. One of the most important stages, which determines not only the stone-free status, but also the risk of complications, is the puncture of the collecting system.

Aim. To determine the efficiency and safety of the novel atraumatic puncture needle "MG" under experimental conditions.

Materials and methods. A series of porcine kidney punctures with a novel atraumatic needle "MG" and standard Chiba and Trocar needles (Coloplast A/S, Denmark) were performed under experimental conditions, followed by a comparative morphological evaluation. The staining of the specimens was done with hematoxylin and eosin. In order to additionally evaluate renal structures after being punctured with an atraumatic needle "MG", a morphological evaluation of the parenchyma stained with van Gieson's picrofuchsin solution was carried out.

Results. Morphological evaluation of the parenchyma after puncture with Chiba and Trocar needles showed the puncture tract with multiple large fragments of desquamated (damaged) epithelium. However, when an atraumatic needle "MG" was used, the puncture tract had clear contours. The damaged epithelium inside the puncture tract was virtually absent. Moreover, with additional staining with van Gieson's picrofuchsin solution (x200), unaffected adjacent blood vessels were visualized.

Conclusion. The novel atraumatic puncture needle "MG" is an innovative development in the urology. Its design with an atraumatic spring-loaded mandrel allows to significantly reduce the trauma to the kidney structures and perirenal tissues during tract dilation.

K e y w o r d s: percutaneous nephrolithotomy; kidney puncture; puncture nephrostomy; atraumatic needle; surgical complications; urolithiasis

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Introduction. For the first time, percutaneous nephrolithotomy (PCNL) as a surgical procedure was described by I. Fernström and B. Johansson in 1976 [1]. Owing to J. Wickham et al., who published the first successful series in 1981, PCNL almost completely replaced open and then laparoscopic interventions in the treatment of nephrolithiasis [2]. During 40 years of active implementation of PCNL as a surgical procedure, it has continuously undergone modifications, which combines scientific and technological innovations. Currently, PCNL is the first-line treatment for large or complex renal stones [3].

Due to the high stone-free rate (SFR), PCNL clearly demonstrates its efficiency, but it is not without complications [4]. According to international and domestic publications, the rate of complications ranges from 20.5 to 23.3%. The most common intrarenal complications are bleeding, perforation of the collecting system and urinary tract infections [4-6].

The puncture of the collecting system is one of the most important steps of the procedure, during which up to 18% of complications may occur [7]. Creation of optimal access is the most demanding initial stage. To date, many techniques and methods have been proposed to minimize complications during puncture: ultrasound tips for precise needle guidance, laser and electromagnetic guidance, and robotic systems for needle insertion [8]. All of them aim to reduce damage to intra- and extrarenal structures by increasing the accuracy of puncture. Despite the measures taken over the past 30 years to reduce the intrinsic traumatic ability of the needle, the proposed options for sharpening the cutting tip did not have significant influence. In this publication, we describe experimental studies and results of puncture of the porcine kidney with a novel atraumatic needle "MG" with pathomorphological comparative evaluation of the results.

Aim. To determine the efficiency and safety of a novel atraumatic puncture needle "MG" in experimental conditions.

Materials and methods. In order to reduce the trauma to the kidney structures during puncture, it is proposed to use a novel atraumatic puncture needle "MG" (patent PM RF No. 201910), shown in *figure 1.* The needle is two-component, consisting of a cannula and mandrel, with a working length of 20 cm and an outer diameter of the cannula of 18 G (1.219 mm). It allows comfortable puncture and is consistent with needles used in routine practice for puncture of the collecting system during PCNL or placing nephrostomy tube. Special markings on the needle (in 1 cm increments) with a thickening on every 5th mark (5 cm) facilitate a measurement of the inserted part of the needle. In addition, ultrasonic marking on the cannula and mandrel allows for better visualization.

The innovative distinguishing feature of needle "MG" is its design. The distal end of the needle consists of a pointed

Fig. 1. Atraumatic "MG" needle for puncture

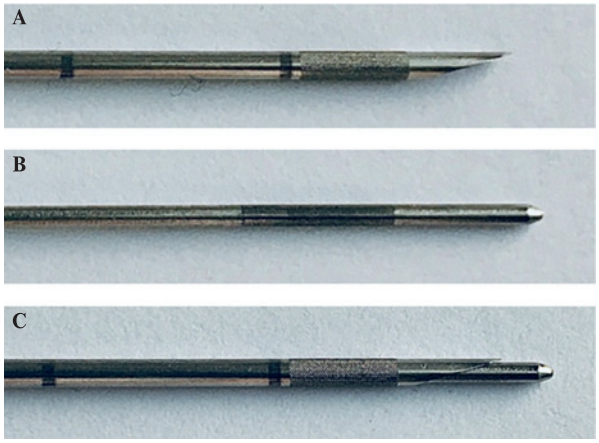

Fig. 2. The distal end of the atraumatic "MG" needle. (A) tapered cannula; (B) atraumatic mandrel bulb; (C) assembled needle

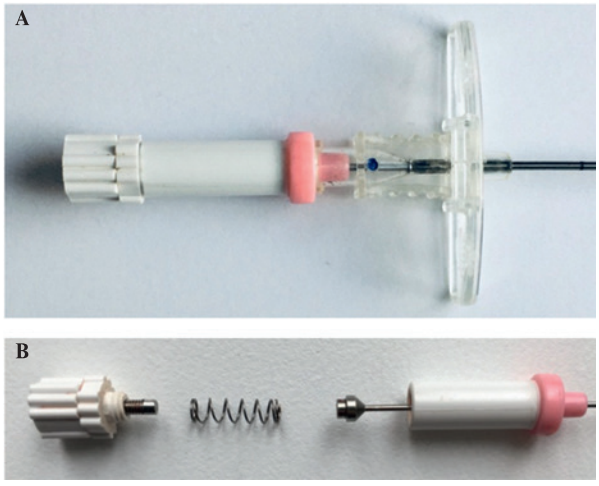

Fig. 3. The proximal part of the atraumatic needle. (A) Connection of different parts of a needle; (B) atraumatic spring-loaded mandrel

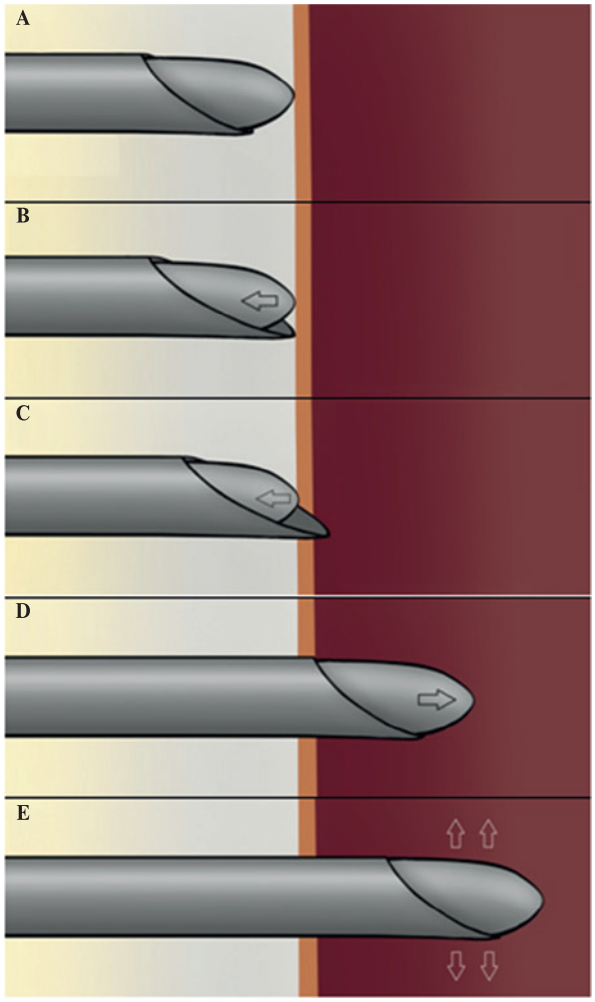

Fig. 4. Scheme of puncturing by atraumatic needle "MG". (A) Needle touches dense structure; (B) spring-loaded mandrel goes behind a sharp cannula; (C) the needle pierces the dense structure; (D) returning the mandrel to its original position; (E) dilation of the soft tissues

cannula, sharpened as Quincke-type, and an atraumatic bulb mandrel * without cutting edges (*fig. 2*). Due to the principally different structure of the mandrel, which has a mobile rounded bulb, protruding beyond the sharp cannula, needle "MG" has atraumatic properties.

The proximal part of the needle is represented by the connection of pavilions with a special handle for fingers in order to provide tight fixation during puncture (*fig. 3*). A special feature of this needle is the design of the mandrel pavilion, in which the spring mechanism is hidden. Due to the spring, the atraumatic part of the bulb mandrel has the ability of reciprocating movements with a total amplitude of 5 mm (*fig. 3*).

When performing a puncture of the collecting system, the needle touches a number of anatomical structures of different thickness and density (skin, aponeurosis, Gerota's fascia, renal capsule, veins, intrarenal structures, mucosa of the collecting system).

Depending on the design of the needle tip (Chiba needle of Quincke-type, Trocar needle of Pencil-Point), dissection of anatomical structures during needle passage occurs differently. However, regardless of the designs, tissues are dissected (cut) by the needle. Unlike its counterparts, during puncture of the collecting system with needle "MG", bulb mandrel first touches dense anatomical structures (*fig. 4*). If the density of the structures does not allow them to be displaced (to dilate), bulb mandrel is displaced beyond the pointed part of the cannula, and the latter pierces the structure. As soon as the needle passes through dense structures owing the cutting tip of the cannula, the atraumatic tip automatically returns to its original position. Passage of the needle due to bulb mandrel allows for spreading soft tissue structures of the kidney, rather than dissect them by the cutting part of the needle. Creating the effect of soft tissue dilation contributes to minimal trauma during the puncture.

To determine the efficiency and safety of puncture needle "MG", a comparative morphological evaluation of porcine kidneys was performed. In the study, in addition to needle "MG", standard Chiba and Trocar needles were also used (Coloplast

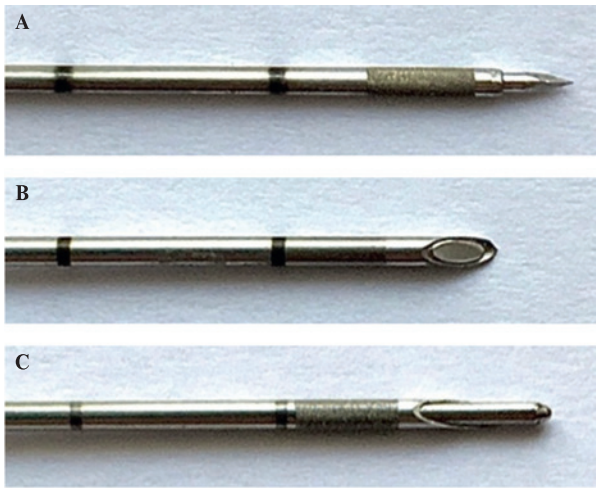

Fig. 5. Puncture needles for access to the collecting system. (A) Needle Trocar; (B) Chiba needle; (C) atraumatic "MG" needle

A/S, Denmark). All needles had an equal outer cannula of 18 G (*fig. 5*).

For the morphological study, a porcine kidney was initially prepared. To perform an accurate puncture of the collecting system (avoiding the entry between pyramids), an artificial dilatation was created by introducing 15 ml of normal saline under ultrasound control through the ureter. At the second stage, punctures of calyces under ultrasound guidance using a linear probe were performed with three different needles (*Fig. 6*). Then, the porcine kidney, punctured with an atraumatic needle "MG", Chiba and Trocar needles (*Fig. 7*), was fixed with 10% formalin solution and sent for morphological examination.

After fixing the kidney in 10% formalin solution, three fragments of kidney tissue were taken from each area (after puncture by Chiba, Trocar and atraumatic needle "MG"). When preparing histological slides, dehydration with alcohol solutions was performed to avoid wrinkling. Then, embedding into paraffin pre-impregnated with xylene at a temperature of 54–56ºC in three portions for creating paraffin blocks was done.

For the study, 9 paraffin blocks were prepared from the porcine kidney, from which histology slides were done. Each slice was up to 4 micrometers thick (4 microns or 0.004 mm). Staining of histological slide preparations was carried out in three stages: staining of nuclei with Mayer's hematoxylin; staining of the preparation with eosin; staining by carbol-xylene as clearing agent. To perform complete pathological and morphological assessment of the parenchyma punctured with an atraumatic needle "MG", an additional assessment of the micropreparation stained with van Gieson's picrofuchsin was done, which allows to evaluate more precisely structural changes in the tissue, especially collagen fibers. The slides were initially stained by Weigert's iron hematoxylin. After water washing, staining with picrofuchsin (picric acid solution and 1% aqueous acid fuchsin) was performed with subsequent washing in water, 96% alcohol and xylene solution. The material is represented by 15 preparations. The histological study was performed using a Leica DM2000 microscope. The microphotographs presented in the publication were taken using a Leica EC3 camera.

Results. During examination of the parenchyma of a porcine kidney punctured with a Chiba needle (*fig. 8*), the needle tract was visualized, in which there was a parenchyma with small fragments of desquamated damaged epithelium and free red blood cells. Large fragments of the parenchyma in the needle tract, clearly visible at x50 magnification, were the result of a large cutting plane of the puncture needle.

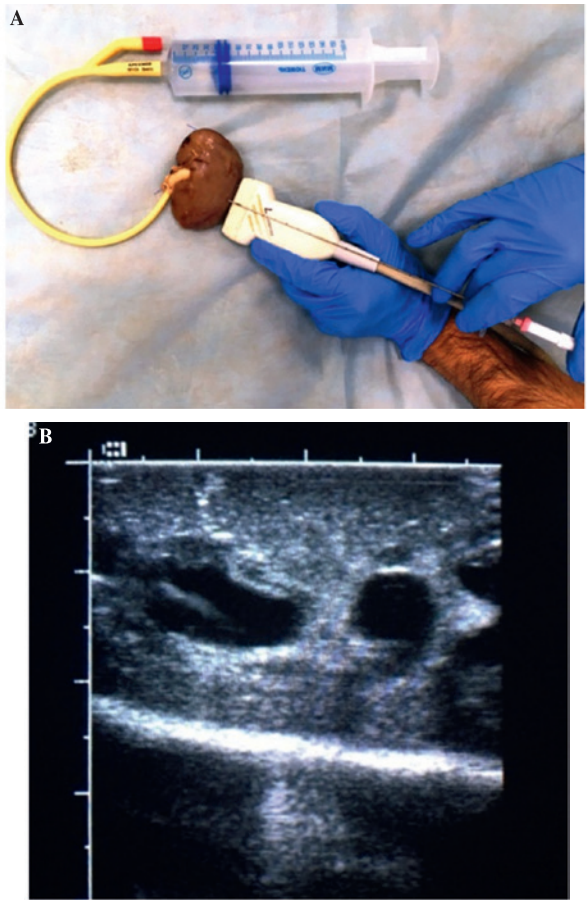

Fig. 6. (A) Puncture of the collecting system of a porcine kidney under ultrasound control; (B) ultrasound picture

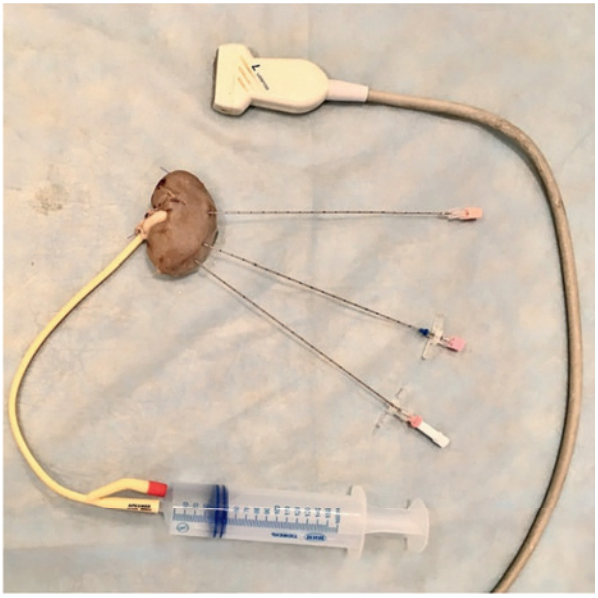

Fig. 7. Gross picture of a porcine kidney after puncture with Chiba, Trocar and atraumatic "MG" needles (from top to bottom)

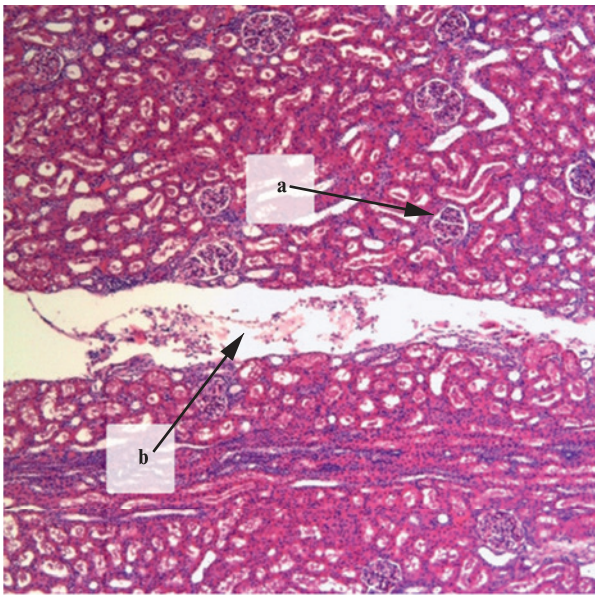

Fig. 8. Micropreparation after puncturing by Chiba needle through the parenchyma (hematoxylin and eosin, x50 magnification). (a) Preserved renal glomeruli; (b) needle tract; damaged, desquamated epithelium and free red blood cells are detected

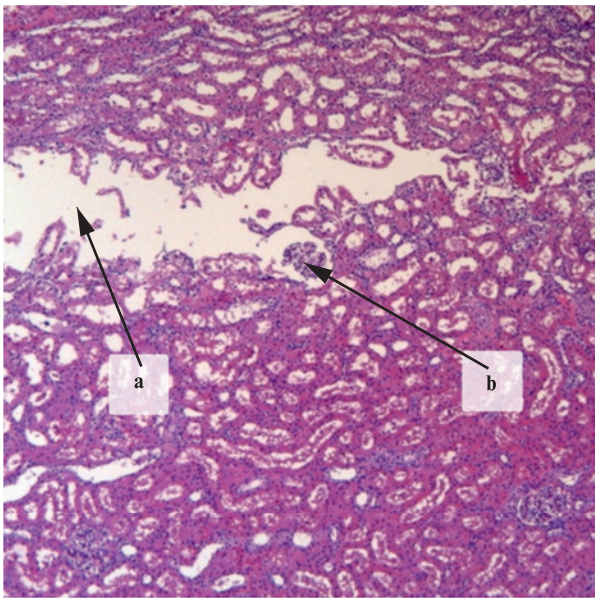

Fig. 9. Micropreparation after puncturing by trocar needle through the parenchyma (hematoxylin and eosin, x50 magnification). (a) needle tract; (b) fragments of convoluted tubules and desquamated, damaged epithelium

On the specimen (*Fig. 9*) after a puncture with a trocar needle at x50 magnification, a desquamated epithelium with uneven edges of the needle tract was observed. With an additional magnification of x200, damaged tubules and large fragments of the glomerulus were clearly visualized.

The study of the parenchyma after puncture with atraumatic "MG" needle (*Fig. 10*), stained with hematoxylin and eosin, at x50, x100 and x200 magnification, showed the needle tract with clear contours and preserved structure of the tubules. There was virtually no damaged epithelium, which was represented only by single small fragments.

According to the results of staining with van Gieson's picrofuchsin (*fig. 11*) on specimens of the parenchyma at x200 magnification, there was a small amount of desquamated epithelium inside the needle tract. The defect had clear edges with preserved integrity of nearby structures. The renal capsule, formed by connective tissue fibers, slightly turned toward the defect. When examining gross specimen, the integrity of the structure of a full-blooded vessel near the needle tract was clearly visualized.

Owing to the atraumatic bulb mandrel, parenchyma puncture was carried out by dilation (spreading) of the structures.

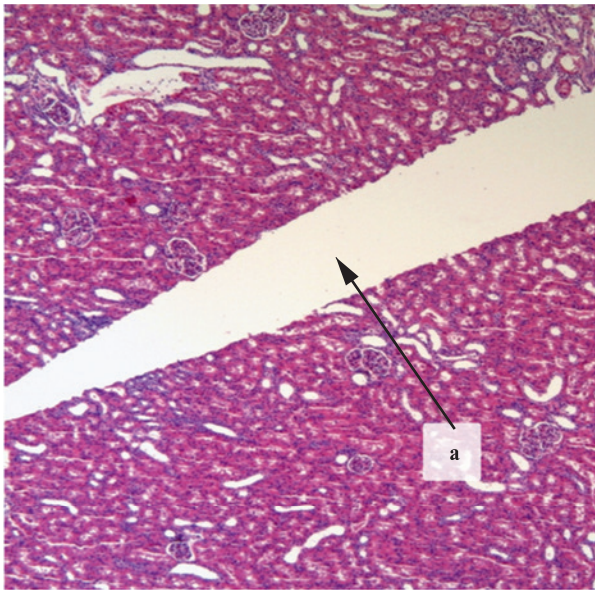

Fig. 10. Micropreparation of the parenchyma after passage of the atraumatic MG needle (hematoxylin and eosin, magnification X50). (a) Needle insertion site with clear contours

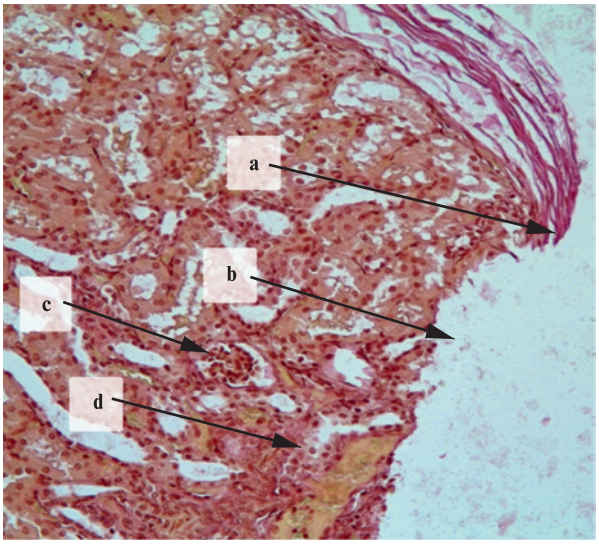

Fig. 11. Micropreparation of the parenchyma after passage of the atraumatic MG needle (van Gieson's picrofuchsin, x200 magnification). (a) Connective tissue capsule, damaged by the needle; (b) desquamated epithelium at the site of needle penetration; (c) preserved glomerulus; (d) blood-filled vessel

Compared to the Chiba and Trocar needles, needle "MG" better preserved the integrity of the parenchyma, avoiding unnecessary trauma to the nearby glomeruli, tubules, and also helped to avoid the injury of full-blooded vessels.

Discussion. PCNL is a minimally invasive surgical procedure for patients with renal stones. However, like any surgical intevention, it is associated with a number of complications. The most common intrarenal complications are bleeding, perforation of the collecting system and upper urinary tract infections [4].

It is very important to precisely plan the upcoming surgery by performing computed tomography (CT) [9]. If a patient has a complex stone, as well as anatomical features that can complicate PCNL and puncture, it is possible (based on contrast-enhanced CT) to perform 3D modeling. Three-dimensional reconstruction of the kidney with vessels and collecting system allows planning the procedure [10].

Close attention to the renal anatomy is associated with many large blood vessels and capillary network, due to which the kidneys have a good blood supply, which constitutes up to 1200 ml (25% of the total cardiac output circulates through the kidneys) [11].

Bleeding is one of the most common complications during PCNL, which can occur intra- and postoperatively. Injury to large segmental vessels can lead to massive bleeding. According to C. Seitz et al., transfusion rate in the postoperative period reaches 7% [4].

Hemorrhage that does not require additional therapy, although not a complication, however, may worsen visibility intraoperatively and affects the SFR [12]. Such bleeding occurs due to microtrauma of the parenchyma during tract dilation, excessive bending of the nephroscope, and puncture (especially with repeated attempts) [13, 14].

Puncture of the collecting system is one of the most important stages of PCNL [7]. The original description of the technique was provided by Seldinger in 1953 for the implementation of vascular access through a guidewire [15]. To put the guidewire in the collecting system during PCNL, the puncture is carried out from the posterolateral position through avascular plane (Brodel line), which has low vascular density in order to possible reduce the risk of injury of the large vessels [16]. To decrease the likelihood of damage to the interlobar arteries and excessive tearing of the parenchyma with a rigid nephroscope, the trajectory of the needle should be directed to the center of the pelvis when it enters the calyx, bypassing the Bertine columns [17]. Reducing the number of punctures helps to preserve the parenchymal structures, decrease the likelihood of bleeding, and also improves the quality of the procedure [18].

When performing a puncture, many techniques have been described using X-ray and ultrasound guidance. A hybrid technique using the C-arm has become one of the most innovative ones. By combining the advantages of biplanar methods (triangulation and "bull's eye"). G. Sharma et al. achieved the highest puncture accuracy (single-attempt puncture was possible in >95% cases) [19].

Owing to the development of modern technologies, auxiliary means for guiding and fixing the puncture needle have been developed to improve the safety of the puncture:

- ultrasonic navigation systems projecting a three-dimensional image by several sensors [20, 21];
- robotic navigation systems that automatically align the puncture needle to the desired calyx for comfortable and correct puncture under C-arm guidance [22];
- an electromagnetic tracking system that facilitate aiming due to initial retrograde placement of the sensor along the ureteral sheath [23];
- devices for comfortable fixation of the needle during aiming under C-arm guidance [24] [25];
- a mobile device for augmented virtual reality that overlays a three-dimensional image of the kidney anatomy on the patient in real time [26].

The above techniques minimize the likelihood of complications owing to accurate access to the collecting system. To reduce trauma to the parenchyma, an alternative Chiba puncture needle with an outer diameter of 21G (0.8 mm) can be used. On the one hand, the small diameter of the needle helps to reduce the area of injury, which is especially useful for unexperienced urologists who have difficulties with single-attempt puncture. However, one of the main disadvantages of such a needle is its rigidity. Due to its dimensions, the needle has a more flexible structure, which affects its trajectory (especially in patients with obesity or fibrous scars around the kidney). Another disadvantage of thin needle is the inability to use standard 0.035-inch guidewires. To perform comfortable dilation over a standard guidewire, after puncture with a 21G needle the surgeon has to initially put a 0.018-inch guidewire and then replace it with a standard one [27]. The atraumatic needle "MG" is devoid of these disadvantages, since it achieves reduced traumatization due to the atraumatic bulb mandrel, and the standard diameter of the outer cannula (18G) additionally gives necessary rigidity during the puncture.

Conclusion. A puncture of the collecting system is the most important stage of PCNL. To date, many techniques have been proposed that can reduce trauma to parenchyma during the puncture. The atraumatic needle "MG" is an innovative tool that allows to dilate soft tissues due to the atraumatic bulb mandrel. Demonstrating high safety for parenchymatous structures under experimental conditions, the needle is of high interest for further clinical research.

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CHANGES OF EPITHELIAL CELLS OF THE NEPHRON IN UNILATERAL URETERAL OBSTRUCTION (EXPERIMENTAL STUDY)

Rostov State Medical University, Rostov-on-Don, Russia

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Introduction. The high prevalence of kidney diseases caused by urinary tract obstruction has led to the need for experimental studies of the dynamics of pathological processes. Despite the fact that the general patterns of development of obstructive uropathy are known, the features of renal tissue damage, in particular structural and molecular biological changes in this pathology, remain insufficiently studied.
Aim: study the dynamics of changes in the phenotype of the epithelial cells of the nephron in unilateral ureteral obstruction in an experimental model.
Materials and methods. The experimental study was carried out on the basis of the Rostov State Medical University. The model of unilateral ureteral obstruction was reproduced in adult rabbits. The studies were carried out on the 7th, 14th and 21st days of complete obstruction of the left ureter. Immunophenotyping of obstructive kidney tissue samples was performed for markers of epithelial phenotype (cytokeratin 7, E-cadherin) and mesenchymal phenotype (vimentin, α- smooth muscle actin).
Results. The sequence of changes in the phenotype of nephron epithelial cells during ureteral obstruction has been established. The first signs of an epithelial-mesenchymal transition (EMT) appeared by day 7 in the form of a decrease in visualization of markers of the epithelial phenotype. On the 14th day, the expression of both epithelial and mesenchymal markers was noted. Significant changes in the phenotype of nephron epithelial cells, such as loss of epithelial markers (cytokeratin 7, E-cadherin) and the acquisition of mesenchymal markers (vimentin, α- smooth muscle actin), were noted by the 21st day of the experiment.
Conclusion. An experimental model of unilateral ureteral obstruction revealed the transformation of the nephron tubule cell phenotype from epithelial to mesenchymal.

K e y w o r d s: *experimental model, unilateral ureteral obstruction, obstructive uropathy, epithelial-mesenchymal transition, immunophenotyping.*

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Introduction. Ten percent of the world's population suffers from chronic kidney disease [1], and ureteral obstruction remains one of the most common causes. The wide prevalence and recurrent nature of renal disorders have determined the main areas for scientific studies, which are aimed at understanding the mechanisms underlying the development of the fibrotic process in kidney damage [2].

Currently, epithelial-mesenchymal transition (EMT) is considered as one of the leading pathways for the progression of chronic kidney diseases as a reflection of the morphological and functional plasticity of epithelial differentiation [3]. EMT is a unique biological process that includes clear molecular reprogramming and phenotypic changes characterized by the transformation of polarized epithelial cells into scattered mesenchymal cells, which leads to an increase in their mobility [4–9]. It is established that epithelial cells are closely interconnected, which prevents their possible migration from the epithelial layer. Mesenchymal cells do not form cell layers or intercellular adhesion complexes; the elongated shape and polar plasticity of mesenchymal cells contribute to their migration ability. In this regard, epithelial cells exposed to EMT acquire a fibroblastic character and lose their functions [10].

Understanding the origin of myofibroblasts in the kidneys is of interest, since these cells are responsible for renal fibrosis [11–12]. Under physiological conditions, a mass of myofibroblasts accumulates at areas of inflammation and repair. In such cases, myofibroblasts act as reparative cells, producing and organizing the extracellular matrix, as well as restoring the integrity of tissues after damage [13]. However, in pathological healing process, activated fibroblasts create a hard, collagen-rich scars that disrupts tissue structure, changes the biochemical and biophysical microenvironment, and leads to tissue dysfunction [14]. There are evidences that EMT is one of the pathways for the development of activated fibroblasts from epithelial cells [15]. The mechanisms underlying the regenerative ability of the kidneys are also under discussion, and EMT is considered as one of such mechanisms [16–18]. In this regard, studies of the dynamics of changes in the epithelium of different nephron parts in obstructive uropathy, as the most common the upper urinary tract pathology, are of relevance. The model with unilateral ureteral obstruction (UUO) allows to quickly achieve the terminal stage of the process [19].

Aim. To study the dynamics of changes in the phenotype of the epithelial cells of the nephron in UUO in an experimental model.

Materials and methods. The content, nutrition, care and euthanasia of animals were carried out in accordance with the regulations for working with laboratory animals at the Central Research Laboratory of Rostov State Medical University. The studies were approved by the local independent ethical committee (protocol No. 21/15 dated 12/10/2015). The

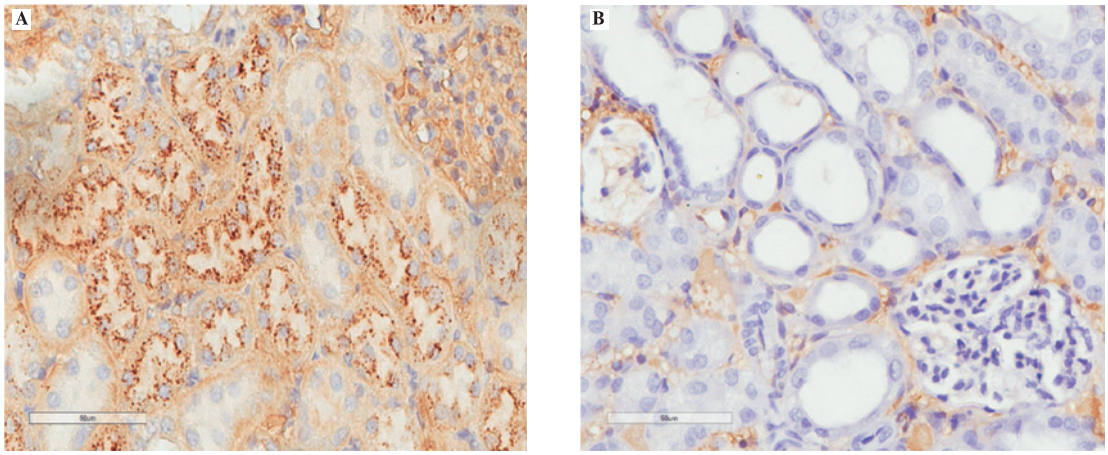

Fig. 1. The expression of E-cadherin in the epithelial cells in nephron in the control (A) and on the 21st day after acute ureteral obstruction (B); magnification × 400

experimental model of UUO on 24 adult rabbits (males aged 3.5 months weighing 2.4–2.75 kg) was reproduced according to the method of E. Giamarellors-Bourbalis et al. [20]. The animals were divided into 4 groups of 6 animals each: one control group and three experimental groups, depending on the duration of the obstruction (days 7, 14, and 21). Kidney tissue samples for light microscopy and immunohistochemical studies were fixed with 10% buffered neutral formalin and embedded in paraffin according to the classical method [21].

Immunophenotyping of tissue samples obtained in the experiment was carried out for markers of epithelial phenotype (cytokeratin-7 [CK7] and E-cadherin) and mesenchymal phenotype (vimentin [Vim], smooth muscle actin [α-SMA]). Primary mouse monoclonal antibodies were used, including Anti-Cytoceratin-7 (Abcam 9021, USA, dilution 1:200), Anti-E-cadgerin (Abcam 233766, USA, dilution 1:150), Vimentin antibody (Biorbyt 317381, United Kingdom, dilution 1:200), Smooth Muscle Actin antibody (Biorbyt 334169, United Kingdom, dilution 1:250). The formed antigen-antibody complexes were visualized using detection system EnVision FLEX (Dako, Denmark).

Microscopy and photography of the samples were carried out on an Automated Upright Microscope System with LED Illumination for Life Sciences Leica DM4000 B LED (Germany). The results of immunohistochemical studies were evaluated by the intensity of staining for each of the markers.

Results. From days 7 to 21, there was a progressive decrease in cytoplasmic staining of CK7 epithelial cells of the proximal and distal tubules and collecting ducts. Therefore, the first signs of a change in the phenotype of epithelial cells of the nephron appeared on the 7th day of a complete UUO. By the 14th day of obstruction, the dilatation of the collecting ducts and tubules of the nephron was determined. By the 21st day, tubular lumen was significantly dilatated in most nephrons, and it was lined with a severely flattened epithelium, slightly stained for CK7.

Concentration of E-cadherin, which belongs to calcium-dependent cell adhesion molecules and is involved in the regulation of intercellular adhesion, cell motility and proliferation of epithelial cells, also decreased during the experiment; however, the expression of the marker was more pronounced in the epithelium of the distal convoluted tubules and collecting ducts, while in the epithelium of the proximal convoluted tubules the expression was weak (*fig. 1*). A decrease in the amount of the E-cadherin protein in the epithelium of the tubules during the experiment led to the dissociation of epithelial cells due to the weakening of intercellular contacts.

The study of the expression of the marker of the early stages of EMT, vimentin, which provides cell strength and their resistance to mechanical stress [23], showed a significant increase in the number of vimentin-positive cells with prolonged UUO, which confirms the progression of fibrosis of the kidney interstitium.

Starting from the 7th day of UUO, the appearance of the actin protein α-SMA, which is a marker of the mesenchymal phenotype, in the distal convoluted tubules and the glomerular capsule was seen (*Fig. 2A*). With an increase in the duration of obstruction, an increase in the number of α-SMA-positive cells was also observed, which indicates an active synthesis of cytoskeletal proteins and dysfunction of epithelial cells (*Fig. 2 B*).

In addition, by the 14th day of UUO there was another sign of EMT, namely the transition from cytokeratin intermediate filaments to vimentin ones. The simultaneous presence of markers of both epithelial and mesenchymal phenotypes in the nephrons of an obstructive kidney indicates the possibility of reversibility of the processes in case of eliminating the damaging factor. However, by the 21st day of UUO, gross structural changes were recorded in the nephrons of the affected kidney, such as dilatation of the tubular lumen, epithelial flattening, while in the distal tubules cystic or pseudocystic transformation with pronounced epithelial atrophy was seen. In the interstitium, where large areas of interstitial fibrosis developed, cells expressing Vim and α-SMA were predominated.

Discussion. The physiological adaptation of the nephron to maintain homeostasis in UUO included a change in the size of the nephron parts, a limitation in the effective surface area for reabsorption, and a change in the length of the tubules that create resistance to urine flow. There are evidences of changes in the proximal tubules in acute kidney injury and during progression of kidney disease [25]. The development of atrophy and fibrosis of hypertrophied nephrons is non-adaptive since it is regarded as a deviation from the normal homeostatic response [26]. The study of variations in the genotype and phenotype of nephrons in UUO provides a new understanding of their susceptibility to stress during the obstruction.

Chronic obstructive uropathy caused by prolonged UUO in the rabbit is morphologically characterized by interstitial fibrosis and tubular dilatation. Damage to the epithelium leads to disruption of intercellular connections and connections between

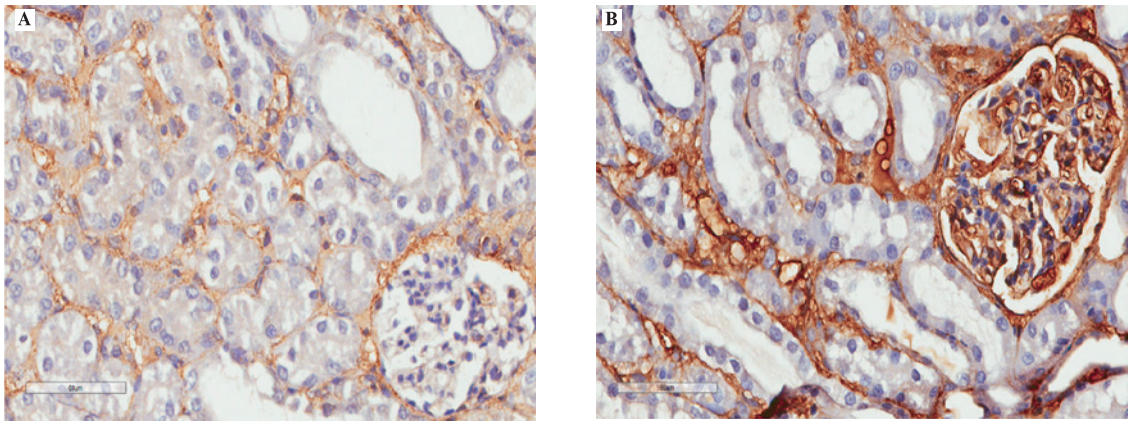

Fig. 2. The expression of mesenchymal marker α-SMA in the epithelial cells in nephron in the control (A) and on the 21st day after acute ureteral obstruction (B); magnification × 400

cells and the basement membrane, which leads to disruption of intercellular interaction, intercellular transport and exposure of cells to mechanical stress.

According to our results, the first signs of EMT appeared by the 7th day as a decrease in content of epithelial phenotype markers with preservation of nephron functions. By the 14th day of the obstruction, profound restructuring of the morphological and functional properties of the epithelial cells of the nephron kidney was found. By this time, markers of both epithelial (CK7, E-cadherin) and mesenchymal (α-SMA and Vim) types were visualized in the renal tubules, which indicates an active process of EMT of epithelial cells of the renal tubules and their conversion into myofibroblasts with fibrous changes. The progressive nature of the transition of epithelial into mesenchymal cells was determined by the 21st day, which was confirmed by the almost complete loss of epithelial markers and the appearance of mesenchymal markers.

Based on our results, we can conclude that long-term UUO provokes the activation of apoptosis and atrophy of the epithelium or leads to EMT with a change in the phenotype not only of nephron cells, but also of the interstitium. During immunophenotyping of kidney tissue under obstruction, the following molecular biological changes were observed, confirming active remodeling of the epithelium: 1) a decrease in the content of molecules and structures that determine the epithelial phenotype, such as E-cadherin (intercellular adhesion protein) and CK7 (cytoskeleton protein); 2) the appearance of the structures that point to mesenchymal phenotype, including cytoskeletal proteins (Vim, α-SMA). Visualization of α-SMA and Vim in the areas of fibrosis indicates an increase in the number of mesenchymal cells (fibroblasts) during the experiment, which ultimately results in renal tissue dysfunction.

Conclusion. The experimental model of UUO showed a transformation of the epithelial cells of renal tubules to mesenchymal phenotype.

Despite a plenty of studies on the role of EMT in the pathogenesis of kidney disease, there was a revolution in our understanding of kidney cell plasticity in the last decade. However, the question of the role and degree of EMT in renal fibrosis remains unclear. Understanding this mechanism can shed light on the pathogenesis of this process. The study of early molecular biological markers of EMT opens up prospects for the development of targeted therapy for renal fibrosis.

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MORPHOFUNCTIONAL CHARACTERIZATION AND DNA STABILITY OF SPERMATOZOA IN PRIMARY EXAMINATION OF MEN FERTILITY

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Aim. To assess the morphological and functional characteristics of spermatozoa and DNA stability in patients with normal seed parameters and with teratozoospermia index exceeding the threshold value.
Patients and methods. 85 males were studied for semen quality parameters. The morphological anomalies of head, middle piece, and tail of spermatozoa were estimated and presented in illustrations. Due to results elaborated the index of teratozoospermia was calculated. Then the Comet assay was performed.
Results. It has been found that spermatozoa from patients with high teratozoospermia index possessed decreased motility as compared to ones from patients with low teratozoospermia index. There was no difference in concentration of spermatozoa between males with low and high index of teratozoospermia. The spontaneous frequency of DNA damage estimated by means of single cell gel electrophoresis followed by silver staining in spermatozoa from healthy donors did not exceed 3,1±0,6. The frequency of DNA damages in sperm of patients with abnormal index of teratozoospermia exceeded 39,6±7,4% ($p<0,01$).
Conclusions. Our results suggest that minor morphological changes in spermatozoa do not affect their motility, while significant morphological abnormalities with ITZ exceeding threshold values are closely associated with impaired DNA stability and dramatically reduce the quality of patient's seed.
Summary. In the initial assessment of patient fertility, the attention should be focused on a comprehensive study of sperm DNA morphology and stability.

K e y w o r d s: morphology of spermatozoa, teratozoospermia index, comet assay

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Introduction. The problem of the reproductive health is one of the factors of the national security of the state. Poor reproductive health of one of the parents makes it impossible to achieve a pregnancy. Based on studies in specialized reproductive centers, it was found that the proportion of men who have difficulties in conceiving a child reaches 30%. Various hypotheses have been proposed to explain the decline in male fertility. According to a number of authors, infectious agents are one of the leading causes of impaired reproductive health in men [1, 2]. The environmental influence on reproductive health is regularly discussed, and if the radiation exposure has been studied sufficiently with the exception of the « radiation-induced bystander effect», the influence of xenoestrogens, pesticides and heavy metals on male fertility still are subject to intensive research. For example, exogenous estrogens disrupt not only the prenatal development of testicles, but also their postnatal development, and the process of spermatogenesis [3]. Along with xenoestrogens, pesticides may damage the hormonal regulation of spermatogenesis [4]. The toxic effect of heavy metals on spermatogenesis cells has been studied extensively [5]. Lifestyle and addictions are also suggested to affect reproductive health. A measurement of seminal plasma cadmium level revealed that it is significantly increased in smokers [6].

As follows from these examples, a myriad of factors may potentially have a deleterious effect on both the process of spermatogenesis and spermatogenesis. In addition, they can affect both the functional characteristics of spermatozoa (mostly, motility) and their morphological structure. Currently, it is possible to standardize the morphological assessment of sperm cells, which resulted in the development of the so-called strict criterion [7]. This allowed not only to performed morphological study in a standardized fashion, but also to develop informative criteria and coefficients (for example, the teratozoospermia index [TZI]) in order to accurately assess the reproductive health based on structure of sperm cells. At the same time, the data on the TZI and other parameters reflecting the sperm quality are still contradictory [8]. Therefore, the aim of this study was to assess the morphological and functional characteristics of sperm cells and DNA stability in patients with normal sperm parameters and with TZI exceeding the threshold value.

Patients and methods

During the period from 2017 to 2019, a total of 85 patients of average age 32 years were evaluated for infertility (no pregnancy within a year). Abstinence period was at least 3 days (determined during the interview). Sperm analysis was performed on average

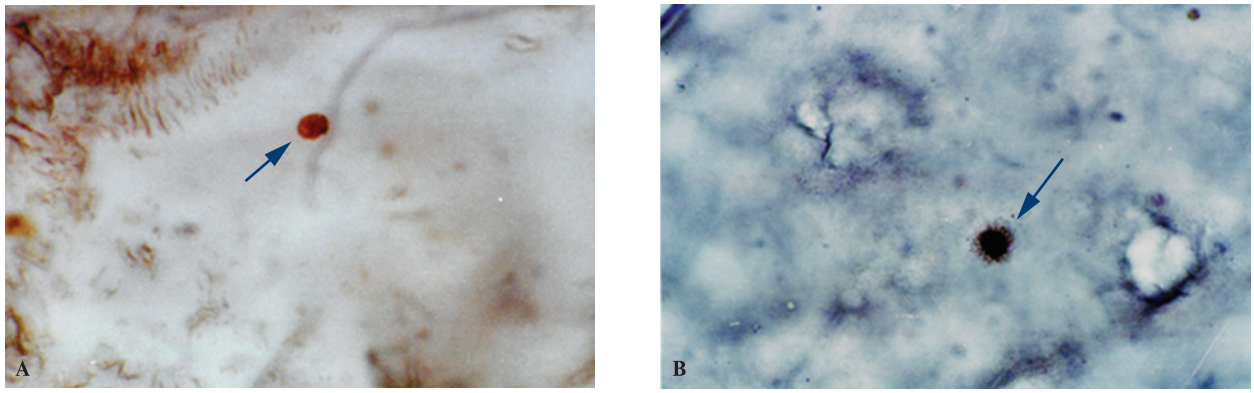

Figure. Sperm cell with normal (A) and impaired (B) DNA stability. Sperm cells are shown by arrows. Microelectrophoresis of DNA of single spermatozoa. Silver staining. Magnification 1000x

on the 4th day. The ejaculate was collected in sterile dry transparent container by masturbation and immediately sent to the laboratory for analysis. The container was stored at 37°C. According to the routine conditions, not only the structural and functional characteristics of spermatozoa were analyzed, but also physicochemical parameters, such as liquefaction time, viscosity, pH, etc. [1, 9]. Depending on the motility, spermatozoa were divided into three classes: active (speed of more than 16 μm/s), inactive (speed of less than 16 μm/s) and immobile. The concentration of spermatozoa was determined in the Goryaev's chamber (sperm without tail were not considered). Immobilization and dilution of spermatozoa were carried out with Barbagallo's solution. To study the morphology of sperm cells, samples were prepared on glass slides, which were dried at room temperature and fixed in absolute alcohol for 10 min. The staining of Romanowsky-Giemsa was carried out. The preparations were analyzed with transmitted light microscopy using an immersion objective of Leica DM4000B with a magnification of 1000x. For the quantitative analysis of the sperm morphology, the program «Videotest-Morphology» was also used. At least 200 cells were evaluated for morphological abnormalities. Each sperm cell was examined for anomalies of the head, middle part (neck) and tail according to standard morphological criteria [10]. Based on the results, the TZI was calculated, which varies from 1 to 3, but normally should be less than 1.6 (N<1.6). In all patients whose TZI exceeded the normal value on the basis of the morphological study, teratozoospermia was diagnosed.

To determine of whether the spermatozoa differ in normal conditions and with TZI exceeding the threshold values, single cell gel electrophoresis was carried out, according to the previously described method, in order to assess DNA stability [11]. To detect sperm DNA, silver staining was performed. In preparations stained with silver nitrate, at least 150 cells were analyzed. Statistical analysis was performed using Student's t-test.

Results and discussion. Morphological analysis showed that spermatozoa with morphological abnormalities were present in the ejaculate of each man. In one patient, up to 34% of globozoospermic cells were found. However, there was no cases with complete globozoospermia. The most severe abnormality referred to sperms carrying two heads.

An increased size of the cytoplasmic droplet can definitely be attributed to the anomaly of the middle part of the sperm cells. Apparently, the acute angle between head major axis and the tail (bent tail) is not an artifact, but a true anomaly of the middle part. The tail anomaly, probably, included spermatozoa with coiled tail. These cases can be considered as tail-to-head anomaly. Sometimes tail has bizarre shapes and resembles a «cobra hood». Based on the revealed anomalies in all 85 patients, the TZI was calculated, which allowed to divide patients into two groups. In 11 men average TZI was 1.807±0.090, which exceeded the threshold value of 1.6, and teratozoospermia was diagnosed. In 74 of 85 patients, the average TZI was 1.243±0.017 (normal value). The amount of sperm with progressive motility in patients with normal TZI (<1.6) was on average 56.34 ± 2.84%, while in the group with TZI more than 1.6, and the mean amount did not exceed 31.1±4.21% (p<0.01). It should be noted that in 42 out 74 men with TZI <1.6, asthenozoospermia was diagnosed. The study of this subgroup in relation to TZI showed that average TZI was 1.262 ± 0.023 (i.e., within normal limits). The analysis of total sperm count showed that the average concentration in 85 patients was 77.92 ± 5.95 million per mL. At the same time, the number of sperm in the ejaculate of patients in whom the TZI was normal (N<1.6) or above the threshold value (N>1.6) did not reveal significant differences. It is generally accepted that an increased amount of morphological sperm abnormalities directly affects male fertility [10, 12]. According to our results, an increase in TZI above the threshold value is always associated with a decrease in sperm motility. However, the opposite is not a rule: we identified a subgroup of 42 patients in whom an increased amount of sperm cells with reduced motility was present, but TZI was within normal limits (<1.6).

When carrying out comet assay in patients with TZI exceeding the threshold values, it was revealed (see *Figure*) that the rate of sperm with genomic instability was 39.6±7.4%, while in patients with normal TZI, this value was also within the normal range, namely 3.1±0.6% (p<0.01).

Conclusions. Our results suggest that mild morphological changes in spermatozoa do not affect their motility, while severe teratozoospermia with TZI exceeding the threshold values are closely associated with DNA instability and significantly reduce the sperm quality.

Final remarks. During the initial assessment of fertility, one should focus mainly on a comprehensive study of the morphology and sperm DNA integrity.

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FEATURES OF THE COURSE OF NOVEL CORONAVIRUS INFECTION AND OPTIONS OF THERAPY DEPENDING ON THE ANDROGENIC STATUS ("FOUNDER" [ОСНОВАТЕЛЬ]): ANDROGENIC STATUS IN MEN WITH COVID-19 AND ITS RELATIONSHIP WITH THE DISEASE SEVERITY

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Aim. To analyze androgen status in men hospitalized with a moderate COVID-19 and its relationship with the severity of the disease.

Materials and methods. The study included 152 males with a confirmed diagnosis of COVID-19 based on the results of a positive PCR for the SARS-CoV-2 virus and/or CT of the lungs hospitalized at the MSU University Clinic due to the moderate and severe COVID-19. Examination of the level of biochemical blood parameters (CRP, creatinine, urea, glucose, total testosterone (T)); CT of the lungs. To objectify the severity of the clinical symptoms, NEWS2 score and the original score for assessing the severity of COVID 19 (SHOKS–COVID) were used.

Results. The median T levels in 152 examined patients was 2.14 [1.21; 3.40] ng/ml. In patients with T levels below the median, the CRP level was more than two times higher, and the D-dimer value was almost two times higher than in patients with T levels above median. The duration of treatment in the hospital was longer in men with COVID 19 and initial T levels below the median than in patients with T about the median (13 days vs 10.5 days, p=0.003). Low T levels was correlated with lung damage by lung CT. After improving the clinical condition, there was a linear increase in the level of T regardless of its initial level.

Conclusion. Among men with moderate and severe COVID-19, a decreased T levels is detected in 46.7% of cases. Patients with low T levels at admission have more severe COVID-19. A significant increase in T levels was observed after successful COVID-19 treatment without any special hormonal manipulations.

K e y w o r d s: *COVID-19, testosterone, androgenic status, SHOCS–COVID, NEWS2*

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Introduction. The rapidly growing third wave of novel coronavirus infection in the Russian Federation is associated with the emergence and spread of a new, more contagious strain of SARS-CoV-2. The delta strain contributes to the rapid progression of the disease and lung damage in different age groups, including young patients without severe comorbidities. Therefore, a critical analysis of the available data on the manifestation of COVID-19 in various subgroups in order to find additional treatment approaches is of particular importance. Definitely, vaccination is and will remain the fundamentally important and main approach to combat the pandemic, and it should be in the center of attention of physicians and become the main topic promoted among all people. But, unfortunately, even vaccinated patients are at risk of infection, including new variants of the SARS-CoV-2 virus, and universal vaccination of the population will take some time.

From the very beginning of the development of the COVID-19 pandemic, almost all over the world, attention was paid to the more frequent incidence and more severe course of infection in men [1, 2]. The theoretical rationale was associated with androgenic function. Testosterone (T), which is converted into dihydrotestosterone (DHT) under the influence of the 5-alpha reductase, can significantly increase the activity of both ACE-2 (angiotensin-converting enzyme) receptors and transmembrane serine protease 2 (TMPRSS-2), which are necessary for the rapid penetration of the virus into cells [3]. Moreover, very rapid progression of novel coronavirus infection is observed in men with a high level of androgens, which was determined by clinical signs [4]. Observations from Italy indicated a possible positive effect of androgen deprivation therapy (ADT), which reduced the content of DHT and its stimulating effect on androgen receptors, and decrease the chance of getting COVID-19 in patients with prostate cancer [5]. However, subsequent retrospective studies carried out by oncologists in the United States on a much larger number of patients with benign prostatic hyperplasia did not confirm the ability of ADT to prevent infection with SARS-CoV-2 [6, 7].

Based on early studies, attempts have been made to use different types of antiandrogen therapy for novel coronavirus infection in men. Small studies of blocking androgen function with progesterone have shown clear clinical improvement [8]. Unique results were also obtained in a Brazilian randomized controlled study (publication of a preprint on June 22, 2021), in which the androgen receptor antagonist proxalutamide demonstrated a 128% higher recovery rate by day 28 and a 77% reduction in mortality than those treated with placebo [9]. However, already on July 7, a criticism of this study was published in the journal "Science" under the heading "Too good to be true" [10]. The results on patient mortality were questioned and, according to most experts, it was not yet possible to draw final conclusions. Currently, controlled study of novel androgen receptor antagonist bicalutamide is ongoing (NCT04374279), as well as Swedish registry with another androgen receptor antagonist, enzalutamide, and gonadotropin-releasing hormone antagonist degarelix (NCT04397718). There are no other studies of ADT.

Several trials, including one at the Moscow State University clinic, have evaluated mineralocorticoid receptor antagonist (MCA) spironolactone and its active metabolite Canrenone, which have additional antiandrogenic properties, as a non-specific treatment. They demonstrated a decrease in a period of viremia and the risk of admission to the hospital, and improvement the prognosis in hospitalized patients with COVID-19 [11–13]. The results of randomized trials, evaluating spironolactone (NCT04345887), will allowed to draw final conclusions about the possible benefit of this class of drugs in the treatment of COVID-19.

But the situation is complicated by the possible impact of novel coronavirus infection on androgenic function in men with low T levels, which can negatively affect the prognosis of the disease [14, 15]. There is evidence of inflammatory and structural changes in the testes and epididymis leading to a deterioration of spermatogenesis in men with COVID-19 [16]. In addition, the more severe the course of the disease, the higher the levels of cytokines (IL-6), the lower T levels may be, as was shown in the pre-Covid-19 period for various diseases [17,18]. Moreover, high levels of T and DHT are associated with better muscle function, including respiratory muscles [19], and improving lung function in men [20].

A small study carried out in Moscow showed a decrease in T levels and, possibly, deterioration of reproductive function in men with COVID-19 [21]. Original studies have also been published [15], as well as reviews of the relationship of low T with a more severe course and a negative prognosis in male patients with COVID-19 [22].

In order to try to understand these associations, we decided to carry out a series of studies within the program: Features of the course of a NOVEL coronavirus infection and TREATMENT OPTIONS for patients depending on androgenic status (**FOUNDER; ОС**обенности течения **НО**вой коронавирусной инфекции и **ВА**рианты **ТЕ**рапии бо**ЛЬ**ных в зависимости от андрогенного статуса (**ОСНОВАТЕЛЬ**).

Aim. To analyze the androgen status in hospitalized men with moderate COVID-19 and its relationship with the course of the disease.

Material and methods. A total of 152 male patients with moderate or severe course of COVID-19, confirmed by a positive PCR test for SARS-CoV-2 and/or CT of the lungs, admitted to the University Clinic of Moscow State University, were included in the study. In Table 1 baseline data of patients are shown (the most complete examination was performed in 82 patients).

Research methods. Serum levels of C-reactive protein (CRP), creatinine, urea and glucose was studied on the automatic chemistry analyzer AU480 Beckman Coulter, Germany; complete blood count (5 diff) was performed using a hematological analyzer XN 2000 Sysmex Corporation, Japan; coagulation indicators (fibrinogen, D-dimer) were determined on the automatic hemostasis analyzer STA-Compact Diagnostica Stago SAS, France; the study of IL6 level was carried out on the immunochemical analyzer Cobas 6000 Roche Diagnostics GmbH, Germany.

CT scan of the lungs and chest was performed on a 32-row Somatom Scope CT scanner manufactured by Siemens (Germany) with a slice thickness of 1 mm.

The research methodology is described in detail in our previous articles (ПУТНИК, БИСКВИТ, КОЛОРИТ) [13, 37, 41]. The quantitative analysis of infiltrative changes in the lungs in patients with COVID-19 pneumonia was performed using COVID-Multivox (Gammamed, Moscow) and Botkin.AI (Intelodzhik, Moscow).

Two scores were used for objective evaluation of disease severity and effects of the therapy, including NEWS2 score [23] updated for patients with COVID-19 [24], and our original scale for assessing the clinical condition of patients with coronavirus infection (SHOKS–COVID), published earlier [25].

At the University Clinic of Moscow State University, all patients underwent determination of total T level on the automatic analyzer Roche Cobas 6000 using Elecsys Testosterone II assay testing procedure. The test is based on a competitive test principle using monoclonal antibodies with high binding capacity and specifically directed against testosterone. Endogenous T isolated from the sample with 2-bromoestradiol competes with the added ruthenium complex-labeled T derivative for binding sites on the biotinylated antibody.

Reference values, as well as all other information on Elecsys Testosterone II testing system for determining total T level, were obtained from the manufacturer's instructions. The lower limit of T levels, in accordance with the instructions, was 2.49 ng/ml in patients under 50 years (ranging from 2.49 to 8.36 ng/ml) and 1.93 ng/ml in those over 50 years (ranging from 1.93 to 7.40 ng/ml).

Statistical analysis. The assessment of the normality distribution was carried out using the Shapiro-Wilk test. Description of quantitative data is presented as median and interquartile range (median and 25%; 75%) in case of non-parametric distribution and as mean and standard deviation if there was normal distribution. Comparison of quantitative characteristics between groups was performed using Mann-Whitney U test for a non-parametric distribution and Student's t-test for a normal distribution.

Qualitative data are presented as absolute and relative values. The significance of differences between groups in qualitative characteristics was assessed based on the chi-square (χ^2) test, as well as the two-tailed Fisher's test.

To compare the changes of parameters within each group, the Wilcoxon signed-rank test was used in case of nonparametric distribution, while for dependent samples with a normal distribution and for qualitative indicators Student's t-test and McNemar's test were chosen, respectively. Spearman correlation coefficient was used for ranking the correlation. Multivariate analysis was performed using linear regression. The significance level in hypotheses tests was equal to 0.05. Statistical analysis was carried out using the R programming language.

Results. The results of the baseline examination of men with confirmed COVID-19 and lung damage at admission to the University Clinic of Moscow State University are presented in *table 2.*

The median age of patients was 58 years (23-88). Median BMI was 29.4 kg/m^2 and 43.8% of patients had obesity. The severity of the disease ranged from moderate to severe. Almost 40% of patients had an oxygen saturation of less than 94%, which required oxygen support in 43.2% of those admitted, while 12.7% were treated in the ICU and 4.9% required invasive mechanical ventilation. The median volume of lung injury according to CT (data are available for 77 patients) was 19.7% and corresponded to grade 1 in 58.4% of patients, grade 2 in 26%, and grade 3 in 15.6% (according to the classification of the Ministry of Health of Russian Federation). A pronounced autoimmune inflammatory response was characterized by a more than 12-fold increase in the level of CRP and a significant decrease in the ratio of lymphocytes/CRP to 19.1, with a reference level of more than 100. An increase in the level of D-dimer was moderate, but did not exclude the risk of thrombotic and thromboembolic complications. The average level of T was 2.14 ng/ml.

The mean total NEWS2 score was 4 points (ranging from 1 to 7), which corresponds to the average risk and, in some cases, high risk of being transferred to intensive care units. The total SHOKS–COVID score was 7 points, which also corresponds to the moderate severity of COVID-19 pneumonia.

Treatment for all patients was carried out according to the protocols of the University Clinic of Moscow State University and included (it is necessary to consider the early period of the pandemic [April-June 2020] and the limitations of our knowledge and understanding):

– almost a quarter of patients received hydroxychloroquine, which is currently not recommended for the treatment of novel coronavirus infection;
– all patients received antibiotics, reflecting the fear of a bacterial infection in the early stages of studying COVID-19. From today's positions, this tactic can be considered redundant (erroneous);
– 43.3% of patients received a combination of bromhexine with spironolactone to improve the clinical course (dyspnea, cough, stuffiness and chest pain) and reduce viremia;
– in 23.5% of cases glucocorticosteroids (GCS) were used;
– proactive anti-inflammatory treatment in 51.8% of patients (pulse steroid therapy, colchicine, ruxolitinib or secukinumab);
– low-molecular-weight heparin in 94.3% of cases (in 69.8% of patients, prophylactic doses were used);
– NSAIDs to reduce fever in 42.4% of cases.

The mean hospital stay was 11.5 days. 2 patients died (1.3%) and 4 (2.6%) had thrombotic and thromboembolic complications. Median T in 152 examined patients was 2.14 [1.21; 3.40] ng/ml, which corresponds to the lower limit of normal. On *fig. 1*, patients are divided by median T; normal values are shown separately for younger (2.49 ng/ml) and older patients (1.93 ng/ml). Despite significant differences in T levels, the age of the patients was almost the same. A detailed comparison of men with COVID-19 and T levels above and below the median is presented in *table 2*.

As can be seen, the groups differed significantly in most of baseline parameters (highlighted in bold in the table). In patients

Table 1

Baseline data of men with COVID-19

	All patients	N
Age, years, median [25-75‰]	**58.0 [43,0; 69,2]**	**152**
50 years or more	101 (66,4%)	
Less than 50 years old	51 (33,6%)	
Body Mass Index (BMI) kg/m2, median [25–75‰]	**29,4 [25,8;32,6]**	**80**
Obesity (BMI> 30 kg/m2)	35 (43,8%)	
No obesity (BMI<30 kg/m2)	45 (56,2%)	
Arterial hypertension, N (%)	**41 (50,0%)**	**82**
Coronary artery disease, N (%)	**10 (12,2%)**	**82**
Diabetes, N (%)	**11 (13,4%)**	**82**
Concomitant diseases (AH, IHD, DM), N (%)	**43 (52,4%)**	**82**
t°, average (SD)	*37,3 (0,79)*	*82*
RR in 1 min, median [25–75‰]	*18,0 [17,0;21,0]*	*82*
HR, in 1 min, median [25–75‰]	*82,5 [78,0;99,8]*	*82*
SBP, mm Hg, median [25–75‰]	*120 [116;130]*	*82*
SO2, %, median [25–75‰]	*95,0 [90,0;97,0]*	*82*
SO2 less than 94%, N (%)	*31 (39,7%)*	*82*
Any oxygen support, N (%)	**35 (43,2%)**	**81**
ICU stay, N (%)	**8 (12,7%)**	**81**
Initially on mechanical ventilation, N (%)	**4 (4,94%)**	**81**
CRP mg/dl, median [25–75‰]	*62,8 [19,3;113]*	*149*
D-dimer μg/mL, median [25–75‰]	*0,56 [0,31;1,03]*	*149*
Lymphocytes/CRP, median [25–75‰]	*19,1 [9,27;72,1]*	*149*
% lung involvement on CT, median [25–75‰]	**19,7 [5,90;36,9]**	**77**
Stage 1 (0-25%)	*45 (58,4%)*	
Stage 2 (25–50%)	*20 (26,0%)*	
Stage 3 (50–75%)	*12 (15,6%)*	
NEWS-2 scores, median [25–75‰]	**4,00 [1,00; 7,00]**	**78**
SCHOKS-COVID scores, median [25–75‰]	**7,00 [4,00;10,00]**	**77**
Testosterone ng/ml, median [25-75‰]	**2,14 [1,21; 3,40]**	**152**
Length of stay, median [25–75‰]	**11,5 [9,00; 15,0]**	**152**

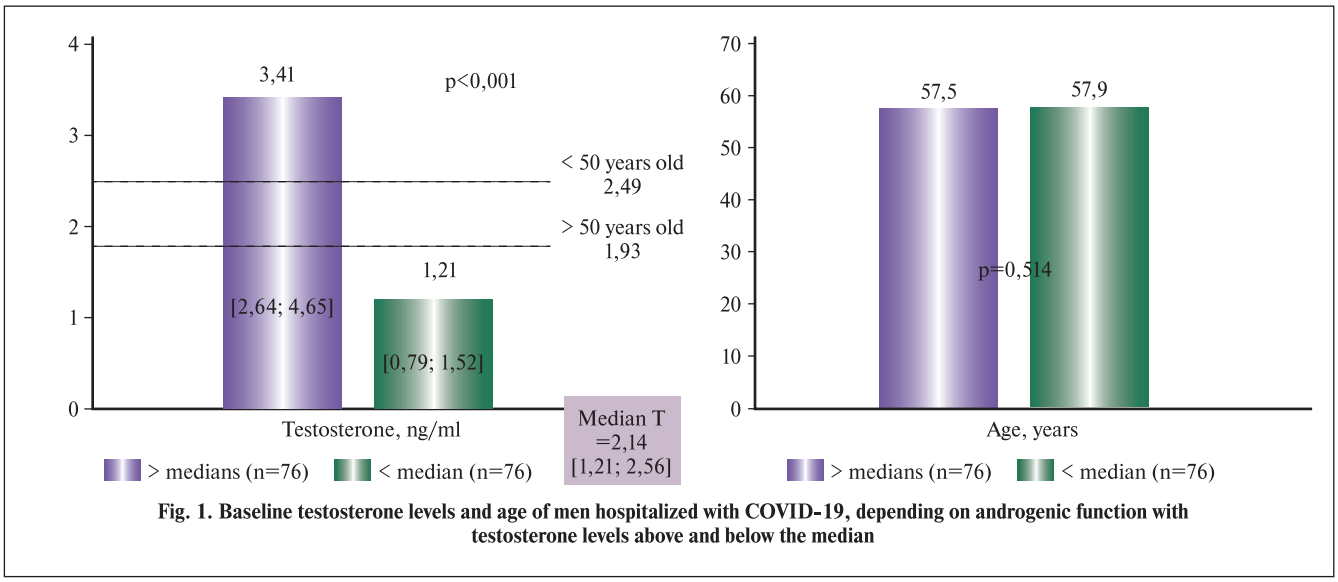

Fig. 1. Baseline testosterone levels and age of men hospitalized with COVID-19, depending on androgenic function with testosterone levels above and below the median

with T levels below the median, the CRP level was more than two times higher and the D-dimer value was almost two times higher than in the comparison group. In other words, the level of inflammation, the likelihood of developing a cytokine storm, and the risk of thrombosis and thromboembolism were significantly increased in patients with T levels not above (as expected at the beginning of the COVID-19 pandemic), but below the median. The length of stay was significantly longer in men with T levels below the median at baseline (13 days vs. 10.5 days in the subgroup with T levels above the median; p=0.003).

During intensive care, the reduction in inflammation and CRP was even more significant in unfavorable subgroup of patients with initially low T levels. Despite this, at discharge, plasma CRP level returned to normal (<5 mg/dL) only in patients with the initially normal T levels and the ratio of lymphocytes to CRP remained two times higher in this subgroup.

Given the possible differences in male androgenic function and T levels depending on age, the *fig. 2* shows separate data on normal or low T levels for patients younger and older than 50 years.

As can be seen from the *fig. 2*, T levels above the normal value (3.84 ng/ml) was detected in 26 patients (51%), while it was below the median (1.42 ng/ml) in 25 (49%) out of 51 patients under 50 years of age (33.6% of a whole group). In 101 patients older than 50 years old (66.4% of those examined), the normal T levels (2.83 ng/ml) was found in 55 men (54.5%), compared to 46 (45.5%) patients with low T levels (1.00 ng/ml). In total, a decreased T levels was seen in 71/152 (46.7%) patients, which characterizes a significant decrease in the androgenic function in large proportion of men with COVID-19.

Additionally, we performed a separate analysis of T levels in groups of patients younger and older than 50 years with and without obesity and with or without concomitant cardiovascular

Table 2

Comparative characteristics of men with COVID-19 and testosterone levels above and below the median

	Testosterone above the median, *n*=76	Testosterone below the median, *n*=76	*p*
Testosterone ng/ml, median [25; 75‰]	**3.41 [2.64; 4.65]**	**1.21 [0.79; 1.52]**	**<0.001**
Age, median [25; 75‰]	57.5 [38.2; 70.4]	57.9 [47.8; 67.9]	0.514
Initially			
CRP mg/dl, median [25; 75‰]	**39.5 [10.7; 83.4]**	**88.1 [46.8; 142]**	**<0.001**
D-dimer μg/ml, median [25; 75‰]	**0.39 [0.28;0.83]**	**0.74 [0.42; 1.12]**	**0.008**
Lymphocytes 109/L, median [25; 75‰]	**1.19 [0.92; 1.65]**	**1.18 [0.83; 1.61]**	**0.321**
Neutrophils 109/L, median [25;75‰]	**3.55 [2.23; 4.54]**	**4.32 [2.96; 6.05]**	**0.003**
Lymphocytes/CRP, median [25; 75‰]	**34.0 [14.3; 106]**	**14.6 [6.85; 29.8]**	**<0.001**
Discharge from the hospital			
CRP mg/dl, median [25; 75‰]	**4.20 [2.97; 7.86]**	**8.79 [4.19; 21.2]**	**0.011**
D-dimer μg/ml, median [25; 75‰]	0.40 [0.22; 0.92]	0.68 [0.36; 1.09]	0.114
Lymphocytes 109/L, median [25; 75‰]	1.77 [1.37;2.34]	1.85 [1.49;2.19]	0.974
Neutrophils 109/L, median [25;75‰]	2.85 [2.24;4.81]	3.22 [2.47;4.10]	0.925
Value Neutrophils/Lymphocytes			
Lymphocytes/CRP, median [25; 75‰]	**423 [180; 818]**	**185 [87.7; 446]**	**0.020**
Changes in treatment			
Δ CRP mg/dl, median [25; 75‰]	**-11.8 [-65.6; -5.40]**	**-65.5[-128.2;–19.3]**	**0.009**
Δ D-dimer μg/ml, median [25; 75‰]	0.02 [-0.23; 0.20]	-0.06 [-0.26; 0.18]	0.278
Δ Lymphocytes/CRP, median [25; 75‰]	337 [133; 643]	155 [52.6; 415]	0.053
Length of stay, median [25;75‰]	**10.5 [7.00; 14.0]**	**13.0 [10.0; 17.2]**	**0.003**

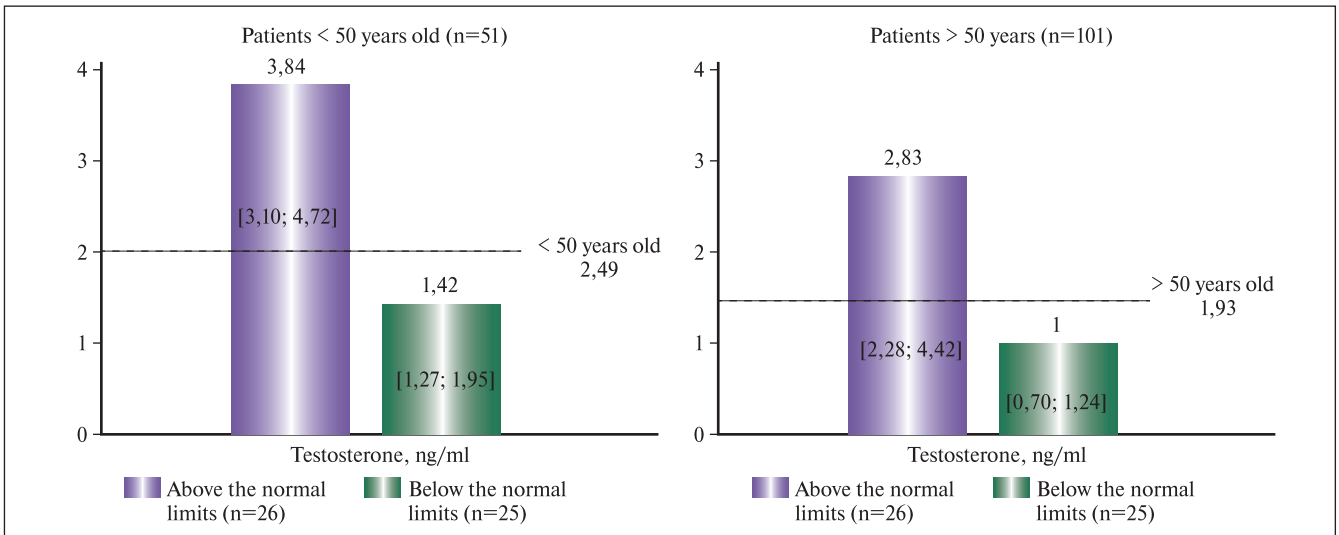

Fig. 2 Analysis of men with COVID-19 with testosterone levels above and below normal limits depending on age (under and over 50 years)

diseases (arterial hypertension, coronary artery disease) and type 2 diabetes mellitus. The results are presented in *table 3*.

Even when distinguishing subgroups of patients younger and older than 50 years, the differences have marginal significance. At the same time, there was no correlation between age and T levels (r=-0.12; p=0.29). Also, there were no significant differences in T levels in patients with and without obesity. There was also no significant correlation between BMI and T (r=-0.18; p=0.11), as well as between T levels in patients with and without comorbidities. Thus, the analysis of possible relationships between the severity of the manifestations of COVID-19 and androgenic function was obvious.

In *table 4*, a comparison of main parameters in men with COVID-19 with initially normal (n=81) and low T levels (n=71) is presented. As can be seen, the patterns obtained in subgroups with T levels below and above the median were confirmed. Patients with initially low T levels had a significantly higher level of inflammation (CRP level and lymphocyte/CRP ratio). D-dimer as marker of thrombosis risk did not differ significantly, however, in patients with normal T, its value

Table 3

Testosterone Levels in Various Subgroups

Characteristic	Number	Testosterone (ng/ml)	*p*
Under 50 years old	*30*	*2.31 [1.43; 3.84]*	*0.05*
Over 50 years old	*52*	*1.87 [0.96; 2.81]*	
Obese	**35**	**1.95 [1.33; 3.01]**	**0.39**
Without obesity	**45**	**2.14 [1.88; 3.24]**	
Unknown	**2**	**3.04 [2.64; 3.45]**	
With comorbidities	*43*	*1.96 [1.13; 3.24]*	*0.54*
No comorbidities	*39*	*2.17 [1.36; 2.27]*	

Table 4

Comparative characteristics of men with COVID-19 and baseline normal or low plasma testosterone levels

	Normal value, n=81	Below the normal value, n=71	*p*
Testosterone ng/ml, median [25; 75‰]	**3.28 [2.61; 4.54]**	**1.18 [0.77; 1.44]**	**<0.001**
Age, median [25; 75‰]	59.0 [43.0; 70.0]	56.0 [45.0; 67.0]	0.617
Age (> or < 50 years) n (%):			0.816
50 years or more	55 (67.9%)	46 (64.8%)	
Less than 50 years old	26 (32.1%)	25 (35.2%)	
At baseline			
CRP mg/dl, median [25; 75‰]	**50.5 [14.7; 91.0]**	**78.3 [31.7; 141]**	**0.003**
D-dimer μg/ml, median [25; 75‰]	0.46 [0.29; 0.97]	0.64 [0.35; 1.10]	0.235
Lymphocytes/CRP, median [25; 75‰]	**26.0 [11.6; 88.5]**	**17.0 [7.23; 35.1]**	**0.014**
Length of stay, median [25; 75‰]	**11.0 [7.00; 14.0]**	**13.0 [10.0; 16.0]**	**0.016**

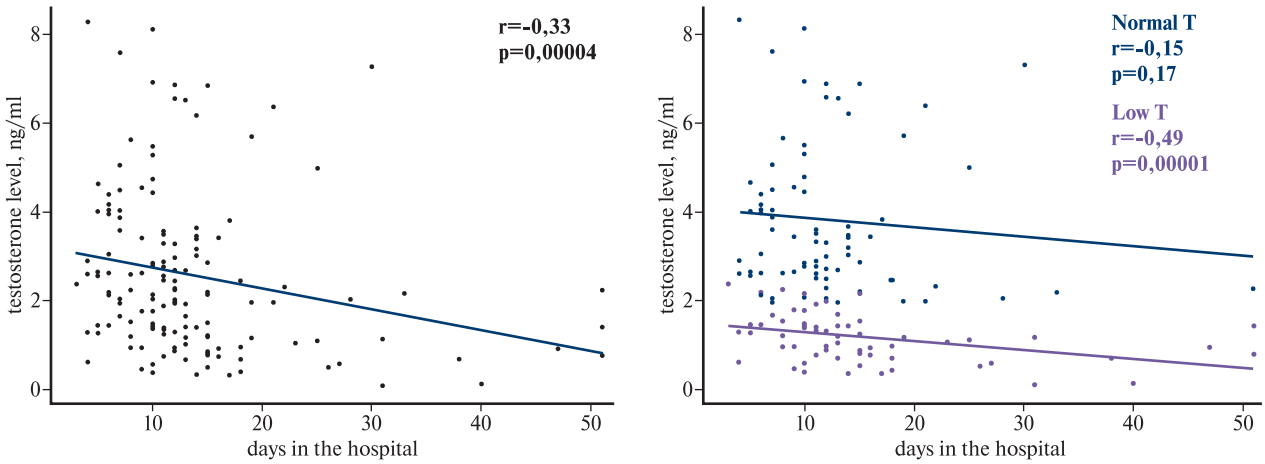

Fig. 3. Correlation between length of stay of men with COVID-19 and baseline total testosterone

was within the normal range, but it was increased in those with low T. Inpatient treatment was significantly longer in patients with low baseline T levels. We analyzed the correlation between length of stay and baseline T levels in men with novel coronavirus infection (*fig. 3*) and found a significant but weak negative association. When analyzed separately, in patients with initially normal T levels, there was no association between androgenic function and the duration of treatment, but in those with initially low T levels, the lower it was, the longer was the duration of the treatment. Hence, a decrease in T levels can be either a marker or a risk factor for the more severe course of COVID-19.

In a subgroup of 82 patients, there were additional data, including oxygen saturation, heart rate and blood pressure plus chest CT, which allowed to integrally assess the severity of the clinical manifestation of the disease (according to NEWS2 score) and the overall severity of COVID-19 according to the SHOKS-COVID score, modified by V.Yu. Mareev, which was proposed at the University Clinic of Moscow State University.

Table 5 shows a comparison of patients with baseline T levels above (n=41) and below (n=41) median, but this almost corresponds to the division into normal (n=40) or low (n=42) T levels. As can be seen, patients with low T levels had a more severe course of coronavirus pneumonia. In this group, lower oxygen saturation, greater dyspnea, and oxygen support were required more often compared to the subgroup with T levels above the median. This was consistent with a larger volume of lung damage according to CT and, at the same time, significantly higher level of markers of inflammation (CRP), thrombosis and thromboembolic complications (D-dimer) in patients with initial T levels below the median. The severity of the disease according to the original SHOKS-COVID score was 9 points in patients with T levels below the median and 5 points in men with T levels above the median (p<0.001).

Table 5

Comparative characteristics of men with COVID-19 who underwent a full examination depending on testosterone level (above and below the median)

	Above the median N=41	Below the median N=41	p
Testosterone, median [25; 75‰]	**3.39 [2.37; 4.98]**	**1.15 [0.85; 1.42]**	**<0.001**
Age years, median [25; 75‰]	52.0 [36.0; 69.0]	58.0 [48.0; 67.0]	0.314
BMI kg/, median [25; 75‰]	28.1 [25.6; 32.3]	29.9 [27.3; 33.4]	0.178
t, mean (SD)	37.4 (0.77)	37.3 (0.82)	0.739
RR in 1 min, median [25; 75‰]	**18.0 [16.0; 20.0]**	**19.0 [18.0; 22.0]**	**0.037**
HR, median [25; 75‰]	82.0 [78.0; 101]	83.0 [74.0; 98.0]	0.941
SBP, median [25; 75‰]	**124 [120; 130]**	**120 [112; 126]**	**0.011**
SO_2, median [25; 75‰]	**96.0 [95.0; 97.0]**	**92.0 [86.0; 96.0]**	**<0.001**
SO_2 less than 94, N (%)	**7 (17.9%)**	**24 (61.5%)**	**<0.001**
Any oxygen support, N (%)	**12 (29.3%)**	**23 (57.5%)**	**0.019**
CRP mg/dl, median [25; 75‰]	**46.2 [13.0; 99.4]**	**110 [44.8; 142]**	**0.008**
D-dimer µg/mL, median [25; 75‰]	**0.45 [0.29; 0.85]**	**0.87 [0.48; 1.37]**	**0.005**
GFR (CKDEpi), mean (SD)	**77.6 (18.7)**	**76.2 (19.1)**	**0.744**
% lung injury (CT), median [25;75‰]	**11.9 [5.42; 34.9]**	**24.6 [7.70; 44.6]**	**0.067**
SHOKS, median scores [25; 75‰]	**5.00 [3.00; 7.00]**	**9.00 [5.00; 11.0]**	**<0.001**
NEWS, median scores [25; 75‰]	**3.00 [1.00; 4.50]**	**5.00 [3.00; 8.50]**	**0.002**
Length of stay, median [25;75‰]	**12.0 [8.00; 15.0]**	**13.0 [11.0; 20.0]**	**0.061**
Death, N (%)	1 (2.44%)	1 (2.44%)	1.000
PE/thrombosis, N (%)	1 (4.76%)	3 (9.68%)	0.633
Death + PE/thrombosis, N (%)	2 (9.52%)	4 (12.9%)	0.675

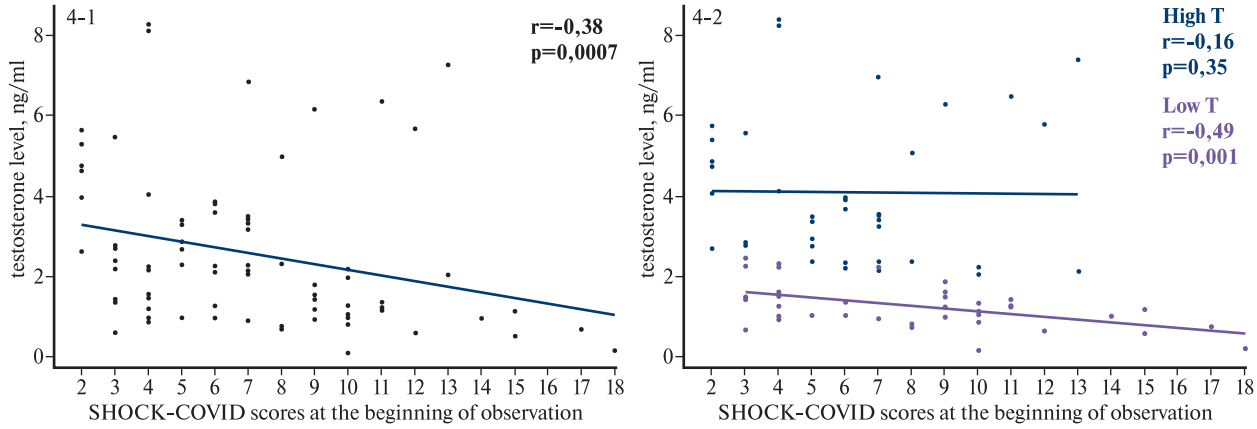

Fig. 4. Relationship between the severity of COVID-19 (according to the SHOX-COVID scale) and testosterone levels in all patients (Fig. 4-1) and in those with testosterone levels below and above normal limits (Fig. 4-2)

Therapy of patients with T levels below the median was significantly more active with more frequent use of corticosteroids (p=0.01), including pulse therapy. In addition, only in this subgroup the doses of anticoagulants were increased from prophylactic to therapeutic (p=0.049), NSAIDs were more often used (p=0.031) and the duration of hospital stay was longer (p=0.061).

In each of groups one patient died, despite the treatment. Thrombotic and thromboembolic complications occurred in one patient with COVID-19 and initially normal T levels and three men with low T levels. The analysis of the correlation between the initial T levels and the total severity of COVID-10, determined by the total SHOKS–COVID score, is presented in *fig. 4*. We found a significant weak negative correlation between the severity of coronavirus pneumonia and T levels, i.e., the lower T levels, the higher total SHOKS–COVID score. However, this association was absent in patients with the initially normal T levels and becomes highly significant (r=0.49; p=0.001) in those with low T levels at baseline.

The regression-correlation relationships between baseline T levels with the volume of lung lesions based on CT at admission (*fig. 5-1* and *5-2*) and before discharge (*fig. 5-3* and *5-4*) are presented on *fig. 5*, since the volume of lung lesions may influence on prognosis in men with COVID-19. Again, the

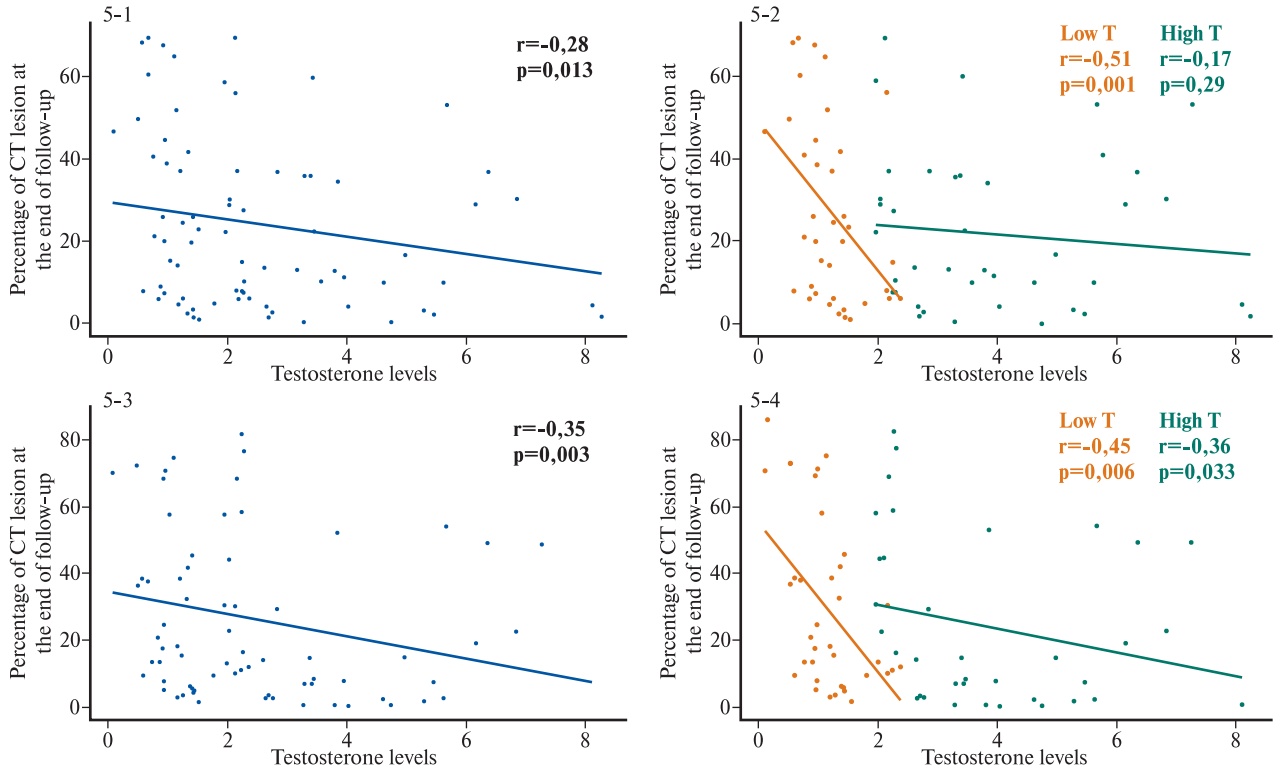

Fig. 5. Relationships between testosterone levels and baseline lung lesion volume on CT in the whole group (Fig. 5-1) and in subgroups with testosterone below (orange) and above (green) normal values (Fig. 5-2) and lung lesion volume after treatment in the whole group (Fig. 5-3) and in subgroups with testosterone below (orange) and above (green) normal values (Fig. 5-4)

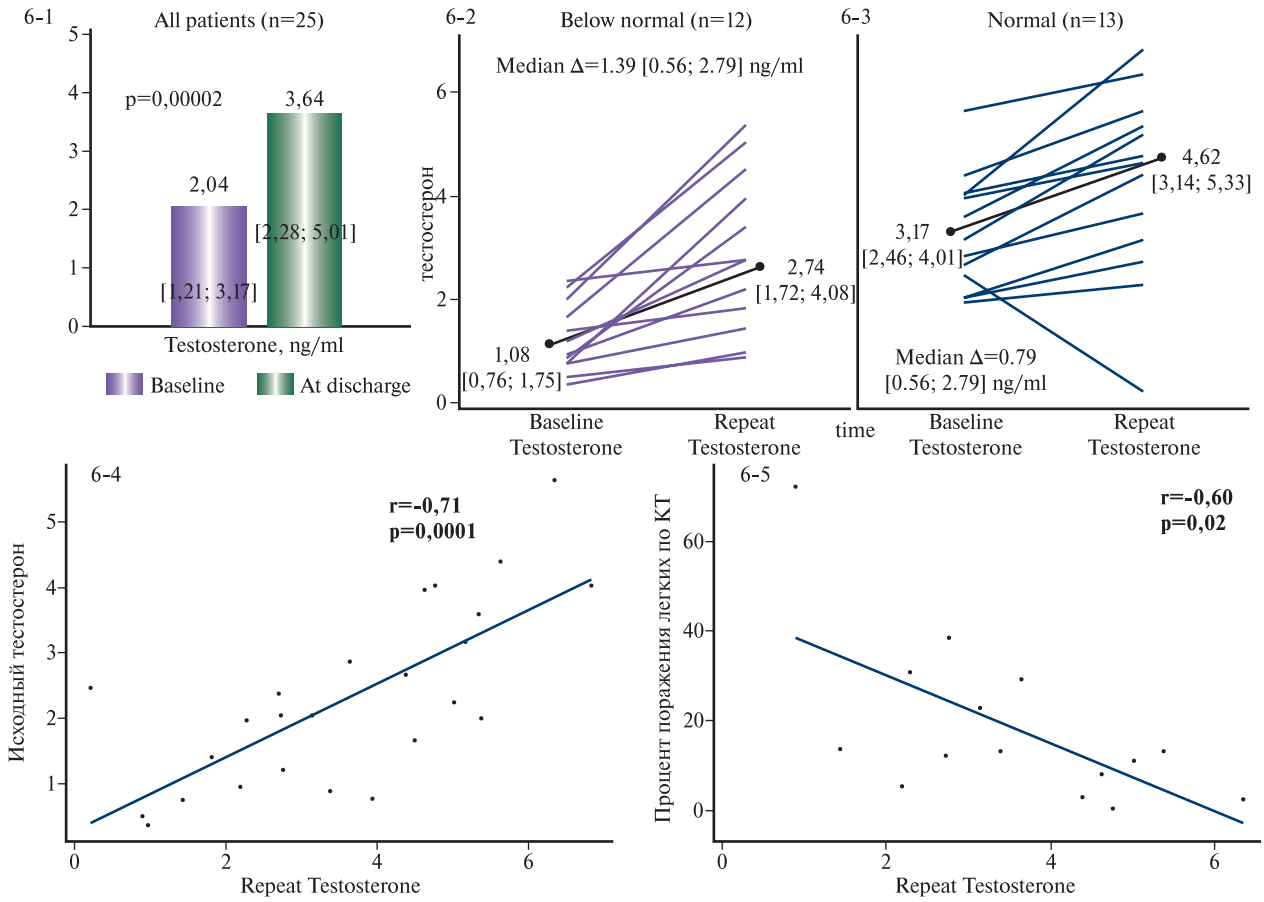

Fig. 6. Dynamics of testosterone levels after treatment of men with coronavirus pneumonia in the whole group (Fig. 6-1), subgroups with baseline low (6-2) and normal testosterone levels (Fig. 6-3) and the relationship of testosterone after treatment vs. baseline (6-4) and testosterone and lung lesion volume according to the results of posttreatment CT (6-5)

similar patterns are shown. There was a significant, albeit weak, negative association between T levels and baseline volume of lung lesions in men with COVID-19 (*fig. 5-1*), with significant difference only in the subgroup with baseline testosterone below normal. This association was moderate (*fig. 5-2*). However, in contrast to the SHOKS-COVID score, there was also a correlation between baseline T levels and volume of lung lesions based on CT at discharge (*figs. 5-3* and *5-4*). A stronger association was found in patients with baseline T levels below normal. Hence, low T levels was significantly associated with lung damage in the examined men with coronavirus pneumonia, and the lower this level, the greater the volume of the lesion and the severity of the pneumonia.

To clarify the role of T levels in determining the severity of COVID-19 in men and the length of stay, multivariate models were constructed. Based on the results of the examination of all 152 patients, a model, which included clinical and biochemical parameters that can influence the length of stay in combination with the natural logarithm of T levels, was built.

Higher values of natural logarithms of T and lymphocytes were associated with shorter hospital stays, while advanced age was a predictor of longer hospital stay. The addition of CRP, lymphocytes/CRP ratio and D-dimer to the model did not lead to an increase in the coefficient of determination R2, therefore, they were not included in the final model. The model explains only 22.5% of the variability in length of stay. According to the results, older the men, more lymphopenia, and lower T levels, the more severe the course of COVID-19.

Based on the data of 82 patients, for whom all examinations were available, including the clinical course, and a volume of lung damage based on CT results, another model was built that included factors, significantly influencing the length of stay of men with coronavirus pneumonia, according to univariate analysis: total SHOKS–COVID score, natural logarithm of T levels, age and heart rate.

The model explains 45.4% of the variability in length of stay. The higher the SHOKS–COVID score and age, the longer the hospital stay. T levels was not a significant prognostic factor. Moreover, excluding it from the model leads to an increase in R2 to 0.46. The replacement of the total SHOKS–COVID score with individual indicators of the severity of COVID-19, including oxygen saturation and the volume of lung damage based on CT, reduced the prognostic value of the model (R2 = 0.331) and none of variants of T levels didn't retain a significant effect on the length of stay for patients with COVID-19.

The addition of lymphocytes count, the logarithm of lymphocytes count, and the ratio of lymphocytes/CRP to the model reduced its predictive value, therefore, they were not included in the final model. The comorbidities also reduced the predictive value. Taking into account the high proportion of patients with low T (46.7%), we tried to analyze its changes after non-specific treatment of novel coronavirus infection in 25 patients, which were repeatedly examined (*fig. 6*).

As shown on *fig. 6-1*, T levels after successful treatment of coronavirus pneumonia significantly increased by almost 80%, and changes were more pronounced in the subgroup with initially low T levels (*n*=12, *fig. 6-2*), than in patients with normal values

at baseline (n=13, *fig. 6-3*). Next, we carried out an analysis of the correlation between baseline T levels and T levels after treatment. As can be seen from *fig. 6-4*, there was a close significant direct relationship between these indicators (r=0.71, p=0.0001). After the clinical improvement of patients with COVID-19, there was a linear increase in T levels, independent of its baseline level. Despite the fact that in the multivariate model T levels lost a significant association with the duration of treatment in patients with coronary pneumonia, our analysis revealed a significant negative correlation not only for baseline level, but also for T levels after treatment, and for the volume of lung damage on CT after successful treatment (*fig. 6-5*). The lower T levels remains, the greater the volume of lung lesion was even after successful treatment of COVID-19. Therefore, our results suggest that T is a marker of the severity of novel coronavirus infection. It cannot be ruled out that initial decrease in T levels is one of the risk factors for the progression of the disease.

Discussion. From the very beginning of the COVID-19 pandemic, serious attention was paid to gender differences [26]. Men had higher risk of getting the disease [27,28] and for a severe course of infection with the development of viral pneumonia, which required more often hospitalization in intensive care units and invasive mechanical ventilation [29]. This could be due to a higher incidence of risk factors and comorbidities, including cardiovascular diseases [30]. At the same time, a possible association between androgenic function and the development of COVID-19 began to be studied. Previously, we discussed in detail the possible relationship between excessive activation of androgen receptors and simultaneous activation of ACE-2 receptors and TMPRSS2, which contribute to invading cells by SARS-CoV-2 [13, 31]. Some studies have demonstrated a more severe course of the disease in men with clinical signs of high androgenic function [32]. In addition, in a number of retrospective limited studies, men with benign prostatic hyperplasia who received 5α-reductase inhibitors has lower risk of getting COVID-19 and for more severe course of infection [5].

At the time the study "Основатель" was planned, all of our early understandings (the same was true for most researchers) of novel coronavirus infection were as follow: high levels of androgens (and the main hormone T) are associated with the severe COVID-19 and spironolactone can slow down and alleviate the course of the disease.

However, directly opposite data about the relationship between low T levels in men and the severity of COVID-19 began to appear [33, 34]. Many mechanisms have also been proposed that can explain the worse course of the disease in patients with low T levels [35, 36].

Our study strongly supports the idea that low (rather than high) T levels in men is associated with a more severe course of novel coronavirus infection. The rate of patients with COVID-19 and low T levels was surprising. Almost half (46.7%) of men had decreased T levels, both young (49% in those younger than 50 years) and older (45.5% in patients older than 50 years). T levels was not associated with age, obesity, body mass index, and comorbidities; therefore, this decrease seems to be associated with novel coronavirus infection itself. Men with COVID-19 and low T levels had a significantly more severe course of the disease, which included severe respiratory failure (significantly greater decrease in oxygen saturation in the blood), an increase requirement for oxygen therapy, high levels of markers of inflammation (CRP), thrombosis and thromboembolism (D-dimer). This resulted in longer and more intensive treatment for these patients compared to those with normal baseline T levels (median hospital stay 13 days vs. 10.5, p=0.003).

A significant negative correlation was found between baseline T levels and the severity of the disease according to SHOKS-COVID scale, which included clinical manifestations, levels of markers of inflammation and thrombosis, and the degree of lung damage based on CT results (r=-0.38, p=0.0007). Similarly, there was a significant negative weak correlation between T levels and the volume of lung lesions (r=-0.28, p=0.013). It is very intriguing and important that these associations were absent in patients with normal baseline T levels. Conversely, in patients with COVID-19 and initial T levels below normal, the correlation with SHOKS-COVID score (r=0.49, p=0.001) became stronger, reflecting the overall severity the disease and the risk of complications, as well as with the volume of lung damage (r=-0.51, p=0.001). Hence, the lower total T levels, the greater the damage to the lungs and the severity of COVID-19, especially (if not exclusively) among patients with abnormal androgenic function at baseline.

Further analysis revealed a significant negative correlation between the initial T levels and the volume of lung damage based on CT results upon admission and at discharge. This relationship was stronger in patients with initially low T levels (r=-0.45; p=0.006), but remained significant in patients with normal T levels at baseline (r=-0.36; p=-0.033), as well as for the whole group (r=-0.35; p=0.003). Thus, the lower T levels in men with COVID-19, the greater the volume of lung damage even after successful treatment.

Multivariate analysis showed that low T levels (its natural logarithm) retained a significant influence on the length of stay (p = 0.0002), as well as lymphopenia and age did. However, the main determinant of the hospital stay was the severity of the disease, determined by SHOKS-COVID scores. Regression with inclusion both SHOKS-COVID (based on CT results and other severity criteria) and T showed that length of stay was determined by disease severity and not baseline T. However, regression cannot distinguish between the two situations (i.e., the prognosis will be the same):

A. Severe coronavirus infection is accompanied by both a significant decrease in T level and longer length of stay (T is one of the markers of the severity of the COVID-19)

B. A low level of T due to some additional mechanisms contributes to the severe course of COVID-19, while severe coronavirus infection requires longer length of stay (T is a risk factor for a more severe course of the disease).

Indirectly, the presence of a close correlation between T and the course of COVID-19 was confirmed by changes of its level during treatment, which we observed in 25 patients. As can be seen from *fig. 6*, as a result of successful treatment of COVID-19 (without specific hormonal manipulations), T levels significantly increased by almost 80%, and in absolute terms this difference was similar in patients with initially low (Δ=1.66 ng/ml) and normal (Δ=1 45 ng / ml) T levels. A profound decrease occurred only in 1/25 patient, despite baseline normal T level. There was a strong association between baseline and follow-up T levels (r=0.71; p=0.001), which is not surprising in the absence of hormonal therapy. The lower T levels at discharge, the greater the volume of lung damage based on CT results (r=-0.6; p=0.02).

Taking into account controversial theories about the relationship between T levels and the severity of COVID-19 and based on our results, we suggested possible scenarios, which are presented in the diagram in *fig. 7*.

As can be seen from the *fig.* and our previous publications [37], it is very important to identify different phases of novel coronavirus infection, since they required different approaches to treatment. In this case, we can talk about the phased of COVID-19 and specific treatment. At the beginning of the

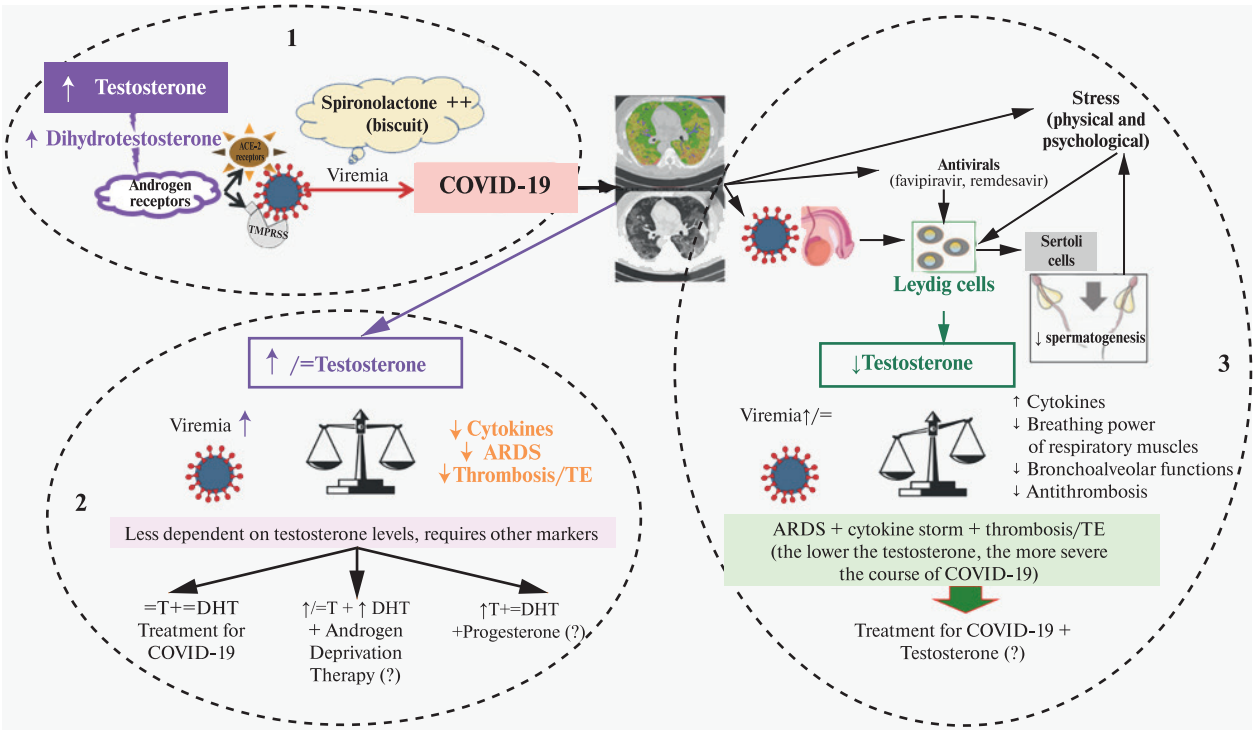

Fig. 7. Hypothesis on the role of testosterone in the development and progression of COVID-19 and possible treatment options for men with a new coronavirus infection

disease (viremia phase), the main aim is to block the entry of SARS-CoV-2 into cells and its replication in order to prevent the progression of the disease. As can be seen from the diagram (*fig. 7, period 1*), male gender and especially elevated androgenic status are adverse factors that can lead to greater morbidity and severe disease [5, 32]. At the same time, T and DHT formed under the action of 5-alpha reductase through the activation of androgen receptors, stimulate ACE-2 receptors and TMPRSS-2, which contribute to invading cells by SARS-CoV-2 [32]. Therefore, men, in particular, with the hyperandrogenic status, are more likely to get COVID-19. At an early stage of the disease, the choice of MCA spironolactone with its numerous but moderate antiandrogenic properties seems justified. This has been confirmed in studies demonstrating both a reduced risk of being hospitalized [12] and a shorter risk of viremia and hospitalization [13]. Moreover, use of canrenone (the active metabolite of spironolactone) is associated with a decrease in the risk of deaths and complications of COVID-19 [11], and in a recent study dedicated to spironolactone, a decrease in mortality was confirmed [12].

Interestingly, the positive effect of spironolactone was achieved with a relatively short course (10-14 days) and the side effects of therapy, including gynecomastia and a decrease in spermatogenesis associated with a compensatory increase in the synthesis and effects of estrogens, did not develop, since they are typical for long-term therapy. The use of more potent specific therapies, such as progesterone [8], ADT [38] or androgen receptor antagonists [12] at this stage of the disease will raise questions about balance risk/benefit and is currently being studied in controlled trials.

With the progression of the disease and the development of viral pneumonia, the next phase begins, requiring proactive anti-inflammatory therapy, primarily with glucocorticosteroids [39–41] and prevention of thrombotic complications with anticoagulants [42]. From the standpoint of androgenic status, the following scenario may occur. As shown on *fig. 7 (period 2)*, in case of typical course COVID-19 with predominant injury of lungs, T levels remain normal and, definitely, much higher in men than in women. It can be assumed that the negative properties associated with T and DHT and resulting in escalation of viremia, weaken and are balanced by important protective properties of male sex hormones. The level of cytokines and the risk of a cytokine storm are reduced [43], as well as likelihood of progression of acute respiratory distress syndrome (ARDS) [44], which is no accompanied by developing thrombotic complications [45]. We have not identified an association between T levels and the severity of COVID-19 and the degree of lung damage in a group of men with baseline normal androgenic function. In this case, the generally accepted treatment seems to be quite sufficient, since after inpatient therapy, T levels significantly increased by 46%, remaining within normal values. However, to answer to this question more precisely, it is necessary to study not only T levels, but also DHT, and preferably the activity of androgen receptors as well. Probably, with normal T levels and a simultaneous increase in DHT, one can assume the benefit of ADT or androgen receptor antagonists, which are currently being investigated in several randomized controlled trials (NCT04446429, NCT04374279, NCT04475601). High T levels in men with COVID-19, viral pneumonia, and severe hypoxia suggest a possible benefit from progesterone. The preliminary data seems to be promising [8], but require confirmation in properly designed randomized study.

Finally, the group of men with severe COVID-19 and initially low T levels is of greatest interest (*fig. 7, period 3*). The possibility of a direct effect of SARS-CoV-2 on the level of male sex hormones and reproductive function has been discussed since last year [46]. The penetration of the virus into the testicles and epididymis can block the synthetic ability of Leydig cells to produce T, reduce stimulation of Sertoli cells and production of normally functioning spermatozoa [47]. Structural changes

in the testicles and epididymis, including congestion, interstitial edema, exudate with high level of erythrocytes, and apoptosis of functionally active cells are described [48]. SARS-CoV-2 was found in the semen of each of the 6 examined men with novel coronavirus infection [49]. In addition to the direct effect of the virus, inflammation, cytokine influence, physical and psychological stress, and asthenia can also be important additional factors for reducing sexual and reproductive function in men [50, 51]. The negative properties of antiviral drugs, including favipiravir, cannot be completely excluded.

In a small Russian study of 50 men with COVID-19, decreased T levels was found in 38% of cases [21]. Changes in the morphology of spermatozoa, an increase in the number of leukocytes in the ejaculate, and a decrease in T levels in patients with COVID-19 were also found. In our study, a reduced level of T was found in 46.7% of 152 males hospitalized with COVID-19, regardless of age and concomitant diseases. A significant negative relationship was found between T levels and the overall severity the disease and the volume of lung damage. The lower the T levels, the more severe the course of the disease and longer length of stay.

Hence, as was mentioned earlier, low T levels can be considered as one of markers of the severity of COVID-19 (regardless of initial course). At the same time, it can be a factor of the progression and severe course of the disease in men, especially those with initially low T as a consequence of COVID-19. These issues are being actively discussed today like “Testosterone in COVID-19: Enemy, friend or sacrifice" [52].

Active and successful inpatient treatment leads to significantly increase in T levels, which was low at baseline, on average by 2.5 times in all patients. Despite this, lower T levels after treatment was associated with greater lung damage. The question of whether additional stimulation of androgenic function is required for these patients remains unanswered. We should not forget about possible complications of testosterone therapy, for example, an increased risk of thrombosis [53]. This may aggravate the course of COVID-19, despite the widespread prescription of anticoagulants in this group.

Conclusion

1. A reduced testosterone level was detected in 46.7% of men with moderate-to-severe COVID-19.
2. Patients with low testosterone levels at admission had more severe course of COVID -19.
3. Successful treatment of COVID-19 leads to a significant increase in T levels, without any additional measures.
4. The absence of an increase in testosterone during treatment is associated with the preservation of a greater volume of lung damage.

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NORMATIVE PARAMETERS FOR MONITORING OF NOCTURNAL PENILE TUMESCENCES: A SYSTEMATIC REVIEW AND ALGORITHM DEVELOPMENT

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Aim: a systematic review of the available literature sources about criteria for nocturnal penile tumescences (NPT) registration, as well as the development our own criteria for evaluating erectograms obtained by Androscan MIT.

Materials and methods: a systematic search of publications describing NPT criteria was carried out in the Medline database. A PRISMA flowchart was used to visually represent the design of the study. The necessary calculations were carried out using the STATISTICA 12 software.

Results: mormal erectile function is characterized by a relative increase in penis diameter of 30% or more with the duration of such NPTs of more than 60 minutes. For a mild ED in the case of a good relative increase (30% or more) with a duration of NPT with such increase less than 10 minutes (the time of one effective erection), it is recommended to determine the duration of NPT with a relative increase of 20% or more. If relative increase is less than 30% it is advisable to use the duration of sufficient erections (with a relative increase in diameter of 20% or more) and the borderline value is 60 minutes or more. Severe ED is characterized by relative increase less than 20% or duration of NPT of less than 10 minutes with any relative increase.

Conclusion: currently there are no uniform criteria for the diagnosis of ED using Androscan MIT. For the unification of ED diagnosing, we first introduced the terms of «effective erection», «sufficient erection», «relative increase» and also developed reference values and an algorithm for evaluating erectograms which will ensure continuity as well as the possibility of comparison of the results from different research groups.

K e y w o r d s: Androscan MIT, RigiScan, reference values, erectile dysfunction

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Introduction. Sleep-related erections (SREs) have been known since antiquity. Even Plato, who lived in the V-IV centuries BC, stated that in men, the nature of the genital organs is naughty and willful, and it tries to dominate, and all because of its mad lust [1]. The first attempts to study SREs were made in the 1940s by a team of neurophysiologists led by P. Ohlmeyer [2]. In the second half of the 20th century a monitoring of SREs, or nocturnal penile tumescences (NPT) was recognized as one of the objective ways to assess the quality of an erection. As the most accessible and cost-effective devices for evaluating nocturnal erections, Rigiscan was developed and introduced into practice in 1985 [3]. However, the diagnostic value of Rigiscan has been controversial for a long time. Initially, normal criteria for NPT measurements were provided by the manufacturer and included in the instructions for use. These criteria included three to six erectile episodes within 8 hours of sleep. During each erectile episode, penile rigidity at the glans and base increased by more than 70% in combination with an increase in penile diameter of 3 cm or more at the base and 2 cm or more at the glans [4]. The duration of one normal nocturnal erection was 10-15 minutes. In 2006, the RigiScan was first included in the clinical guidelines of the European Association of Urology as a method for diagnosing erectile dysfunction (ED) by monitoring NPT [5]. Normal erectile function was recorded in the presence of an erection with a rigidity of the glans penis of 60% or more, lasting 10 minutes or more [5]. The proposed criteria differed significantly from those stated by the manufacturer, but for 5 years they were annually included in guidelines until 2021 [6].

The Russian analogue of RigiScan device is Androscan MIT, which was included in the State Register of Medical Devices in November 2018 [7]. Sensor-recorder is a ring of soft cylindrical spring connected to a miniature recorder with built-in battery and microprocessor with flash memory. Unlike RigiScan, this device does not determine the rigidity of the penis, but only the change in the diameter of the penis at the base, the duration of NPTs, and their number. Normative values for Androscan have not been described in the instructions, and the literature data on the issue are limited and controversial [8, 9].

Aim. To systematically review the available literature on reference values for monitoring NPT using foreign and domestic devices, as well as to develop our own criteria based on the data obtained.

Materials and methods. A systematic search of relevant literature containing information on reference values for RigiScan was performed in the Medline database. The following term was used: "RigiScan". The "related articles" function allowed to expand the search. The reviews, as well as articles in Chinese, Japanese, Portuguese, Spanish, German, French,

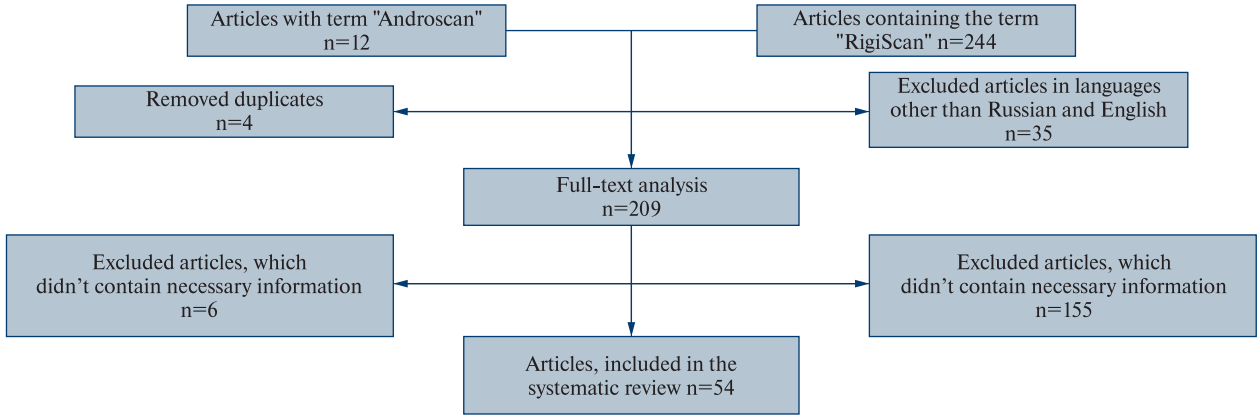

Fig. 1. Flowchart showing selection criteria for systematic analysis

Polish, were excluded from the search due to inability of their full analysis. A systematic search for the necessary literature containing information on the reference values for Androscan MIT device was carried out in the Russian database eLibrary.ru and in the Google Scholar database with a term "Androscan".

Based on the data of our foreign colleagues, the concept of "the best erectile episode" was used in our study, since the detection of even one normal erection indicates the presence of a potential that, under the influence of supraspinal control, can maintain an erection sufficient for penetration and satisfactory sexual activity.

For the routine characteristics of erectograms, we chose the following criteria: the relative increase in the diameter of the penis during the "best erectile episode" and the total duration of NPT with a given relative increase. Based on the experience of foreign colleagues, when we determined the relative increase in the diameter of the penis base, not single maximum value was used, but maximum value, the duration of which was 3 minutes or more, in order to eliminate the artifacts associated with movement and putting on the device.

The PRISMA flowchart was used to visualize the study design. The necessary calculations were carried out using the STATISTICA12 software.

Results. During the literature search, 244 articles containing the term "RigiScan" and 12 articles with term "Androscan" were obtained (*fig. 1*). After excluding 35 articles in languages other than Russian and English and 4 duplicates, 217 papers were selected for full-text analysis. After reading all the publications, 165 articles were additionally excluded since they didn't contain necessary information on the reference values for RigiScan and Androscan MIT. Finally, 52 articles were included in the systematic review. The results are presented in table 1 and 2.

As shown in Table 1, most authors believe that normal erectile function may be present even in the presence of one NPT, called the "best erectile episode". In the presence of at least one erectile episode with a rigidity of the glans and base of the penis of more than 70%, with an increase in the diameter of the glans and base by more than 2 and 3 cm, respectively, erectile function, assessed using RigiScan, was considered normal.

Based on the available data, we first introduced the concepts of "effective erection" and "sufficient erection". An effective erection was defined as an erection with a relative increase in the diameter of the base of the penis of 30% or more. Sufficient erection was regarded as an erection with a relative increase of 20% or more.

It has been shown that normal erectile function is characterized by the presence of effective erections (relative increase of 30% or more) with their total duration of 60 minutes or more. The abnormality of one of the parameters points to mild ED, while a change in both parameters is characteristic of moderate ED.

For mild ED, in case of a good relative increase in diameter (30% or more) and the duration of NPT with such an increase of less than 10 minutes (the time of one effective erection), it is recommended to determine the duration of NPT with a relative increase in diameter of 20% or more, since the patient has the potential associated with an increase in the diameter of the penis, however, the duration of an effective erection is reduced despite the normal duration of "sufficient erection" (with a relative increase in diameter of 20% or more). In the case of an increase in the diameter of the penis less than 30%, it is advisable to consider the duration of sufficient erections with boundary value of 60 minutes or more, since in the presence of so-called "sufficient erection", its duration should correspond to the full cycle of NPT (6 tumescences of 10 minutes or 4 of 15). As discussed above, severe ED on the erectogram is characterized by a relative increase in the diameter of the penis less than 20% or NPT duration of less than 10 minutes with any relative increase in diameter (*Fig. 2*).

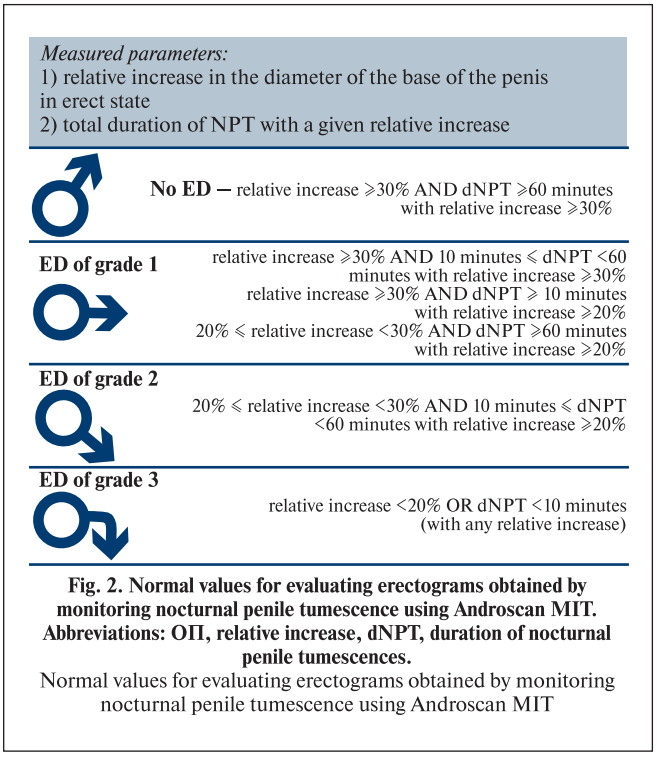

Fig. 2. Normal values for evaluating erectograms obtained by monitoring nocturnal penile tumescence using Androscan MIT. Abbreviations: ОП, relative increase, dNPT, duration of nocturnal penile tumescences.

Normal values for evaluating erectograms obtained by monitoring nocturnal penile tumescence using Androscan MIT

Table 1

Reference values for monitoring nocturnal penile tumescence using RigiScan

Author	Year	Reference values				
		Number of erections	Rigidity of the base and glans, %	Diameter of the glans, cm	Diameter of the base, cm	Duration of the erection, min
Tongyan Liu et al. [9]	2020	1	⩾70	⩾2	⩾3	⩾10
Yong Zhang et al. [10]	2020	3	⩾60	-	-	⩾10
Pan D et al. [11]	2020	-	⩾60	-	-	-
Zhi-He Xu et al. [12]	2019	1	⩾60	-	-	⩾10
Zi-Jun Zou et al. [13]	2020	1	⩾60	-	-	⩾10
Zhi-He Xu et al. [14]	2019	1	⩾60	-	-	⩾10
Zaher Bahouth et al. [15]	2015	1	⩾70	⩾2	⩾3	⩾10
Elhanbly S. et Elkholy A. [16]	2012	1	⩾70	⩾2	⩾3	⩾10
Iacono F. et al. [17]	2012	1	⩾70	-	-	⩾10
Fabrizio Iacono et al. [18]	2012	1	⩾70	-	-	⩾10
Khafagy A.H. [19]; patients suffering from erectile dysfunction (ED)	2012	-	⩾80	-	-	-
Mizuno I. et al. [20]	2010	-	⩾60	-	-	-
Elhanbly S. et al. [21]	2009	1	⩾70	⩾2	⩾3	⩾10
Sim H.G. et al. [22]	2006	1	⩾70	-	-	⩾10
Pu X. et al. [23]	2006	1	⩾55	-	-	⩾10
Greenstein A. et al. [24]	2007	1	⩾70	-	-	⩾10
Yang C.C. et al. [25]	2006	1	⩾70 ⩾60	- -	- -	⩾10 ⩾10
Melman A. et al. [26]	2006	1	⩾70	-	-	⩾10
Taher A. [27]	2004	1	⩾55	-	-	⩾10
Elhanbly S. et al. [28]	2004	1	⩾70	⩾2	⩾3	⩾10
Karadeniz T. et al. [29]	2004	2	⩾60	-	-	⩾5
Yaman O. et al. [30]	2004	1	⩾60	⩾2	⩾3	⩾10
Ichiro Mizuno et al. [31]	2003	1	⩾70	-	-	⩾10
Meinhardt W. and Horenblas S. [32]	2003	2	⩾70	-	-	⩾10
Donald D. Suh et al. [33]	2003	-	⩾7	-	-	⩾10
Suzuki K. et al. [34]	2001	-	⩾70%	-	-	⩾10
Guay A.T. et al. [35]	2002	3-4	⩾50 (glans) ⩾60 (base)	⩾2	⩾3	⩾10
Basar M.M. et al. [36]	2001	3	⩾60	⩾2	⩾3	⩾10
Yang C.C. et al. [37]		1	⩾70	-	-	⩾10
Kim H.S. et al. [38]	2000	3-6	⩾40	-	⩾2	⩾10—15
Chen J. и.et al. [39]	1999	-	⩾70	-	-	⩾10
McMahon C.G., Touma K. [40]	1999	-	⩾65 (base)	⩾2,5	⩾3,5	-
Wessells H. et al. [41]	1998	1	⩾70	-	-	⩾10
Chen J. et al. [42]	1998	-	⩾60	-	-	⩾10
Hatzichristou D.G. et al. [43]	1998	1	⩾60	-	-	⩾10
De Rosa M [44]	1998	1	⩾70	⩾2	⩾3	⩾10
Helgason A.R. [45]		-	⩾55	-	-	⩾10
Karadeniz T. et al. [46]	1997	1	⩾70	-	-	⩾10
Guay A.T. et al. [47]	1996	-	⩾60 (base) ⩾50 (glans)	-	-	-
Benet A.E. et al. [48]	1996	1	⩾60	-	-	⩾5
Sandler A.D. et al. [49]	1996	4	⩾90	-	-	⩾15
Tay H.P. et al. [50]	1996	3-6	⩾40		⩾2	⩾10—15
Licht M.R. et al. [51]	1995	-	⩾70	-	-	-
Ogrinc F.G. et al. [52]	1995	-	⩾70 ⩾60	-	-	⩾10
Davis-Joseph B. et al. [53]	1995	1	⩾60	-	-	⩾5
C Carani и др. [54]	1995	-	⩾60	⩾3	-	⩾5
Allen R.P. et al. [55]	1993	1	⩾70	⩾2	⩾3	⩾10
Ackermanet M.D. al. [56]	1991	-	⩾70	-	-	⩾10
Kropman R.F. et al. [57]	1991	3-6	⩾70	⩾3	⩾2	⩾10—15
Kessler W.Q. [58]	1988	1	⩾70	⩾2	⩾3	⩾10
Mode (min-max)		1 (1-6)	70 (45—90)	2 (2—3)	3 (2—3,5)	10 (5—15)

Table 2

Reference values for monitoring nocturnal penile tumescence using Androscan MIT

Author	Year	Number of erections	Duration of the erection, min	Increase in the diameter of the base of the penis, cm
Kurbatov D.G. et al.	2017	4—6	10—15 каждая	⩾3 см
Pavlov A.Yu. et al.	2019	⩾3	⩾10	*dэ/dнэ ⩾0,75

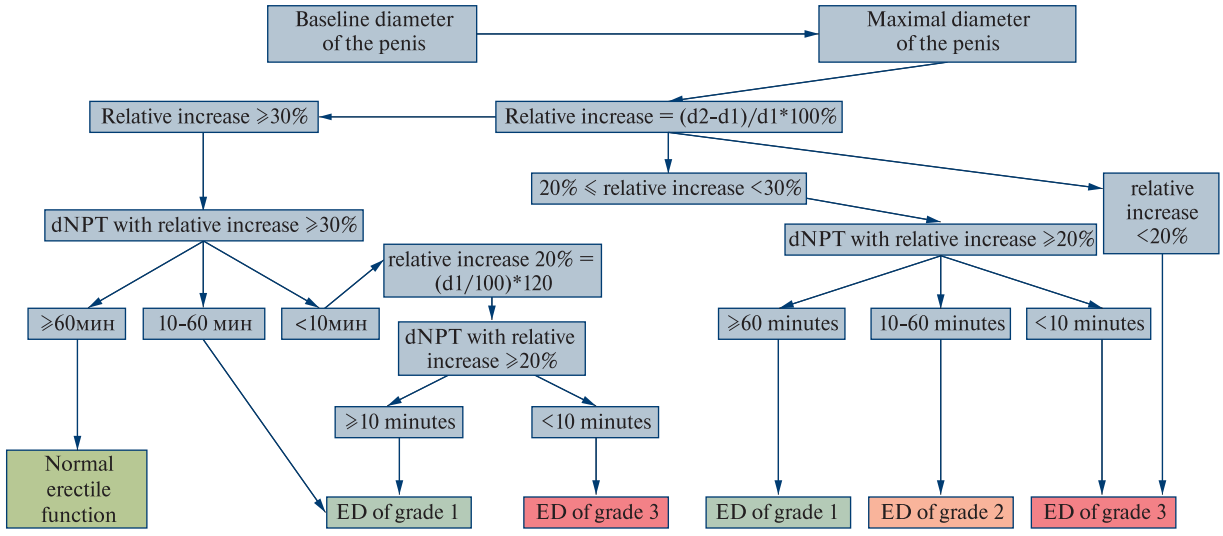

Fig. 3. Algorithm for evaluating erectograms obtained by monitoring nocturnal penile tumescence using Androscan MIT
Abbreviations: ПЧ, penis; ОП, relative gain; dNPT, duration of nocturnal penile tumescence; ЭД, erectile dysfunction.

Based on the developed criteria, we proposed an algorithm for evaluating erectograms obtained using Androscan MIT, which is presented in *fig. 3*.

Discussion. The domestic device Androscan MIT, which is used for registering NPT, does not measure penile rigidity, but allow to directly measure the diameter of the penis in flaccid and erect state. The most objective way, in our opinion, is the calculation of the relative increase in the diameter of the erect penis, as was demonstrated in the work of A. Yu. Pavlova et al. (2019) [59] due to large differences in the absolute values of the penile circumference in the population [60].

The largest study of penile circumference in erect and flaccid state in sexually active men was carried out by Italian researchers in 2021. A total of 4685 men without ED were examined, with a mean age of 19 years. The median penile circumference in the flaccid state was 10 cm, while in the erect state it reached 13 cm [61]. Thus, the diameter of the penis in patients with normal erectile function increases by 3 cm, which corresponds with the criteria proposed by the manufacturers of RigiScan. Relative increase in this case is 30%, which serves as a criterion for the so-called effective erection (*fig. 4*).

Based on most studies on RigiScan (*Table 1*), the duration of one effective erection is 10 minutes or more, however, according to the criteria of manufacturers, the duration of one effective NPT can be more than 15 minutes, and their number ranges from 4 to 6 per night [4]. Thus, in the presence of 4 or 6 effective erections, the duration of which is 10–15 minutes, the total duration of erections is 60 minutes, which was chosen as a criterion for normal erectile function.

To evaluate reference values for RigiScan, researchers led by J. P. Heaton et al. (1995) proposed the so-called RigiScan Number (RN). The measured parameters of the best erectile episode were evaluated on a 9-point scale (*fig. 5*). The change in penile circumference and duration of the erection were regarded as the first parameter, and rigidity was the second one. RN scores for rigidity and tumescence at the glans and base of the penis were summed up. With a maximum score of 36, a score of 16 or more was considered to be sufficient for sexual intercourse [62].

For Androscan MIT, according to the proposed algorithm, only the diameter of the base of the penis considered, which

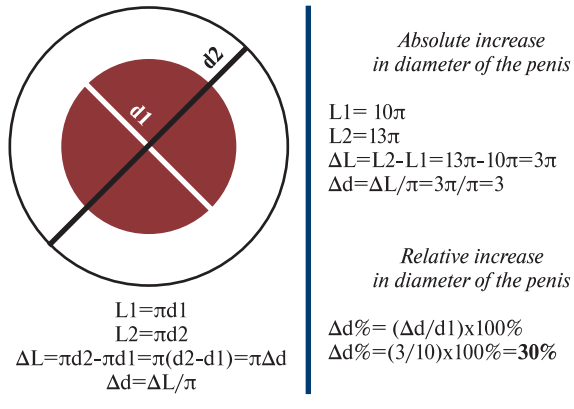

Fig. 4. Calculation of the relative increase in the diameter of the penis during normal erection based on the results of Di Mauri M. et al. (2021)

TABLE I. *Criteria for calculating the Rigiscan Number (RN) used in the analysis of results*

Rigiscan Number Score Sheet			
Best Change in Circumference in Cms	Duration in Mins	Score	Range of Best Rigidity
0.0–0.5	<1	0	00%–10%
0.5–1.0	<1	1	10%–20%
1.0–1.5	<1	2	20%–30%
1.5–2.0	<1	3	30%–40%
2.0–2.5	<1	4	40%–50%
>2.5	<1	5	50%–60%
2.0–2.5	>1	6	60%–70%
>2.5	>1	7	70%–80%
>3	>5	8	80%–90%
>3	>10	9	90%–100%
Circumference	Tip score =		
	Base score =		
Rigidity	Tip score =		
	Base score =		
Rigiscan Number			

Maximal possible RN = 36
MAX Tip score (circumference)=9
MAX Base score (circumference)=9
MAX Tip score (rigidity)=9
MAX Base score (rigidity)=9

Androscan only measures the diameter of the base of the penis, therefore, MAX RN is equal 9 points; increase in the diameter of the base of the penis >3 cm (30% according to the study of Di Mauri M. et al. (2021)

Borderline RN sufficient for sexual intercourse = 16
Tip score (circumference)=4
Base score (circumference)=4
Tip score (rigidity)=4
Base score (rigidity)=4

The boundary value of RN for Androscan MIT = 4 points => increase in the diameter of the base of the penis >2cm (20% based on a study by Di Mauri M. et al. (2021))

Fig. 5. Criteria for calculating relative increase, J. P. Heaton et al. (1995)

reduces the maximum number of points to 9. This corresponds to an increase in the circumference of the penis by 3 cm or more, which serves as a criterion for normal erectile function. The limit value of an erection sufficient for sexual intercourse, based on the algorithm, was reduced to 4 points, which corresponds to an increase in the circumference of the penis by 2 cm or more [62]. Thus, when tumescence is accompanied by an increase in the circumference less than 2 cm, ED should be regarded as severe; borderline relative increase, based on the formulas presented in *fig. 3*, is 20% [62].

The borderline value for differentiating moderate and severe ED, equal to 20%, was confirmed and used in a number of studies as the concept of "recorded erection". In most foreign publications an erection is defined as an increase in the diameter of the penis by 20% or more lasting more than 3 minutes [11, 12, 14, 63, 64]. Extrapolating the obtained data to the Russian device, severe ED is characterized by a relative increase in the diameter of the penis of less than 20%. At the same time, with severe ED, erections with a relative increase in the diameter of the base of the penis above 20% and even above 30% can be recorded, however, the duration of such erections should not exceed that of one "effective erection", which, as can be seen from Table 1, is 10 minutes or more. Thus, severe ED is characterized by a relative increase of less than 20% or an NPT duration of less than 10 minutes with any relative increase.

The differentiating between mild and moderate ED is the most difficult in terms of diagnosis. There are some publications, in which authors attempts to make such a distinction [65], but it is still a controversial issue. Therefore, in order to classify ED according to the results of testing by Androscan, it is necessary to determine the sequence of disorders occurring during the development of ED. Samir M. Elhanbly et al. (2018) showed that the diagnostic accuracy of the tumescence of the base of the penis after taking sildenafil is 72.7% (the area under the ROC curve = 0.722), which exceeds the diagnostic accuracy of NPT duration (68%, area under the ROC curve = 0.674) [66]. As a consequence, it can be suggested that change in the diameter of the base of the penis occurs earlier in the development of ED, followed by a decrease in the duration of NPT. Additional evidences were obtained in a study by F. Montorsi et al. (2000). When analyzing the effect of sildenafil on the quality of NPT in patients with vascular ED, it was found that sildenafil leads to a significant increase in the diameter of the base ($p<0.001$) and glans penis ($p<0.001$), as well as the duration of erectile episodes ($p<0.01$) [67]. Therefore, in the absence of sildenafil, penile diameter will change to a greater extent than duration of erectile episodes. However, in a study by H. Wessells et al. (1998) a different sequence of changes was seen. When studying the effect of the analogue of melanocortin, melanotan-2, on erectile function, it was found that after taking the drug during NPT monitoring, the diameter of the base of the penis and the duration of erections significantly increase ($p=0.0157$ and $p=0.0021$, respectively) [41] Thus, based on the results of several studies, in the absence of pharmacological stimulation in patients with ED during NPT monitoring, both the duration of tumescences and the diameter of the base of the penis may initially change. Hence, abnormality of one of the parameters corresponds to mild ED, while a change in both parameters is characteristic of moderate ED.

Conclusion. Based on the systematic analysis, we developed reference values and an algorithm for evaluating erectograms, which are obtained during monitoring of NPT using Androscan MIT. For the first time, we developed a classification of ED according to severity based on qualitative changes of NPT. For the first time, the concepts of "effective erection", "sufficient erection", "relative increase" were introduced. The relative increase is characterized by a percentage increase in the diameter of the base of the penis during the "best erectile episode" with a total duration of the maximum value of more than 3 minutes. An effective erection was defined as an erection with a relative increase in the diameter of the base of the penis of 30% or more. Sufficient erection is an erection with a relative increase of 20% or more. Introduction of the results into clinical practice will greatly help urologists and andrologists, who use NPT monitoring as an objective method for diagnosing ED. Such unification and systematization will ensure the continuity of examinations in different clinics, as well as provide the possibility of a reliable comparison of the results between research groups.

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LITERATURE REVIEWS

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LOWER URINARY TRACT SYMPTOMS AND COVID-19

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The data presented in the review showed that the coronavirus affects not only the lungs, but also the organs of the urinary system. The new virus causes a mosaic, multi-organ disease with severe consequences after the egg and has a wide organotropism. The role of SARS-CoV-2 in the development of lower urinary tract symptoms (LUTs), which are manifested by frequent, imperative urges, dysuria, nocturia, is not entirely clear. It is assumed that biologically active substances, the activation of which is caused by a virus, play a certain role in the development of SNMP, namely the expression of angiotensin converting enzyme 2 (ACE2), cytokines, activation of toll-like receptor 4 (TLR4), etc.

An increase in cytokines that are released into the urine and / or expressed in the bladder and the presence of SNMP in patients with coronavirus infection have been called de novo urinary symptoms or COVID-19 associated cystitis (CAC) in the literature. Urinary symptoms de novo or associated cystitis COVID-19 (CAC) develops against the background of a complete lack of data for the presence of a bacterial pathogen in the urine. Despite the unusual manifestation of coronavirus infection, similar mechanisms of damage to urothelial cells in viral and bacterial infections give us the right to think about the use of pathogenetically justified prevention of the development of an inflammatory reaction in the urinary tract, as well as short-and long-term consequences of this disease.

For this purpose, it is necessary to recommend drugs that have a multifactorial effect: diuretic, anti-adhesive, anti-inflammatory and regulate the local immunity of the bladder mucosa. We assume that we can expect a decrease in the number of complications from the organs of the urinary system, and more successful rehabilitation of patients with coronavirus infection and in the post-ovarian period. Final conclusions and recommendations will be available after well-planned clinical trials have been conducted.

Key words: *SARS-CoV-2, urinary tract infections, LUTS, cytokines, COVID-19 associated cystitis (CAC), de novo urinary symptoms, D-mannose, proanticyanidins, vitamin D*

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There is a rapid progression of morbidity and mortality in the world from COVID-19 due to the person-to-person transmission, the appearance of new mutations and superimposed bacterial infection that can affect any organs, including the genitourinary system. Current treatments are mainly focused on symptomatic and respiratory support [1], and only recently in the literature there have been published specific recommendations for the rehabilitation of patients with COVID-19 and the prevention of both viral and bacterial infections [2].

One of the most interesting features of this pandemic is that acute respiratory syndrome is not the only symptom of SARS-CoV-2, therefore, all the effects of COVID-19 have to be studied. At the beginning of the epidemic, it was suggested that the virus is not transmitted through urine and practically does not affect the urinary tract [3]. However, the subsequent analysis of clinical and laboratory data showed that the virus invades not only the lungs, but also other organs, such as kidneys, urinary bladder, prostate, testicles, liver, heart, brain and blood vessels [1]. Consequently, SARS-CoV-2 causes mosaic multi-organ disease with severe postcovid complications and may affect different organs [4, 5].

When preparing the review, data from scientific articles published in the PubMed databases (https://www.ncbi.nlm.nih.gov/pubmed/), Scopus and on the websites of professional medical associations were extracted. The search in databases was performed using key words “Coronavirus disease”, “COVID-19”, “Urogenital system”, “Cystitis”, “Inflammation”, “Cytokines”, “SARS-CoV -2”, “D-mannose”, “Vitamin D”, “Proanticyanidins”. After that, based on the relevance of the data, reliability of publications, impact factors of journals and the consistency of results in manuscripts, a total of 34 full-text articles were included in the review.

A few reviews are devoted to secondary bacterial and viral infections that quite often occur in patients with SARS-CoV-2 and represent either true concomitant infections or superinfections [6].

According to R. Nori et al., bacterial coinfection was diagnosed in 46 (19%) patients with COVID-19. The most

frequent affected organ systems were the genitourinary tract (57%), followed by skin (10%) and respiratory tract (8%) [7].

Christophe Masset et al. were among the first to suggest that SARS-CoV-2 increases the risk of replication of *Polyomavirus hominis* (BKV) in posttransplant patients, even with mild course of COVID-19. The early complications of Covid-19 are currently not well studied, especially in the patients after kidney transplantation. Two clinical cases of BKV replication after mild SARS-CoV-2 infection were described in the review. The first patient was a 59-year-old man who had had confirmed slight BKV viremia prior to transplantation, which was performed 3 months ago. In the second case, a 53-year-old woman had a transplantation 15 years ago. She had a history of episodes of BKV cystitis that was cured with therapy. A few weeks after infection with SARS-CoV-2, the patient experienced a recurrence of lower urinary tract symptoms (LUTS) and exacerbation of cystitis. Therefore, infection with SARS-CoV-2, even in non-severe cases, can lead to replication of other viruses. Monitoring of viral replication after COVID -19 in kidney transplant recipients can confirm these preliminary data [8].

The infectious and inflammatory process in the lower urinary tract can be caused by various viruses, including adenovirus and cytomegalovirus, and the SARS-CoV-2 virus is not an exception [1]. The inflammatory process in the bladder leads to development of LUTS, caused by damage to the urothelium with subsequent activation of afferent nerves, as well as by detrusor dysfunction due to local effects [9].

Breyer BN et al. showed that the human immunodeficiency virus is an independent risk factor for the development of severe LUTS [10]. Human T-lymphotropic virus-1 (HTLV-1) can also cause LUTS, including nocturia, urgency, urinary frequency and dysuria [11].

The invasion of the urothelial cells lining the lower and upper urinary tract with the SARS-CoV-2 is currently closely studying. It was previously known that the urothelium lining the urinary bladder, like in the collecting system, expresses angiotensin-converting enzyme 2 (ACE2). According to the recent publications, ACE2 is a receptor for the spike protein of the SARS-CoV-2 virus [1] and plays an important role in its penetration into human cells. In COVID-19 patients, expression of ACE2 has been detected in the genitourinary system, including proximal convoluted tubules, bladder urothelial cells, prostate, and testes [12, 13]. For example, ACE2 was found in bladder urothelial cells in 2.4% patients with COVID-19 with concomitant acute cystitis [14].

However, it is still not confirmed whether the ACE 2 receptor is located on the luminal or basal side of urothelial cells, so the mechanism of damage to the mucous membrane of the bladder and collecting system by SARS-CoV-2 remains unclear. Perhaps, SARS-CoV-2 causes viral cystitis through infected urine either from the lumen of the urinary tract, affecting the urothelium, or from the basal side during the viremic phase [12, 14].

As a result, the urinary tract can become a target for SARS-CoV-2, which provokes an inflammatory process in the bladder and kidneys, as well as LUTS, thus contributing the activation of bacterial and viral infections.

Recent molecular studies of the innate immune response to urinary tract infections have revealed several characteristics, which is common to that in patients with COVID-19. Viral and bacterial ligands drive the activation of toll-like receptor 4 (TLR4). The signal transmitted to the cell through this receptor is functionally close to the interleukin-1 receptor. The studies on TLR4 have showed a strong protein-protein interaction with the SARS-CoV-2 spike (S) glycoprotein, which led to its over-activation. It is also known that P-fimbriae, which are bacterial pathogens causing lower urinary tract infections, may bind to glycosphingolipid receptors and triggers the release of ceramide. The ceramide consists of sphingosine and fatty acids, acting as an intermediate signaling element upon TLR4 activation. In addition, SARS-CoV-2 has an affinity for linoleic acid, which is part of the cell membranes, and this indicates the potential to modulate TLR4 activation like P-fimbriae [15, 16].

An increase in a number of biologically active substances both in the blood and in urine in patients with a new viral infection is described.

There have been publications that urinary level of inflammatory cytokines is elevated in patients with urinary incontinence and interstitial cystitis/painful bladder syndrome in comparison with the controls. Patients with interstitial cystitis and Gunner's ulcers have been shown to have elevated levels of IL-16, IL-18 and other biologically active substances in the bladder wall compared to the control group [17].

For example, in patients with an overactive bladder (OAB), urinary cytokines are elevated compared to controls, including monocyte chemotactic protein-1 (MCP-1), soluble fraction of the CD40 ligand (sCD40L), macrophage inflammatory protein (MIP-1β), IL-12p70/p40, IL-5, epidermal growth factor (EGF), growth-related oncogene GRO-α, sIL-2Rα, and IL-10 [18]. Laura E. Lamb et al. found an increase in urinary cytokines and the incidence of LUTS in patients with COVID-19. The authors considered urinary symptoms as *de novo* COVID-19-associated cystitis (CAC), caused by an increase in inflammatory cytokines that are secreted into the urine and/or expressed in the bladder. They believe that in such cases cystitis is associated with impaired immune response and is a manifestation of ongoing systemic inflammation, which results in chronic inflammation. Development of a systemic inflammatory response syndrome is accompanied by an increase in the level of inflammatory cytokines, which are released into the urine or expressed on the bladder mucosa [19].

Dhar Nivedita et al. evaluated urinary symptoms in patients who were discharged after hospitalization for COVID-19. All patients had a positive molecular diagnostic test for SARS-CoV-2. The main complaints were urinary frequency (⩾13 episodes a day in 85% cases) and nocturia (⩾4 episodes a night in 87% cases). Urinary symptoms were assessed using an OAB questionnaire (0-5 points for each domain, including urgency, urinary frequency, urinary incontinence and nocturia), ranging from 0 to 5. A total of 39 patients with COVID-19 (7 women and 32 men), who developed *de novo* urinary symptoms without urinary tract infection, as confirmed by laboratory tests, were studied. All patients were African American with no fever or other clinical manifestations that would require hospitalization. The mean overall score for OAB symptoms was 18 points (12–20 in men and 15–21 in women). Thus, the results of the study demonstrate the development of *de novo* urinary tract symptoms in patients with COVID-19, and their cause is still unknown [20].

J. N. Mumm et al. were the first who observed a urinary frequency in 7 out 57 patients hospitalized with COVID-19. All patients had positive test for SARS-CoV-2 on nasopharyngeal swabs with viral pneumonia confirmed by computed tomography. At the same time, there was no acute kidney damage or prostatitis, since laboratory data and urine culture were otherwise normal. Patients had on average 13.7 urination on the day of admission and 11.6 on the 5th day of treatment. Researchers found a potentially dangerous overlap between classic urinary tract symptoms and COVID-19 symptoms,

which were not fully described yet. Considering the absence of any other causative factors, frequent urination may be secondary to viral cystitis which is associated with COVID-19. As a result, urinary frequency should be considered as an anamnestic tool in patients with infective symptoms to increase awareness among urologists during the COVID-19 pandemic to prevent fatal implications of misinterpreting urological symptoms [14].

E. Lamb Laura et al. showed that all patients with a positive test for COVID-19 (7 women and 32 men, all were African American) had *de novo* urination symptoms without urinary tract infection and negative urine culture [19].

There are some reports in the literature about the development of atypical hematuria in patients with COVID-19. Moreover, the time from the first contact with a viral infection to the onset of COVID-19 and symptoms of hemorrhagic cystitis in those cases ranged from 5 to 8 days [14].

Haghighi Ramin et al. described a clinical case of acute hemorrhagic cystitis in a 41-year-old woman who worked in the intensive care unit for the treatment of patients with COVID-19. Hemorrhagic cystitis was manifested by total severe hematuria, dysuria, and frequency [21].

Sun et al. for the first time isolated the virus from urine of a patient with COVID-19 [22]. H Kashi Amir et al. performed literature search on the presence of the SARS-Cov-2 in urine of infected patients and its possible predictive factors. A total of 39 studies were found in the systematic review, including 12 case reports, 26 clinical cases and 1 cohort study. Urine samples from 533 patients were examined. In 14 studies SARS-Cov-2 were isolated from urine in 24 patients. The overall detection rate of SARS-Cov-2 in urine samples was 4.5%. In most publications, virus load in urine was lower than in rectal or oropharyngeal swabs. Urinary excretion of the virus was noted in adult patients with moderate to severe COVID-19 and in four children with a mild course of the disease. Excretion of the SARS-Cov-2 in urine was detected from the 1st to the 52nd day after the onset of the COVID-19. One study showed the pathogenicity of urine-derived virus in cell culture, while another study failed to detect replication of the isolated viral RNA in cell cultures. The authors noted that urinary tract symptoms were not associated with urinary excretion of the virus [5].

Y. Cheng et al. found that positive test on glucosuria and proteinuria in patients with COVID-19 plays an important role for determining the severity of upper urinary tract impairment [23].

Yeliz KAYA et al. used validated questionnaires to diagnose LUTS in COVID-19 patients. According to the results, storage symptoms associated with inflammation of the bladder mucosa and psychogenic disorders due to depression and OAB are among early manifestation. Therefore, clinicians should evaluate LUTS and other symptoms if there is a suspicion on COVID-19 [24].

Osman Can et al. evaluated LUTS after COVID-19 in 94 men admitted to the hospital. The patients were divided into two groups depending on their age: more and lower than 50 years. The authors assessed all available data, including IPSS total scale (International Prostate Symptom Score) before and after the disease. Data analysis showed increased frequency in elderly patients after COVID-19. At the same time, the severity of the disease did not correlate with IPSS score. The authors suggested that LUTS may be one of the manifestations of cystitis associated with COVID-19 or OAB. Elderly patients with progression of LUTS should be screened for COVID-19 if the cause is unclear [25].

Therefore, the available data on the relationship between inflammatory disorders of the urinary system and SARS-CoV-2 are obvious. The pathophysiology of organ damaging by SARS-CoV-2, including the urinary system, is still not clear. The lack of a thorough understanding of the mechanisms of affecting certain organs leads to difficulties in studying the ways of transmission of infection, clinical diagnosis, treatment and development of methods for rehabilitation and prevention of complications. Based on basic and clinical studies on the organ damage by SARS-CoV-2, the genitourinary tract can be affected by a virus, which lead to an exacerbation and the development of symptoms of acute cystitis, including hemorrhagic, as well as symptoms of OAB. Despite the unusual manifestation of coronavirus infection, similar mechanisms of damage to urothelium cells in viral and bacterial infections allows to use pathogenetically based prevention of inflammatory reaction in the urinary tract, as well as the short-term and long-term complications. As a result, it is possible to recommend drugs that have a multifactorial effect, such as diuretic, antiadhesive, anti-inflammatory, and regulate the local immunity of the bladder mucosa.

D-mannose has an anti-adhesive effect against opportunistic pathogens of UTI. It is known that regular intake of D-mannose at a dosage of 2000 mg reduces the frequency of UTI recurrence by 4 times compared to standard prophylaxis with nitrofurantoin [26]. According to recent meta-analysis, published in 2020, a relative risk of UTI recurrence in patients receiving D-mannose compared to those taking placebo was 0.23 (95% CI, 0.14-0.37; heterogeneity = 0%; D-mannose, n=125, placebo, n=123). The pooled relative risk of recurrent UTI when comparing D-mannose with antibacterial prophylaxis was 0.39 (95% CI, 0.12-1.25; heterogeneity = 88%; D-mannose, n=163, antibiotics, n=163). Adverse events were reported in two studies that evaluated D-mannose monotherapy (in one study (n=10) there was no cases, while the other reported low rate of side effects). D-mannose appears to protect against recurrent UTI (compared to placebo), possibly as effective as antibiotics [27, 28]. D-mannose also interacts with its receptor, which is important for the treatment of bacterial and viral infections and stimulates the transformation of macrophages into the so-called anti-inflammatory macrophages [29].

In Cochrane Reviews (2004) the role of proanthocyanidins at a therapeutic dosage of 36 mg in the treatment and prevention of recurrent UTIs was clearly demonstrated [30, 31]. The active components of cranberry (salicylic acid and polyphenols) have a proven anti-inflammatory effect, reducing the level of C-reactive protein (a highly sensitive indicator of tissue damage during inflammation) and interleukins, as well as increasing NO synthesis [32]. Cranberry has a diuretic effect and enhances glomerular filtration (due to the regulation of renal vasculature tone), which results in the increased excretion of pathogens and contributes to a more rapid resolution of inflammation [33].

In-vitro studies have shown that the bladder epithelium in women receiving vitamin D3, which stimulates the production of antimicrobial peptides and provides a protective immunoprotective effect of cathelicidin and β-defensins, prevents the development of UTI [34].

In conclusion, we can propose that the prevention of bacterial UTI in patients with COVID-19 at the stage of treatment and rehabilitation is possible with a use of combination of several components that are part of the dietary supplement Uronext (NPO Petrovax Pharm LLC, Russia), which contains D-mannose 2000 mg, pro-anticyanidins 36 mg (or 500 mg of Cran-Max cranberry extract concentrate) and vitamin D3 1 mcg.

Recommendations for use: in adults, 1 sachet QD, after dissolving in a glass of water (100 ml) at room temperature; take

with meals. For pregnant and lactating women: take the product as recommended after discussion with a physician. We propose that this approach allows to decrease the number of urinary tract complications and contributes to more successful rehabilitation of patients in the post-COVID period. In order to give final conclusions and solid recommendations, the results from well-conducted clinical trials are required.

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ANDROLOGICAL ASPECTS OF NEW CORONAVIRUS INFECTION COVID-19

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COVID-19 is a new highly contagious infectious disease caused by the SARS-CoV-2. The World Health Organization (WHO) on March 11, 2020, has declared the novel coronavirus (COVID-19) outbreak a global pandemic.
Increasing attention is paid to the fact that men have a worse prognosis for the COVID-19. In addition, SARS-CoV-2 can infect the testes, potentially affecting testosterone production, as well as having a negative influence on the reproductive potential.
Our aim was to review the current concepts of the possible influence of testosterone levels on the pathogenesis of COVID-19 in men and to present the available data on the impact of COVID-19 on the structure and function of the testis.
Based on the analysis of 72 articles using the MEDLINE database (PubMed), it can be concluded that testosterone is involved in the co-regulation of the synthesis of angiotensin-converting enzyme-2 and transmembrane serine protease-2, facilitating the penetration of SARS-CoV-2 into target cells and promoting easier infection in men. On the other hand, low testosterone levels increase the risk of cardiopulmonary complications. Hypogonadism appears to be an important unfavorable prognostic factor.
Orchitis is a reported complication of COVID-19. Damage to testicular tissue is possible due to direct invasion by a virus, a secondary autoimmune reaction, hyperthermia and thrombosis of testicular microvessels. Prophylaxis of possible vertical and sexual transmission of infection is recommended.
Despite the available data, further studies are required to assess the definite role of androgens in the course of infection and the influence of SARS-CoV-2 on male reproductive potential.

Key words: *SARS-CoV-2, COVID-19, testosterone, hypogonadism, sperm analysis, male infertility*

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COVID-19 is a new highly contagious infectious disease caused by the SARS-CoV-2. Increasing attention is paid to the fact that men have a worse prognosis for the COVID-19 and higher death rate than women in all age groups [1]. Indeed, epidemiological data demonstrate that the proportion of male patients among those who die from COVID-19 reaches 59–73% [2–4].

Some of the hypotheses explaining the gender differences in mortality from COVID-19 highlight the role of testosterone [5]. Due to the fact that the measurement of testosterone levels is not a routine laboratory test for patients with COVID-19, the functional state of the gonads in infected patients has not yet been studied. However, considering that the worse clinical course is observed in elderly patients with one or more concomitant chronic diseases, it can be proposed that they are more likely to have a hypogonadal state [6]. On the other hand, it is known that angiotensin-converting enzyme-2 (ACE2), which is necessary for the penetration of SARS-CoV-2 into host cells, is also expressed in spermatogonia, Leydig and Sertoli cells [7, 8]. Therefore, SARS-CoV-2 can infect the testicles, potentially affecting testosterone levels in young patients. Another possible negative consequence of testicular damage by SARS-CoV-2 may be disturbances of spermatogenesis and male infertility.

In our work, we tried to describe the effect of testosterone levels on various pathogenetic aspects of coronavirus infection in men, as well as to present the available data on the impact of COVID-19 on the structural and functional state of the testicles.

Testosterone and penetration of SARS-CoV-2 in human cell

ACE2, a membrane protein that catalyzes the conversion of angiotensin I to angiotensin II, ensures the entry of SARS-CoV-2 into the host cell, contributing to transmissibility and severity of COVID-19 [9]. ACE2 is normally secreted in the lungs, oral mucosa, intestines, cardiovascular system, testicles, etc. [7, 10] and plays an important role in the regulation of pulmonary homeostasis and protection of the lungs from damage [11]. Low levels of ACE2 have been described in severe acute and chronic pulmonary diseases [12]. On the contrary, with arterial hypertension, diabetes mellitus, and taking certain medications, the level of ACE2 expression in various tissues may increase [12, 13]. These conditions can facilitate the penetration of the coronavirus into the cell and negatively influence on the prognosis of COVID-19 [12, 13]. However, gender differences in ACE2 expression/activity have not only genetic origin, but can also be due to hormonal factors, since estrogens induce enzyme expression [14, 15].

Transmembrane serine protease 2 (TMPRSS2) is a human enzyme whose function is not completely studied [16]. TMPRSS2 is able to cleave the SARS-CoV-2 spike antigen, which is necessary for the virus binding to the host cell membrane. The expression of the TMPRSS2 gene in humans is enhanced through the stimulation of androgen receptors by testosterone [17]. Therefore, its expression may be higher in men, which

causes greater permeability of their target cells for coronavirus [18]. Therefore, inherited hyperandrogenic phenotype may explain rare cases of severe COVID-19 in young patients [19].

Given the role of androgen sensitivity in the pathophysiology of COVID-19, using antiandrogen drugs to disrupt the mechanism of virus entry into the cell is discussed [20, 21]. This approach is supported by the data of Montopoli et al., who found that patients with prostate cancer, receiving androgen deprivation therapy, have a significantly lower risk of affecting by COVID-19 (hazard ratio = 4.05, $p = 0.0059$) [22].

Based on these data, it can be proposed that a physiological difference in circulating androgen levels between the sexes may predispose men to greater lung damage and systemic responses in COVID-19. On the other hand, lower expression of ACE2 in men may be the basis for more severe pulmonary and systemic manifestations.

Testosterone, respiratory and cardiovascular system of patients with COVID-19

Severe cardiovascular impairment with COVID-19 is typically diagnosed in elderly patients and those with chronic diseases such as arterial hypertension, diabetes mellitus, cardiovascular diseases and chronic obstructive pulmonary disease (COPD) [23]. There is a strong correlation between the severity of the infection and the presence of arterial hypertension. It is known that arterial hypertension is a pathological condition characterized by endothelial dysfunction. The endothelium secretes a wide range of substances (in particular, nitric oxide (NO) and interleukin-6 (IL-6)), which are involved in the regulation of vascular tone and cell adhesion, inflammation of the vascular wall, proliferation of smooth muscle cells and have an antithrombotic effect [24]. Endothelial inflammation leads to a loss of physiological balance between these substances, predisposes to the development of thrombosis and atherosclerosis, which contributes to the cardiovascular events. In endothelial cells, an increased secretion of ACE2 is noted, as a result of which SARS-CoV-2 can affect blood vessels [25]. Notably, in patients who initially have higher levels of cytokines (IL-6) and lower levels of NO, COVID-19 may cause a more severe cytokine storm. Keeping this in mind, some authors suggest the possibility of using phosphodiesterase type 5 (PDE-5) inhibitors in the treatment of COVID-19 [26]. In addition to an increase in the level of NO, an inhibitory effect these drugs on virus replication is also noted [27]. Therefore, it seems appropriate to study the possibility of using PDE-5 inhibitors as part of the combination therapy for COVID-19.

Elderly patients, who often have a hypogonadal state, are more susceptible to the progression of atherosclerosis [28]. Normal testosterone levels are required to maintain optimal triglyceride metabolism, glucose levels, blood pressure, left ventricular mass, waist circumference, and concentrations of circulating pro-inflammatory cytokines [28, 29]. In addition, testosterone is a fast-acting coronary vasodilator [30]. It is able to increase systolic output and cardiopulmonary exercise tolerance [30]. Low total and free testosterone levels result in a significant increase in overall mortality, mainly from cardiovascular diseases and especially in elderly patients [31, 32].

Hypogonadism occurs in 22–69% of patients with COPD [33]. Low testosterone levels can reduce respiratory muscle activity, total and reserve lung capacity [34]. A normal serum testosterone level has a protective effect on some spirometric parameters [35]. In addition, testosterone replacement therapy has been shown to increase maximal oxygen uptake [36].

Considering this, men with initially low serum testosterone levels can be expected to have an initially worse function of cardiovascular and respiratory systems, which can increase the risk of cardiopulmonary complications in case of COVID-19.

Testosterone and risk of thromboembolism in COVID-19

One of the characteristic features of the severe COVID-19 is a pronounced lesion of the alveolar epithelium and endothelium. As a result, tissue factor and plasminogen activator inhibitor type 1 accumulate in the lungs, which in turn leads to the deposition of fibrin in the alveoli and determines hypofibrinolytic state, which predisposes to thrombosis [37]. In some cases, the severe course of COVID-19 is complicated by disseminated intravascular coagulation [38]. Patients with a poor prognosis usually have high levels of fibrinogen and D-dimer, an increase in prothrombin time and activated partial thromboplastin time, as well as thrombocytopenia [39].

Published data suggest a higher incidence of thrombosis among men, both in those who were hospitalized and dead [40, 41]. In humans, testosterone is known to increase the expression of thromboxane A2, a platelet receptor, thereby increasing platelet activation and aggregation [42]. On the other hand, the level of serum testosterone affects the activation and reactivity of platelets by a negative feedback mechanism [43]. Testosterone enhances the synthesis and secretion of endothelial NO, which serves as a potent inhibitor of platelet activation. In addition, megakaryocytes and platelets contain estrogen and androgen receptors, which indicates the direct regulation of their activity by sex hormones [44]. It can be proposed that testosterone protects men from excessive platelet activation, but when hypogonadism develops, for example in elderly and comorbid patients, this protective effect loosen. Glueck et al. found that serum testosterone level has a positive correlation with the activity of tissue plasminogen activator and a negative relationship with the activity of fibrinogen and plasminogen activator inhibitor type 1, which also confirms the direct antithrombotic role of testosterone [45].

Thus, hypogonadism may increase the risk of newly diagnosed thrombosis in patients with COVID-19, and this fact should be especially considered in elderly and comorbid patients.

Testosterone and immune dysfunction systems in COVID-19

Different levels of circulating sex hormones seem to determine the gender difference in the response to infection [46]. Testosterone exhibits an immunosuppressive effect, since it suppresses the production of IL-6, IL-1β and TNF-α, and stimulates the secretion of IL-10. Moreover, testosterone suppresses T helper (Th) 17 cells, stimulates the differentiation of regulatory T cells, which contributes to suppression of the inflammatory immune response. At the same time, testosterone promotes the production and secretion of cytokines by Th1 and Th2 in higher proportions through stimulation of T-cell, and also inhibits B-cell proliferation and humoral response. On the other hand, testosterone deficiency can reverse the its effect on the immune system, hence predisposing to systemic inflammation with possible complications in elderly and comorbid patients [46]. An observational study of 331 hospitalized patients (127 men and 204 women) demonstrated that men had a poor prognosis and were less likely to recover (55.6 vs 63.0% in women) [47]. Notably, the authors found significant differences in humoral response between men and women with severe infections. In particular, men have a delayed peak in antibody levels and lower levels of IgG compared to women. This is explained by the different influence of sex hormones on the proliferation, survival and activity of B cells, which are enhanced by estrogens and suppressed by

testosterone [46]. The level of testosterone has also a significant impact on the immune system functions. For example, patients who are transferred to the intensive care unit/died in the intensive care unit, had lower levels of total and calculated free testosterone than men in therapeutic wards or in the intensive care unit, but in a stable condition [48]. Moreover, total and calculated free testosterone levels show a negative correlation with biochemical risk factors (neutrophil count, lactate dehydrogenase, procalcitonin, c-reactive protein, and ferritin) and a positive correlation with lymphocyte count. A sharp increase in the risk of transfer to the intensive care unit and death was noted at the level of total testosterone <5 mmol/l or calculated free testosterone <100 pmol/l [48].

As a result, on the one hand, a normal serum testosterone level predisposes to weak immune response, leading to a generalized spread of the virus and potentially severe complications. On the other hand, testosterone protects men from overproduction of pro-inflammatory cytokines (so-called cytokine storm). Conversely, male hypogonadism may exacerbate already weakened immune response against viral infection and contribute, rather than prevent, the development of a highly dangerous cytokine storm in COVID-19 [49, 50]. The role of low testosterone as a predictor of severe COVID-19 in men is clear but not yet fully studied. Wide screening of testosterone levels in patients hospitalized with COVID-19 may play an important role in assessing the risk of severe disease and patient death [51].

Male obesity, testosterone and COVID-19

Obesity is a common comorbidity in patients with COVID-19, with a prevalence of up to 49% [52]. Patients with an elevated body mass index are more likely to require transfer to intensive care unit and have an increased risk of death from COVID-19 [53, 54]. In particular, patients requiring invasive mechanical ventilation often have a body mass index of more than 35 kg/m2 [55]. Considering that ACE2 is secreted in adipocytes, it is logical to assume that an abundance of adipose tissue can significantly increase the number of available receptors for SARS-CoV-2, and therefore the severity of the systemic response to infection [56]. In addition, obesity and male hypogonadism are interconnected. Obesity-induced hypogonadism is common in overweight males and is characterized by a complex and multifactorial pathogenesis, including adipose tissue dysfunction, changing of testosterone-estrogen ratio and LHRH secretion, insulin resistance, obstructive sleep apnea [57-59]. Notably, adipose tissue dysfunction and male hypogonadism, even subclinical, are associated with increased levels of circulating cytokines (IL-6, -1, TNF-α), endothelial dysfunction, and represent an additional risk factor for thrombosis, possibly having detrimental consequences in patients with COVID-19 [60, 61].

Male obesity should be assessed as a relative risk factor for severe COVID-19. Obesity may impair baseline respiratory function, increasing the risk of requiring mechanical ventilation; cause an imbalance of the hormones involved in the regulation of the immune system and hemostasis, and results in a weak immune response to infection, cytokine storm, endothelial dysfunction and thrombosis.

Impact of SARS-CoV-2 on the testicles

In the testis, ACE2 is expressed predominantly in Sertoli and Leydig cells and spermatogonia. At the same time, the level of ACE2 expression in early and late spermatocytes and spermatids is rather low, while in Sertoli and Leydig cells it is about 3 times higher than in alveolocytes of type II [62].

Based on these data, we can propose the possibility of acute viral damage to the testicles, followed by disturbance of spermatogenesis and the development of hypogonadism. In addition, testicles can serve as a depot of viral particles, which may contribute to prolonged release of the virus after convalescence in men than in women.

The possibility of an acute inflammatory process in the testicles of patients with COVID-19 has been demonstrated in pathological studies by Yang et al. [63]. During immunological analysis, the authors noted that ACE2 was diffusely expressed in Sertoli cells, strongly expressed in Leydig cells, and was absent in spermatogonia. Thus, if testosterone levels in patients with COVID-19 can be affected by direct invasion of Leydig cells by the virus, then gametogenesis may be impaired due to damage not to progenitor cells, but to their microenvironment. In another study, the histological pattern in patients with COVID-19 was characterized by abundant infiltration of the testicular interstitium with CD3+-T lymphocytes, CD20+ B cells, and CD68+ macrophages, as well as by intense precipitation of IgG on the seminal epithelium [64]. It suggests that SARS-CoV-2 may trigger a secondary autoimmune inflammatory response in the testis. SARS-CoV-2 can also negatively affect the testicles due to fever and microthrombosis in testicular vessels, since under the virus provokes hypercoagulable state. There are some reports in the literature, according to which one of the symptoms of a new coronavirus infection may be acute orchitis [65, 66].

Data on the functional manifestations of COVID-19-associated testicular lesions have been published, including evidence that COVID-19 may induce the acute hypogonadism through damage to Leydig cells, as was shown by Ma et al. [67]. According to Holtmann et al., in patients with a moderate course of the COVID-19, there is a significant deterioration in sperm parameters compared to patients with mild COVID-19 and otherwise healthy men [68].

The issue of presence of SARS-CoV-2 in semen and the possibility of sexual transmission is still controversial. Yang et al. didn't reveal viral particles in autopsy testicular tissue using electron microscopy, and polymerase chain reaction for viral RNA was positive in only 1/12 cases [63]. Similar data were obtained in the work of Song et al. [69]. No virus RNA was detected in semen of 34 Chinese patients one month after recovery [66]. On the contrary, in another study, 6 semen samples out of 38 obtained from patients in the acute phase of the disease (39.5%) and convalescents (60.5%) were positive for SARS-CoV-2 [70]. Notably, positive samples were found in 4/15 men in the acute phase of the COVID-19 (26.7%) and 2/23 (8.7%) convalescents. It can be proposed that more severe the disease, the greater viral load in the blood and the higher the chance for SARS-CoV-2 to overcome the hematotesticular barrier [71].

According to literature, there is only a small risk of virus penetration into the seminal fluid. However, even a small risk is unacceptable in the treatment of infertile couples. In this regard, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology recommend avoiding pregnancy or participation in any IVF program for patients with positive diagnostic criteria for COVID-19 [72].

As was discussed above, testosterone is involved in the co-regulation of ACE2 and TMPRSS2 synthesis, enhancing their expression and facilitating the penetration of SARS-CoV-2 into target cells. However, due to versatility of testosterone effects, its normal level is necessary for the proper functioning of different organ systems. Low testosterone causes endothelial dysfunction, shifts coagulation system towards hypercoagulability, provokes hyperinflammatory immune response and leads to a high risk of cardiopulmonary complications. For these reasons, the hypogonadal state should be considered as unfavorable prognostic factor in COVID-19. However, the functional state

of the gonads in infected patients has not yet been thoroughly studied.

Hypogonadism may exist from the baseline or be induced by a virus. Acute orchitis is the described complication of a new coronavirus infection. Viral orchitis, in addition to disturbances in steroidogenesis, can also lead to an impairment of spermatogenesis, causing male infertility. Data on COVID-associated infertility are not yet available, and evidence of pathospermia in convalescents requires further studying.

Another important aspect of viral orchitis is the possibility of penetration of SARS-CoV-2 into semen, with subsequent vertical and sexual transmission of the infection.

In most publications, the presence of viral RNA in semen has not been confirmed. However, before this issue is finally resolved, it seems reasonable to avoid unprotected sex, pregnancy, or participation in any IVF programs while having an active disease.

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A PLACE OF ROBOT-ASSISTED CYSTECTOMY IN TREATMENT OF MUSCLE-INVASIVE BLADDER CANCER

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Bladder cancer occupies one of the leading positions in morbidity in the world and constitutes a serious problem for healthcare system. The muscle-invasive bladder cancer is the most aggressive and more difficult to treat with drug therapy. Radical cystectomy is the standard treatment for muscle-invasive bladder cancer, with the most commonly used open approach. Currently, there is an active introduction of minimally invasive procedures, which is due to their advantages in perioperative care. Laparoscopic procedures have been broadly adopted for the oncourological practice, but the real breakthrough in the field of minimally invasive surgery has occurred after implementing of robotic-assisted interventions. It should be noted that the extensive radical procedures are associated with significant intra- and postoperative complications, which directly affects the patients' condition and quality of life postoperatively. In this regard, robotic-assisted radical cystectomy appears to be a promising treatment method for muscle-invasive bladder cancer. The aim of this review is to collect and analyze current data on the results of robotic-assisted radical cystectomy, with particular attention to the comparison with open and laparoscopic techniques for different surgical and oncological outcomes.

Key words: muscle-invasive bladder cancer, minimally invasive surgery, open cystectomy, laparoscopic cystectomy, robot-assisted radical cystectomy

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Malignant neoplasms of the urinary system are a serious problem for the global health system, as the number of patients is growing every year and their average age is decreasing. Bladder cancer (BC) occupies one of the leading positions in this group of diseases. Bladder urothelial carcinoma is the 10th most common cancer in the world [1]. The muscle-invasive BC is the most aggressive (T2–T4), which determines the clinical behavior of the tumor and the choice of the optimal treatment method. The high risk of metastases and low efficacy of anticancer drugs at these stages make surgical procedures the main treatment method.

Radical cystectomy with pelvic lymph node dissection is the standard treatment for localized muscle-invasive BC in most Western countries [2, 3]. There are several variations of radical surgeries that have different functional results, intra- and postoperative complications. Until 1995, open radical cystectomy (ORC) was the only available surgical treatment for muscle-invasive BC [4, 5]. ORC is associated with high and significant mortality; however, it remains the "gold" standard [6]. In recent years, minimally invasive surgical methods, such as laparoscopic radical cystectomy (LRC) and robot-assisted radical cystectomy (RARC), have been increasingly used, since they are believed to provide good postoperative results due to shorter rehabilitation period and recovery [7]. At this stage in the development of oncological and surgical care, robotic surgery is the most promising, opening up new opportunities for intracorporeal interventions and orthotopic reconstructions of hollow organs, which include the bladder.

Robot-assisted and open radical cystectomy: advantages and disadvantages

The robotic surgical system da Vinci® (Intuitive Surgical Inc., Sunnyvale, USA) has significantly changed the role of minimally invasive surgery and brought it to a new level. Compared to a classic laparoscopy, robotic system gives a number of advantages, including greater mobility, an increased range of motion, tremor filtering, and a three-dimensional view. Robot-assisted pelvic and extraperitoneal surgical interventions has proven their efficacy [8, 9]. The introduction of RARC into surgical practice was accompanied by strong concerns about its surgical and oncological safety [10]. The main competitor of RARC is still ORC, which is the predominant treatment for patients with high-risk muscle-invasive and non-invasive BC [11]. A number of retrospective studies [12, 13] and randomized trial form Memorial Sloan Kettering Cancer Center [14] suggest that there are no significant differences between ORC and RARC in the rate of complications. To date, favorable outcomes in terms of positive surgical margins and the number of removed lymph nodes (lymph node yield) have been published [9, 13, 15, 16]. Early oncological outcomes (recurrence and mortality rate) also appear to be acceptable [16, 17].

According to Kimura et al. [18], RARC is associated with less perioperative blood loss and transfusion rate compared to ORC. However, ORC showed much less operative time. A systematic review and meta-analysis of randomized controlled trials found no difference between RARC and ORC in terms of perioperative complications, mortality, and the quality of life. Non-randomized controlled trials reported better perioperative outcomes for RARC, including shorter hospital stay, low 30-day complication rates and 90-day mortality. It should be noted, that results of non-randomized studies were not confirmed in randomized trials [18].

Novara et al. [19] obtained similar results. Cumulative analysis showed that the operation time was shorter in ORC, while RARC was associated with lower blood loss and transfusion rates. At the same time, RARC outperforms ORC and LRC

in terms of the rate of perioperative complications, although certainty of the evidence is low.

According to the systematic review [20], RARC and ORC are comparable in terms of time to recurrence, serious complications, quality of life, and positive surgical margins (low certainty of the evidence). In addition, the investigators were unable to prove a significant reduction in the rate of perioperative complications in RARC compared with open surgery. However, there was a decrease in the risk of blood transfusion and length of stay after RARC.

According to another large prospective randomized study [21], the incidence of 90-day perioperative complications of grades II-V (according to the Clavien-Dindo system) after RARC was 62%, compared to 66% after ORC. Such a small discrepancy doesn't show a significant advantage of RARC over ORC. Robot-assisted approach provided the same oncological results as open surgery. Moreover, assessment of quality of life 3 and 6 months after the surgery did not demonstrate the advantages of RARC over the traditional ORC.

We performed the evaluation of oncological results after ORC and RARC [22]. A total of 118 patients with Ta-T3N0-3/M0 BC were included in the study. Along with radical cystectomy, the patients underwent pelvic lymph node dissection. There was no significant difference in overall recurrence rates and survival among patients after ORC and RARC. At the same time, the analysis of the first recurrence showed an insignificant increase in the number of metastatic sites in patients who underwent ORC.

Nguyen et al. [16] assessed relapses after ORC and RARC. The relapse-free period for patients after ORC was 30 months, compared to 23 months after RARC. Over 2 years, there was no significant difference in the number of local relapses between two groups (ORC, 15/65 [23%], RARC, 24/136 [18%]). In addition, the similar location of local relapses was found (no port site metastases). The frequency of distant relapses in ORC and RARC groups was comparable (26/73 [36%] vs. 43/147 [29%], respectively). The frequency of extrapelvic lymph nodes metastases differed significantly: 10/43 (23%) and 4/26 (15%) in the RARC and ORC groups, respectively. Peritoneal carcinomatosis was diagnosed in 9/43 (21%) patients after RARC and in 2/26 (8%) after ORC. In conclusion, there was no association between RARC and adverse oncological outcomes, i.e., robot-assisted approach was not an independent predictor of recurrence.

The aim of the largest phase III randomized controlled trial (RAZOR), conducted from July 1, 2011 to November 18, 2014, was to compare RARC and ORC in terms of progression-free survival and postoperative adverse events in patients with T1-T4/N0-N1/M0 BC. Three hundred and two patients (150 in RARC group and 152 in ORC group) were included in the study. Two-year progression-free survival was 72.3% (95% confidence interval [CI]: 64.3-78.8) after RARC and 71.6% (95% CI: 63.6-78.2) in ORC group. The difference of 0.7% (95% CI: -9.6 to 10.9; p for non-inferiority = 0.001) between groups indicates an insignificant predominance of RARC over ORC. Analysis of adverse events showed similar results between the two groups. In RARC group, adverse events were seen in 101 (67%) of 150 patients, compared to 105 (69%) patients in ORC group. Urinary tract infections and postoperative bowel obstruction were the most common adverse events in both groups. Thus, an important conclusion can be made about the similar efficiency of RARC and ORC in two-year progression-free survival. The authors also emphasized that with the widespread introduction of robot-assisted procedures in surgical practice new randomized trials will be carried out for obtaining a reliable assessment of RARC.

Comparison of open, robot-assisted and laparoscopic radical cystectomy

The greatest interest among all the publications devoted to RARC in advanced BC is the Single-centre Early Phase Randomised Controlled Study (CORAL) [24]. It was the first study to compare postoperative complications, perioperative, pathological, oncological outcomes and quality of life of patients after ORC, RARC and LRC. A total of 59 patients met the selection criteria and were divided into three groups: ORC (*n*=20), RARC ((*n*=20), LRC ((*n*=19). Primary end points were 30- and 90-d complication rates, assessed by the Clavien-Dindo system. On day 30, 14 (70%) patients after ORC, 11 (55%) after RARC, and 5 (26%) after LRC had at least one complication of any grade, with significant differences between groups. However, at day 90, the differences between the groups were not significant. The secondary endpoints of the study were mostly the same. It should be noted that the mean operative time was longer for RARC compared to ORC and LRC. Prolonged ileus was more often after ORC. In addition, patients in ORC group had longer length of stay. There was no significant difference in terms of positive surgical margins between groups. The mean number of lymph nodes removed in ORC group was 18.8, compared to 16.3 and 15.5 after RARC and LRC, respectively. Differences between ORC and LRC were significant (p=0.01). Oncological efficacy was assessed after 12 months. The recurrence was detected in 10 patients (ORC: 2/19, RARC: 5/19, LRC: 3/18; p=0.5) and 4 patients died (ORC: 0/19, RARC: 1/20, LRC: 3/18; p=0.1), including 2 due to BC (both after LRC; p=0.1). There was no significant association between cystectomy approach and outcomes in terms of relapse, overall mortality, or disease-specific mortality. Survival differences were also not significant (p=0.7). The quality of life was assessed using the questionnaire, which was completed by 53 patients. No significant associations were found between the type of cystectomy and the quality of life.

Benefits of intracorporeal neobladder reconstruction

Of great importance for radical cystectomy is the reconstruction of the lower urinary tract, i.e., neobladder formation. Hussein et al. [25] noted an annual increase in intracorporeal reconstructions compared to extracorporeal ones. The authors attribute this trend to an increased number of ileal conduits, and to a lesser extent with orthotopic neobladders.

A retrospective analysis of extracorporeal (n=1031) and intracorporeal reconstruction (n=1094) was carried out. In the group of intracorporeal urinary diversion shorter operative time (357 vs. 400 min), less blood loss (300 vs. 350 ml), and fewer blood transfusions (4 vs. 19%; all p<0.001) were seen. Nevertheless, the number of serious complications after intracorporeal urinary diversion was markedly higher, which over time decreased [25].

Currently, there are many publications describing various aspects of robotic surgical procedures. The use of RARC in the treatment of muscle-invasive BC, although seems to be a promising, still requires further evaluation of its efficiency. This review of the literature identified several advantages of RARC, including less intraoperative blood loss, transfusion rate, and length of stay. In terms of oncological results, as well as the quality of life, RARC is comparable to ORC and LRC.

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LECTURES

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CURRENT APPROACHES TO TARGETED PROSTATE BIOPSY

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Currently, prostate cancer (PCa) is the second most common malignancy in men after lung cancer and the fifth leading cause of death worldwide. According to world and national statistics, over the past 20 years, there has been a steady increase in both incidence and mortality from PCa. Prostate biopsy is the cornerstone of the PCa diagnosis. However, recently, systematic transrectal biopsy as a standard approach has been questioned, since it has significant drawbacks that reduce the quality of PCa diagnosis.
Considering the clinical importance of accurate PCa staging, MRI-guided targeted biopsy has been developed, which is currently the most accurate technique for taking a sample of tissue from suspicious areas. The optimal approaches to targeted prostate biopsy and the potential possibilities of including multiparametric MRI in the primary diagnostic algorithm are highlighted in this review, based on the results of large studies. The method allows to increase the overall PCa detection rate, the detection rates of clinically significant PCa, reduce the frequency of diagnosis of low-risk tumors and increase the overall accuracy of PCa detection, which has an outmost importance for the patient selection for active surveillance and to control disease progression.

Key words: *Fusion-biopsy, prostate cancer, MRI, targeted biopsy, diagnosis of the prostate cancer*

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Prostate cancer (PCa) is the second most common cancer in men and the fifth leading cause of death worldwide [1]. According to GLOBOCAN (the International Agency for Research on Cancer and WHO database), 1.276 million new cases of PCa were diagnosed worldwide in 2018, with a higher prevalence in developed countries. Mortality was estimated at 358,000 cases, which accounted for 3.8% of deaths from cancer in men [2, 3]. Although it is supposed that incidence rates will rise to 2.293 million by 2040, mortality rates will change slightly: an increase of 1.05% is predicted.

The incidence and mortality of PCa are closely related to age, with older adults at highest risk (over 65 years of age). Mortality from PCa also increases with age, reaching almost 55% over the age of 65. African American men have the highest incidence and risk of developing a more aggressive PCa than Caucasian men [4].

In Russia, 42,500 new cases of PCa were recorded in 2018, which consist 62.4 per 100,000 males. Almost 13,000 men died from PCa (19.1 per 100,000). From 1999 to 2018, there has been a steady increase in both incidence and mortality from PCa, and the growth also persists for age-standardized values [5].

Although PCa incidence rates in the world are high, most cases are detected at the stage of a localized tumor. The five-year survival rate in the United States for men diagnosed with PCa is about 98%. According to the EUROCARE-5 study, the 5-year survival rate of patients with PCa from 2003 to 2007 was 83% [1]. According to other publications, this value ranges from 76% in eastern countries to 88% in southern and central Europe [6].

The most common complaints include difficulty voiding, increased frequency and nocturia. These symptoms can also occur with BPH. A later stage of the disease may be accompanied by chronic urinary retention and loin pain, since the spine is the most common site of bone metastasis [4].

PCa usually can be suspected in case of an elevated (> 4 ng/ml) total prostate-specific antigen (PSA) concentration in the serum. However, since PSA levels are also found to be elevated in benign pathology, biopsy is the standard diagnostic test for PCa [7].

Traditionally, the diagnosis of PCa has been based on the template prostate biopsy with transrectal ultrasound (TRUS) guidance, in which 10–12 biopsies are taken from different zones of the prostate. Despite the systematic approach, TRUS-guided biopsy does not detect about a third of clinically significant tumors and is associated with the risk of overdiagnosis of clinically insignificant PCa (cancer in situ) [8]. Given the clinical importance of precise PCa staging, attempts have been made to more accurately stratify risk, including using magnetic resonance imaging (MRI). Currently, according to the recommendations of the European Association of Urology

(EAU), in the case of a negative result of the previous biopsy and persistence of suspicion of PCa, the patient should undergo multiparametric MRI (mpMRI) and subsequent targeted biopsy [9].

Further improvements in the diagnosis of PCa will lead to better oncologic results, reduce the incidence of treatment-related side effects, optimize the use of health care resources, and improve survival rates.

History of development and classification of biopsy techniques

Since 1922, when the first ever transperineal prostate biopsy was described, its technique has been improved considerably [9, 10]. The first transrectal biopsy was performed in the 1930s. Since 1950, transrectal biopsy under finger guidance has been used, and the first clinically important transrectal ultrasound images of the prostate was done by Watanabe et al. in 1968 [11].

In 1989, Hodge et al. proposed a sextant biopsy of the prostate, in which the samples were taken from six areas. Subsequently, the method was refined, expanded and became the standard in the diagnosis of PCa [11]. Currently, the «gold» standard is TRUS 12-core prostate biopsy, which allows to detect PCa in 38.7% of patients [12]. Moreover, complications rates are comparable to that of sextant biopsy [13]. Biopsy can be performed in two ways, including transperineal route and more widely used transrectal approach. According to a number of prospective studies, the incidence of PCa detection and complications rates do not differ between two approaches [14].

Recently, however, role of TRUS guided systematic biopsy as a standard has been questioned, since it is associated with significant drawbacks, such as a false negative result, detection of clinically insignificant tumors, missing of clinically significant PCa, underestimation of PCa grade [15, 16]. TRUS-guided biopsy does not allow for sampling tissue from the anterior and apical parts of the prostate, which may result in missing clinically significant PCa [17]. According to the PROMIS study, in 26% of men with previous negative result of TRUS-guided biopsy, PCa was subsequently detected on a targeted biopsy [18]. In order to overcome these shortcomings, some experts advocate the use of transperineal mapping of the prostate with 5 mm sampling. While this technique can improve detection rates, it is also associated with a significant risk of complications such as urinary retention (22.5%), urinary tract infections (9.2%), negative effects on urinary and sexual function, and overdiagnosis [19].

Significant improvements in imaging studies have contributed to the development of MRI-guided biopsy, which can be performed either alone or in combination with ultrasound guidance [20]. The technique allows to visualize prostate lesions using MRI prior to biopsy in order to determine their volume and extent. Further, targeted biopsy from the suspicious lesions is performed. MRI of the prostate has a sensitivity of 44–87% with a negative predictive value of 92–94% for detecting advanced PCa [21].

General characteristics and main advantages of targeted prostate biopsy

mpMRI provides excellent tissue contrast and serves as a method of choice for evaluating local extent of the tumor [22]. According to a large meta-analysis, the overall PCa detection rate in MR-guided biopsy did not differ significantly from that in TRUS-guided biopsy, however, MR-targeted biopsy allowed for detect significantly more clinically significant cancers (sensitivity of 91%) and increase the percentage of positive biopsies [23]. Other studies have showed comparable data [24-26].

The American Urological Association (AUA) and the Society of Abdominal Radiology (SAR) recommend pelvic MRI followed by an MRI-targeted biopsy for men who remain at high risk for PCa despite a previous negative biopsy [22].

There are several approaches to using MRI results during prostate biopsy: MR-targeted (direct) biopsy, cognitive biopsy, and MR/TRUS-combined or fusion biopsy. These methods will be discussed in more detail below.

Technique and methods for targeted prostate biopsy

MR-targeted biopsy of the prostate. At the beginning, the patient is placed inside of the MRI system to visualize any suspicious lesions previously seen on mpMRI. Tissue samples are taken through a transrectal or transperineal approach using additional MR images to confirm needle placement. MRI-guided biopsy provides a higher detection rate of intermediate and high-risk PCa with a lower rate of diagnosing low-risk tumors [2, 27]. In one study of patients with a prior negative biopsy, the incidence of PCa was 51%, with 65% of these men having a clinically significant PCa [28]. In another study of 148 patients with elevated PSA level, MR-targeted biopsy revealed PCa in 55.6% of cases in primary settings and 43.1% of men with prior negative biopsies. In 82.4% of cases, clinically significant PCa was detected [29].

The advantages of the MRI-guided biopsy are fewer punctures and higher precision in needle positioning, while disadvantages include the need to place the patient inside of the MRI machine during the whole procedure, which may be impossible in persons suffering from claustrophobia.

Cognitive biopsy. Among all MRI-guided techniques, this method is the simplest to perform, since it does not require additional equipment.

Initially the specialist evaluates the MR images of the prostate and then performs a targeted biopsy from the suspicious area, which was found and described in the protocol of the diagnostic ultrasound study. Cognitive fusion-guided biopsy can be performed in the urology department as standard biopsy, since it does not require additional hardware or software besides an ultrasound machine. However, this technique largely depends on the experience of the urologist and the extent of the lesion, since the extrapolation of MRI results to real time TRUS images requires specialized knowledge in the field of reading and interpreting MRI and performing TRUS [30]. Experienced professionals can obtain the same results for PCa detection with the cognitive biopsy technique as with computer-software-based fusion [31]. The only limitation of this method is the detecting precise location of the lesion. In addition, small tumors can be missed without using special programs for evaluating MRI and ultrasound images [30, 31]. For example, in a study by Cool et al. the cognitive fusion-guided biopsy did not reveal more than 50% of clinically significant PCa [32].

MRI/TRUS fusion biopsy. A major advance that has allowed urologists to quickly and easily introduce mpMRI into clinical practice is the ability to combine MRI images with real-time ultrasound images to perform accurate sampling from MRI-detected lesions [16]. Several software platforms have been developed to evaluate these images, the first of which was UroNav (Invivo, Gainesville, Florida, USA) in 2004.

In the first stage, one should analyse MR images using special software (UroFusion [Biobot Surgical], DynaCAD [Invivo], McDraw [Koelis], ProFuse [Eigen] platforms), which

is usually provided by the manufacturer. The resulting images are carefully evaluated to identify and stratify lesions using PI-RADS v2 (Prostate Imaging Reporting and Data System version 2). Then the images of the prostate and the affected areas are mapped. During this process, a radiologist outlines the contours and foci of prostatic lesions on T2-weighted images [33]. Then three-dimensional (3D) images are recreated, which are transferred to the image fusion system [34].

The second stage is TRUS with the acquisition of images in different axes, followed by image mapping. Finally, images from both methods are fused using specially designed software. This allows to align the suspicious area, detected on MRI, with the TRUS image, which enables real-time navigation and orientation using TRUS probe. However, accurate alignment is demanding, since the geometry and orientation of the prostate are not similar between the two imaging modalities due to differences in position and timing of imaging. In particular, TRUS is performed in the left decubitus position, while MRI is performed in the supine position. This causes progressive and rotational differences, as well as distortion of the geometry due to variable bladder distension and the use of a transrectal probe [4].

The final step is to perform the biopsy itself. By combining MR and TRUS images, real-time navigation is possible. The software must receive feedback from the ultrasound probe to track a path of biopsy needle, for which several strategies have been developed. One of the methods is to use an electromagnetic field, which is created by a device on the patient's body, connected to an ultrasound sensor. The electromagnetic field can perceive the movements performed when the sensor is moved by the operator [15]. This type of feedback mechanism is used in UroNav, Virtual Navigator and BK Fusion systems [30, 35, 36]. Artemis, Biojet, Fusion Bx and Biopsee systems use the mechanical lever technique, which, when manipulated by the operator, provides feedback to the system [30, 36].

A unique method of providing feedback is used by Urostation/ Trinity systems. The technique relies solely on ultrasound images obtained during the primary scan. During the biopsy procedure, these images are recreated and allow to track the needle position in real time [30, 37].

Fusion biopsies can be performed as an outpatient procedure. Patient preparation is similar to TRUS guided biopsy. In the morning on the day of the procedure, it is recommended to perform a cleansing enema. A few hours before the procedure, a single prophylactic dose of oral fluoroquinolone is prescribed [38]. Fluoroquinolones are the preferred group of antibacterial drugs because they penetrate well into the prostate and reduce the risk of gram-negative bacterial infections typical for transrectal route.

Patients taking antithrombotic drugs deserve special consideration. Due to the increased risk of minor bleeding, patients on dual antiplatelet therapy (low-dose aspirin and clopidogrel) should stop taking clopidogrel 1 week before the procedure. Patients receiving antiplatelet monotherapy can continue to take the drug. In special cases, for example after coronary artery interventions (2 weeks after coronary angioplasty, 6 weeks after stent placement and 12 months after placement of a drug-eluting stent), dual antiplatelet therapy should be continued. Although we are usually discontinued oral anticoagulant 5 days before the procedure, and intravenous heparin therapy should be considered for patients at high risk of venous thromboembolism [39].

Typically, a biopsy is performed under local anesthesia. A total of 10 ml of 1% lidocaine solution is injected at the base and apical part of the prostate within Denonvilliers' fascia [40]. For the best effect, it is important to inject the anesthetic solution to the posterior edge of the prostate toward the seminal vesicles. It is necessary to avoid accidental air injection, which can lead to shadowing artifact during the biopsy. After the a while, biopsy is performed using an automatic biopsy gun [34].

Fusion biopsy is associated with the same intra- and postoperative complications as conventional TRUS guided biopsy. Within a few days after biopsy, transient hematuria and hematochezia can develop, which do not require specific treatment [34]. In the ProtecT (Prostate Testing for Cancer and Treatment) study, complications included hemospermia (92.6%), hematuria (65.8%), pain (43.6%), hematochezia (36.8%), and fever (17.5%) [41]. In most cases, rectal bleeding resolves within 48 hours. To stop rectal bleeding, one should immediately tamponade the rectum after the biopsy using a probe or balloon. In patients with coagulopathy or artery tears, profuse bleeding may occur, which requires clipping or coagulation during sigmoidoscopy.

In addition, there is a risk of infectious complications due to contamination via transrectal route. It is possible to minimize the risk of infectious complications by antibacterial prophylaxis, however, strains resistant to fluoroquinolones can lead to infectious and septic complications. In such cases, parenteral third-generation cephalosporins or aminoglycosides are prescribed [42, 43].

Prostate edema after biopsy can cause acute urinary retention and worsen lower urinary tract symptoms [44]. A small number of patients may develop erectile dysfunction and syncope [34].

Currently, PCa is the second most common cancer in men after lung cancer and the fifth leading cause of death worldwide. In Russia, over the past 20 years, there has been a continuous increase in both incidence and mortality from PCa.

Prostate biopsy is the cornerstone of PCa diagnosis and determining of treatment tactics. Despite the systematic approach, TRUS guided biopsy as «gold» standard is associated with a high number of false-negative results, detection of clinically insignificant tumors, missing of clinically significant PCa, and underestimation of the PCa grade.

Considering the clinical significance of PCa staging, a number of methods of targeted biopsy under MRI guidance has been developed. MRI of the prostate is currently the most accurate imaging technique for detecting suspicious lesions. The method allows to increase the overall PCa detection rate, the detection rates of clinically significant PCa, reduce the frequency of diagnosis of low-risk tumors and increase the overall accuracy of PCa detection, which has an outmost importance for the patient selection for active surveillance and to control disease progression. Targeted prostate biopsy has already found its application in patients with a negative result of a previous biopsy with continued clinical suspicion of PCa.

Although some questions remain to be answered, for example the optimal technique of targeted biopsy or a role of MRI as an initial imaging study, the wider use of targeted biopsy may improve the diagnosis of PCa and will optimize the use of health care resources, improving patient survival rates.

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PROSTATE CYSTS

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The classification of prostate cysts, a description of the typical clinical features and current treatment methods are presented in the article, based on modern literature data.

K e y w o r d s: prostate cysts, endoscopic treatment, infertility, impaired spermatogenesis

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A prostate cyst is a hollow, encapsulated, in the vast majority of cases, benign lesion of the prostate or prostate-vesicular complex, filled with a liquid component. The cysts can cause urination disorders. The treatment of the prostate cysts remains an urgent problem of modern urology. The prostate cyst is a rare disease, accounting for 7.0 to 8.6% of all benign lesions of the urogenital tract [1]. Since they are most often diagnosed in people of working age, the development and improvement of contemporary treatment methods remains a top priority for leading urological clinics.

Symptoms of prostate cysts include hemospermia, ejaculatory pain, obstructive urinary symptoms and urinary tract infection (UTI). Prostate cysts can cause recurrent epididymitis and urinary incontinence, as well as male infertility. Often, they are associated with various anomalies of the genitourinary system, such as hypospadias, cryptorchidism, and ipsilateral renal agenesis [2]. The severity of symptoms depends on different factors, including the location, a size, and persistence of the uropathogen. However, it should be noted that in most cases, prostate cysts do not cause pronounced clinical manifestations and are most often incidentally diagnosed during instrumental examination.

Classification. Clinical manifestations

There are several classifications for prostate cysts, which reflect the anatomical and topographic features and allow to choose the optimal surgical treatment. In 2008 R Nayyar et al. proposed the classification, according to which all cysts were divided into three groups [3]:

1 – median cyst that does not communicate with the urethra (typical prostatic utricle cyst); 2a – median cyst that communicates with the urethra (cystic dilatation of the prostatic utricle); 2b – cystic dilatation of the prostatic utricle, communicating with the vas deferens; 3 – cystic dilatation of the ejaculatory duct.

In 2009, A. Galosi and R. Montironi published an article, in which they presented a new extended classification of prostate cysts [4]:

1. Median cysts: a - prostatic utricle cyst (formerly called the Mullerian duct cyst), located in the midline above the verumontanum; b - cystic dilatation of the prostatic utricle, which has a histologically confirmed communication with the urethra, but is not detected by transrectal ultrasound (TRUS); c - an enlarged prostatic utricle (persistent Mullerian duct tissue, or the male vagina [vagina masculinus]), which is a congenital pathology, when the verumontanum may be absent, but due to the wide communication with the urethral lumen, it cannot be regarded as prostate cyst. This entity is often associated with other malformations, such as hypospadias.
2. Cysts of the ejaculatory duct.
3. Parenchymal cysts, which are also divided into: a - simple cysts and b - multiple cysts: I - ductal ectasia (microcysts), II - a small cystic nodule, III - a large cystic nodule.
4. Complicated cysts: a - infectious, b - hemorrhagic.
5. Cystic tumor: a – cystic carcinoma, b – cystadenoma, c – cystic carcinoma, d – dermoid cyst.
6. Secondary cysts due to other pathologies: bilharzia and echinococcosis.

R. Furuya et al. presented their classification of the prostate cysts in 2017 [5]:

1. Parenchymal cysts: a - median, b - paramedian, c - lateral.
2. Extraprostatic cysts: a - seminal vesicles cyst, b - vas deferens cyst, c - Cowper's duct cyst.

All prostate cysts, depending on their location, are divided into intraprostatic and extraprostatic. By origin, cysts are divided into congenital and acquired. Median cysts are located in the midline, behind the upper half of the prostatic urethra. They include prostatic utricle cysts and Mullerian duct cysts *(fig. 1)*.

Prostatic utricle cysts result from intrauterine dilatation of the prostatic utricle and has a communication with the posterior urethra. They are usually diagnosed in the 1st or 2nd decade of life. Prostatic utricle cysts are small and typically have a size of 8–10 mm (*fig. 2*). Prostatic utricle cysts contain white or brown fluid and spermatozoa may be detected (a cytological marker of these cysts). They are reported to occur in 1–5% of the population [6, 7]. Prostatic utricle cysts are associated with various congenital anomalies of the genitourinary system, including hypospadias, cryptorchidism, and ipsilateral renal agenesis [7, 8]. They have a variable clinical presentation, including UTI, pain, post-micturition urinary

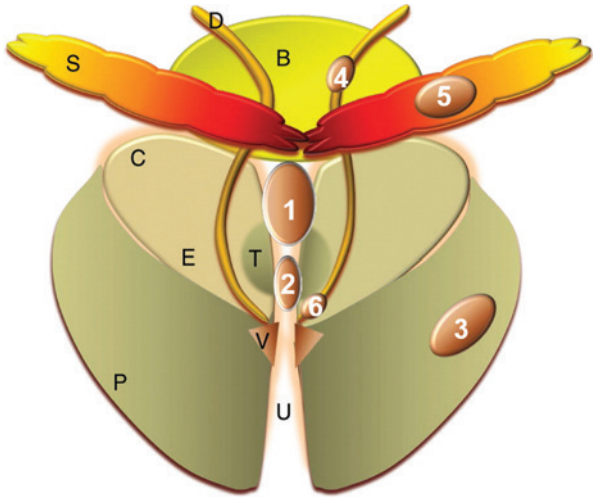

Fig. 1. Scheme of prostate cysts (adapted from [7])
B - bladder, C - central zone of the prostate, D - vas deferens, E - ejaculatory ducts, P - peripheral zone of the prostate, S - seminal vesicles, T - transitional zone of the prostate, U - urethra, V – verumontanum.
1 - Mullerian duct cyst; 2 - prostatic utricle cyst; 3 - retention cyst; 4 - vas deferens cyst*; 5 – seminal vesicle cyst*; 6 - ejaculatory duct cyst (* extraprostatic cysts)

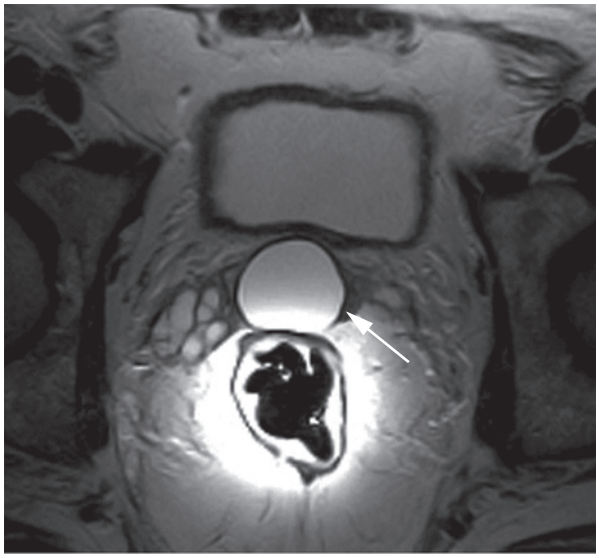

Fig. 3. Mullerian duct cyst (indicated by an arrow) on an MRI T2-weighted image (axial section) [7]

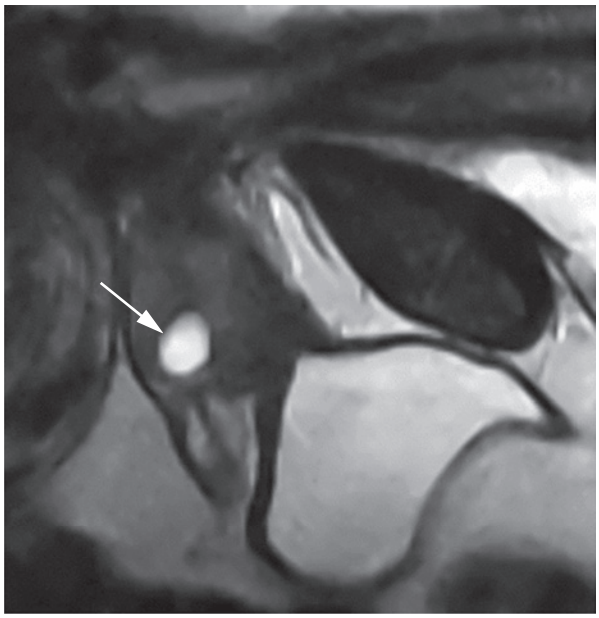

Fig. 2. Prostatic utricle cyst (indicated by an arrow) on an MRI T2-weighted image (sagittal section) [7]

incontinence, recurrent epididymitis, and hemospermia [6]. Since they communicate with the urethra, they can cause urinary incontinence after voiding. Prostate cysts can become infected and contain pus or blood, making it difficult to visualize, because they look like abscesses and cystic prostate tumors [9].

Mullerian duct cysts develop from the remnants of the paramesonephric (Mullerian) duct due to its incomplete involution at the 3rd month of fetal development. Mullerian duct cysts are connected to the verumontanum by a stalk, but have no communication with the urethra (*fig. 3*). They often diagnosed in the 2nd to 4th decades of life, but there are reports of a few cases manifested in infancy [10]. According to the autopsy studies, the prevalence of the Mullerian duct cysts among men is about 1%, but it may be underestimated, since some authors report the values up to 5% [9]. Mullerian duct cysts are usually asymptomatic, but may present in adulthood with urinary retention and UTI [11]. They can also cause impaired ejaculation due to obstruction of the ejaculatory duct in the midline. Like prostatic utricle cysts, Mullerian duct cysts can become infected; on imaging, they resemble abscesses or cystic prostate tumors [12]. Development of prostate carcinoma in prostatic utricle and Mullerian duct cysts are described [12]. They content brown or green fluid with serous or mucosal consistency, usually heterogeneous due to small stones, blood or pus. There are no spermatozoa in these cysts. The comparative characteristics of prostatic utricle cysts and Mullerian duct cysts are presented in the table [7].

Ejaculatory duct cysts are located laterally (paramedian cysts), close to the midline and posterior to the prostatic urethra (*fig. 4*). They are rare and form as a result of congenital or acquired obstruction of the ejaculatory duct [13]. Obstruction of the ejaculatory duct is one of the main causes of male infertility. In the sperm analysis, predominantly oligospermia or azoospermia, as well as hemospermia, are found. However, despite pathospermia, this group of patients usually has otherwise normal physical examination, and the serum level of gonadotropins is within normal range. When aspirated, ejaculatory duct cysts may contain fructose or sperm, often stones, and sometimes pus or blood. Cystic dilatation of the ipsilateral seminal vesicle has been described [14].

Lateral prostate cysts are located at some distance from the midline and can affect any area of the prostate. They include retention cysts, degenerative cysts, and cysts, associated with tumors.

The pathogenesis of retention prostate cysts is unclear, but they are associated with age and are observed in patients with benign prostatic hyperplasia (BPH). It has been established that they occur more often in infertile than in fertile males [8]. Retention cysts result from dilation of glandular acini due to acquired obstruction of prostatic ducts, resulting in cysts of 1–2 cm in

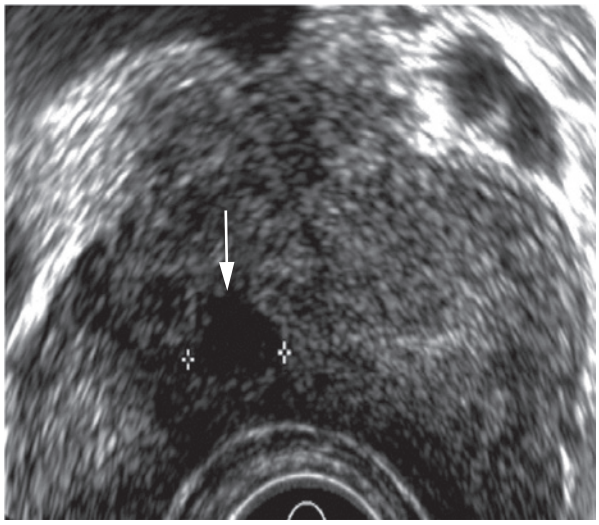

Fig. 4. Ejaculatory duct cyst on TRUS of the prostate. The arrow indicates a small anechoic cyst located laterally [7]

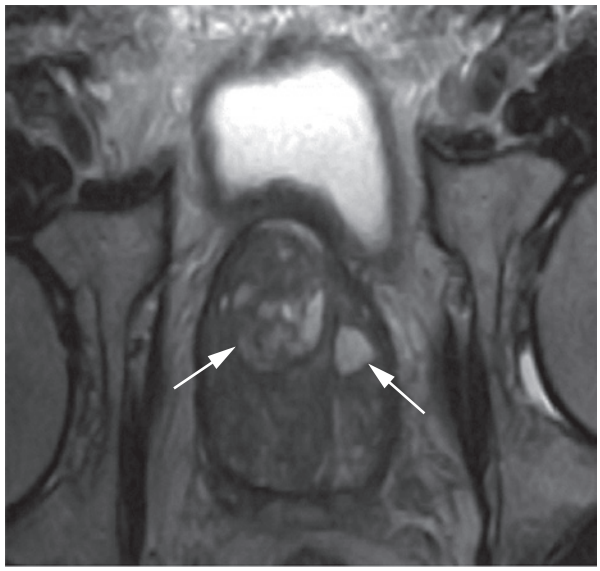

Fig. 5. Degenerative cysts of the prostate (indicated by arrows), which cause an increase in the size of the central zone of the prostate and a pronounced intravesical protrusion [7]

diameter containing clear fluid. These cysts are common, appear in the 5th to 6th decade of life, and are rarely symptomatic [9]. Retention cysts are unilocular lesions with smooth walls, usually round in shape, which can occur in any area of the prostate. They are identical in appearance to cysts, associated with BPH. The diagnosis is based on their location in the peripheral zone or the absence of other evidence of BPH [9].

Degenerative prostate cysts constitute the majority of cystic lesions of the prostate. They are located in the transition zone along with BPH nodules. These cysts are irregular in shape and variable in size, and may also contain blood or stones. Patients with these cysts usually present with symptoms of urinary tract obstruction due to BPH (*fig. 5*) [5].

Tumor-associated cysts are benign or malignant neoplasms of the prostate that contain cystic components. Cystadenoma is a rare benign tumor consisting of cysts lined with simple columnar epithelium with loose fibrous or fibromuscular stroma and signs of chronic inflammation, which can often be large (*fig. 6*) [16]. Cystic carcinoma of the prostate is predominantly a cystic mass with nodular walls, with hard components being a neoplasm [17, 18]. Magnetic resonance imaging (MRI) in T2-weighted images may show cystic content and the extent of the lesion. The pathogenesis of cystic carcinoma of the prostate can be associated either with a pseudocyst due to central necrosis or hemorrhage in the malignant tumor, or with malignant degeneration of the retention cyst. Most reported cystic carcinomas are pseudocysts with intralesional bleeding; only 17% of them arose due to the degeneration of retention cysts [18]. In cystic prostate lesions, the presence of blood should raise suspicion of malignant tumor. Other prostate tumors that may present with a cystic lesion include papillary cyst adenocarcinoma and transitional cell adenocarcinoma. In rare cases, leiomyoma and prostate liposarcoma may have cystic elements. On MRI, heterogeneous signal intensity of cystic components and the presence of soft tissue suggest a neoplastic nature.

Extraprostatic cysts arise outside the prostate from nearby structures, including seminal vesicles, vas deferens, and Cowper ducts.

Seminal vesicle cysts can be congenital or acquired. They are usually found in patients aged 10 to 40 years [19, 20]. Congenital seminal vesicle cysts can be subdivided into isolated cysts, cysts associated with abnormalities of the upper urinary tract, and cysts, which are related to autosomal dominant polycystic kidney disease [20]. Several cases of seminal vesicle cysts have been reported, most of which are associated with ipsilateral renal agenesis and are included in the Zinner syndrome [1, 21].

Seminal vesicle cysts are usually small (<5 cm) and are either asymptomatic or present with irritative symptoms and recurrent infections. Large cysts (>8–10 cm) are less common and may present with symptomatic bladder or bowel obstruction [1,

Table

Comparative characteristics of prostatic utricle cysts and Mullerian duct cysts

Characteristic	Prostatic utricle cysts	Mullerian duct cysts
Patient age, years	0–20	10–30
Origin	Intrauterine dilatation of the prostatic uterus	Incomplete involution and local saccular dilatation of the Mullerian duct
Configuration	Pear-shaped	Drop-shaped
Bulging over the prostate base	No	Yes
Communication with prostatic urethra	Yes	No
Presence of spermatozoa	Yes	No
Malignant potential	Yes	Yes

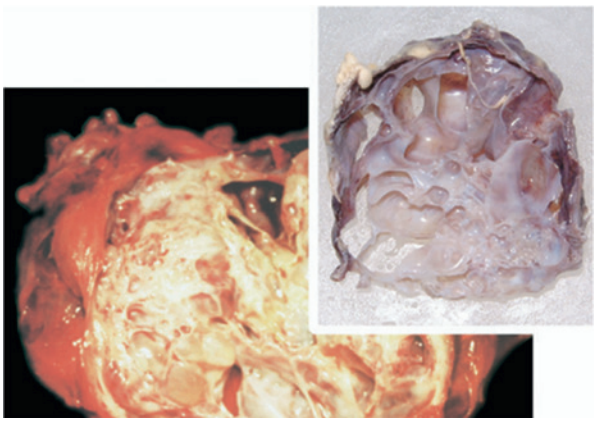

Fig. 6. Macropreparation of cystoadenoma of the prostate clearly shows its polycystic nature [16]

20]. Patients with seminal vesicle cysts may also have chronic recurrent prostatitis and epididymitis, painful ejaculation, urethral discharge, urgency, hematuria, acute urinary retention, defecation pain, tenesmus, constipation, pelvic discomfort, perineal or testicular pain, abdominal or small pelvis mass, infertility or hemospermia [22].

Vas deferens cysts are located along the vas deferens and above the prostate. Congenital anomalies of the vas deferens are the most common finding in men with azoospermia and low ejaculate volume [14]. Infection, obstruction, and neoplasia are possible causes of acquired vas deferens cysts.

The ducts of Cowper's gland open into the bulbar urethra. Obstruction of these ducts may result in retention cysts. Cowper's duct cysts can be congenital or acquired. Most of these cysts are asymptomatic, but larger ones can cause hematuria or urinary tract obstruction and possibly male infertility [12]. Several cases of intrauterine and early postpartum death have been reported as a result of urinary tract obstruction due to large Cooper's duct cysts. In adults, acquired Cowper's duct cysts result from infection or trauma [8].

Treatment

Currently, in the world literature, there are virtually no studies devoted to a comparison of treatment methods for prostate cysts. The available publications are clinical observations, which describe various surgical interventions. In the domestic literature, there is a limited number of publications, especially those related to endoscopic procedures.

One of the first descriptions of transurethral endoscopic intervention for prostate cysts dates back to 1987. Japanese authors H. Takatera, H. Sugao and T. Sakurai described a clinical case of prostate cyst in a 51-year-old man who admitted to the hospital with complaints of perineal discomfort, periodic episodes of gross hematuria and a decrease in ejaculate volume [23]. Ultrasound revealed an intraprostatic cyst, and transurethral incision was performed. According to the follow-up ultrasound 4 months postoperatively, there was no residual cyst and symptoms resolved completely within 3 months. A. Six et al. in 1989 described three cases of prostate cyst in men with obstructive urinary symptoms who underwent transurethral endoscopic resection of the anterior wall of the cyst in order to provide adequate drainage [24]. In 1990, J. V. Rodriguez and E. Ruiz-Castane carried out a study that included 12 patients with prostatic utricle cyst, 6 of whom suffered from infertility [25]. Transurethral resection of the prostatic cysts in all cases eliminated the distal obstruction of the seminal duct, and in half of the cases the mobility and concentration of spermatozoa increased. Successful endoscopic treatment of retention prostate cysts was also described by M. Tamaki et al. in 1994 [26].

The advantage of intraoperative use of sonography and fluoroscopy for transurethral incision of the prostate cysts was demonstrated in the work of T. Manohar in 2008 [27]. From 1997 to 2005, 25 patients with ejaculatory disorders, including hemospermia, underwent transurethral incision of the ejaculatory ducts under TRUS and X-ray control. Improvement in symptoms was noted in 96% of patients with ejaculation disorders. All patients with painful ejaculation and hemospermia reported complete resolution of symptoms within 3 months after procedure. In addition, 3 men developed transient epididymitis, managed conservatively. None of the patients experienced retrograde ejaculation or urinary incontinence. The development of hemospermia in a 51-year-old man caused by prostate cyst and obstruction of the ejaculatory ducts was described by Japanese scientists H. Fuse and R. Nishio in 2003 [28]. The cyst wall was dissected with a urethrotome. The cyst contained bloody fluid and two openings of the ejaculatory ducts were identified inside. There were no intra- and postoperative complications. After 1 month after surgery, hemospermia was completely absent, and there were no relapses within 7 months.

One of the first articles comparing the efficiency of endoscopic methods for the treatment of prostate cysts was a study by Xiong-Bing Zu et al., published in 2009 [29]. The authors retrospectively analyzed the clinical data of nine patients, five of whom presented with frequency and urgency, three with difficulty urinating with a weak urine stream, and one with hemospermia. Transurethral incision was performed in three patients with small cysts located near the prostatic urethra, and six patients with larger cysts located deeply underwent laparoscopic excision. The duration of transurethral removal ranged from 30 to 50 min and blood loss was 20–70 ml. With laparoscopic access, the respective values were 100-150 min and 30-50 ml, respectively. All patients were followed for 3-12 months and didn't have any erectile and ejaculation dysfunction as well as obstructive and irritative symptoms. The authors concluded that for small prostate cysts located close to the prostatic urethra, transurethral incision is the preferred treatment method. Due to its safety, efficacy, minimal invasiveness, few complications, and rapid recovery, laparoscopic excision can be used as a first-line treatment for large prostate cysts. A similar comparative study was carried out by Xiang-Zhou Sun et al., who analyzed the medical records of 48 patients with Mullerian duct cysts and compared diagnostic methods (TRUS and MRI) and treatment procedures [30]. Seven patients underwent transperineal ultrasound-guided puncture of the cyst, followed by aspiration. One patient with a large Mullerian duct cyst underwent open surgery and another patient underwent laparoscopic removal of the cyst. In 39 cases, small cysts were treated with transurethral incision. All patients underwent TRUS after 3 months. There were no cases of cysts regrowth. Within 6 months after surgery, 10 out of 39 patients with obstructive azoospermia conceived a child. In 9 out of 29 patients, spermatozoa were detected in the ejaculate, and in one 19-year-old patient symptoms of chronic pelvic pain were resolved. According to the results, patients with Mullerian duct cyst most often complained of infertility caused by obstructive azoospermia, as well as chronic pelvic pain. TRUS is the preferred imaging modality for Mullerian duct cysts, and pelvic MRI can provide more accurate information about the location

and size of the cyst. The authors noted that transurethral access is the most effective method for treating Mullerian duct cysts, while laparoscopic excision may be the method of choice for large ones.

Despite the introduction of endoscopic methods for the treatment of prostate cysts, there are still some indications for open surgery. In 2009, a group of Indian urologists described a successful open removal of a large prostate cyst in 13-year-old patient (11.5 x 4.4 cm) that caused burning during voiding and postmicturition dribbling [31]. Complete excision of the cyst was performed through the posterior sagittal approach with lateral mobilization of the rectum. There were no intra- or postoperative complications. Complete resolution of symptoms was noted 3 months after the intervention.

The role of vesiculoscopy in the diagnosis and treatment of prostate cysts was studied by Korean scientists led by Pil Moon Kang [32].

The study included 61 patients with symptomatic median prostate cysts and complaints of hemospermia, chronic pelvic pain (perineal pain, discomfort in the scrotum and testicular pain) and ejaculatory disorders, who did not receive medical therapy for 4 weeks. All patients underwent transurethral vesiculoscopy using a 9.0 Fr rigid ureteroscope and a Bugbee electrode. According to endoscopic examination, hemorrhage in the dilated prostatic utricle and seminal vesicle were observed in 11 (18.0%) and 21 (34.4%) men, respectively. Stones in the cavity of the enlarged prostatic utricle were observed in 12 (19.7%) cases, while stones in seminal vesicle were found in 6 (9.8%) patients. Hemospermia resolved in 29 (90.6%) of 32 men after surgery, while in 29 patients with chronic pelvic pain syndrome and ejaculatory dysfunction, Chronic Prostatitis Symptom Index (NIH-CPSI) score after treatment improved from 19.0 ± 3.8 up to 11.8±3.6 ($p<0.001$). Acute epididymitis developed postoperatively in 2 (3.3%) patients. This study showed that the role of vesiculoscopy in reducing symptoms was associated with endoscopic fenestration, removal of blood clots, stones, purulent contents, and, if necessary, electrocoagulation of intracystic hemorrhage. This endoscopic method has combined diagnostic and therapeutic role in the treatment of symptomatic median prostate cysts.

The first laser lithotripsy of seminal vesicle stones in a 25-year-old patient with complaints of painful ejaculation was described by S.P. Cuda et al. in November 2006 [33]. A 7 Fr rigid ureteroscope was used to perform transurethral vesiculoscopy, which revealed a group of loose stones 1–3 mm in diameter that were located in the seminal vesicles and ejaculatory ducts. The stones were fragmented with a holmium laser (Ho:YAG) and removed by irrigation and a stone basket. The patient was discharged the next day after the operation, the symptoms completely absent after 3 months. Laser incision of a prostate cyst in a 35-year-old man with a complaint of primary infertility was described by J. Ethan et al. even earlier, in 2000 [34]. When conducting TRUS, a median cyst of 14×9×10 mm, located above the verumontanum, was detected, which have communication with the dilated ducts of the seminal vesicles. The patient underwent urethrocystoscopy with laser neodymium (Nd:YAG) incision of the anterior wall of the cyst under ultrasound guidance. Since the cyst was inaccessible for visualization during standard urethrocystoscopy, sonography facilitated a precise dissection of the wall without the risk of trauma to adjacent structures.

A combined (laser and electrosurgical) endoscopic procedure for the treatment of prostate cyst with obstruction of the ejaculatory duct was demonstrated by a group from Seoul led by Joo Yong Lee in 2012 [35]. The authors described a clinical case of a 32-year-old man with oligo- and azoospermia, who had a median prostate cyst of 2 cm, as well as dilated seminal vesicles. Using a holmium laser, a transurethral opening of the cyst cavity was performed with subsequent monopolar resection of the cyst. One month after the intervention, an increase in ejaculate volume up to 3 ml was seen and the concentration of spermatozoa elevated to 15.2×106/ml. In conclusion, the authors noted that the use of a holmium laser for combined endoscopic treatment of midline prostate cysts has some advantages, including precision opening of the cyst, preventing unnecessary coagulation and reducing the risk of damage to adjacent structures. The advantages of holmium laser for prostate cysts and obstruction of the ejaculatory ducts have also been described in other publications [36, 37].

Thus, the majority of authors confirm the high efficiency and safety of endoscopic methods of surgical treatment of prostate cysts. Transurethral marsupialization remains the most common endoscopic procedure for medium-sized cysts. However, a small number of publications emphasizes that transurethral endoscopic interventions for prostate cysts are still under development, and require additional researches.

Prostate cysts are a relatively rare disease. The choice of optimal treatment method has not been determined yet and should be directly dependent on the size, the anatomical and topographic features, as well as on the manual skills of urologists and the available equipment. When choosing a treatment method, one should consider the concomitant comorbidities of the patient, which may affect the choice of anesthesia and the patient's position on the operating table.

In our opinion, transurethral endoscopic marsupialization or resection of symptomatic prostate cysts of small size is the preferred method of surgical treatment, if the cyst is not deeply located inside the prostate. Large cysts, as well as cysts located deeply from the prostatic urethra, it is recommended to manage with laparoscopic (extraperitoneal) access, which can prevent the development of such potential complications as rectal perforation, erectile dysfunction and urinary incontinence. At the same time, one should not completely refuse to perform open surgical or puncture, since in a number of clinical situations, they may be an alternative to endoscopic procedures or be the method of choice. In conclusion, we would like to emphasize that the choice of treatment method for prostate cysts should be discussed with the patient, adhering to an individualized approach.

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