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## DIAGNOSTICAL ASPECTS OF TUBOPERITONEAL INFERTILITY ASSOCIATED WITH GENITAL TUBERCULOSIS

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### Abstract

The article discusses the genital tuberculosis (GT) diacrisis, which causes infertility in 10-27,8% of cases. In order to determine the character of tuboperitoneal infertility associated with GT, the authors examined 44 patients with infertility, placed for endoscopic treatment. The study was conducted in two stages. The first stage implied evaluation of the clinical and endoscopic features of tuboperitoneal infertility factor (TPIF) associated with GT (n = 32): the first main group (n = 16) included women with detected (4) and clinically treated GT (12), the comparison Group 1 (n = 16) included patients with TPIF along with the lack of GT data.

The second stage implied assessment of local specific humoral immunity with a view to determine the level of specific A, M and G immunoglobulins related to TB mycobacteria (TBM) in optical density (OD) units by ELISA method in the peritoneal fluid (n = 39): Group 2A included 21 patients with TPIF, Group 2B - 11 patients with infertility, not associated with damage to the fallopian tubes, Group 2C included 7 patients with TPIF and GT in past history. Among patients with a long TPIF history ( $5,33 \pm 0,91$  years) the set GT history made 25%. Patients with TPIF and GT regardless of a particular process stage (active or old) showed prevalence of proximal tubal occlusion type, compared with TPIF regardless of GT, along with dominance of distal occlusion, pronounced adhesive process of uterine appendages, increased frequency of chlamydial infection in past history ( $p < 0.05$ ).

During TPIF associated with active GT, specific features of local humoral immunity implied the increase in IgM ( $2,950 \pm 0,22$  OD) in the intact PF, among patients with old GT – increase in IgA ( $1,646 \pm 0,16$  OD) and IgG ( $1,565 \pm 0,32$  OD) to TMB, in contrast to the comparison group ( $p < 0.05$ ). Thus, the definition of significant clinical,

endoscopic, and immunological features typical for tuboperitoneal infertility associated with genital tuberculosis is recommended at suspicion on GT and laparoscopic surgery in patients with a long tubal infertility. This will shorten the time of disease disclosure, and therefore increase the effectiveness of treating patients with GT.

**Key words:**

Infertility, Tuboperitoneal factor, Genital tuberculosis, Local specific immunity, Diacrisis.

**Introduction:**

In the current demographic conditions, the problem of infertility is extremely topical: there are approximately 10% of infertile couples in Europe, in the United States - 8-15%, in Canada - about 17% in Australia - 15.4%, in Russia - 18-25,8%; this tendency does not tend to decrease (15% is a critical level according to WHO) [1, 2, 3, 4, 5]. The most significant reason of female infertility is the ascending genital infection, which leads to the TPIF development, which is a "leader" in the female infertility structure - 30-85% [4, 6, 7, 8]. Tuberculosis still remains the global public health problem, its incidence rate among women has increased by 81,8% for the past decade [9, 10]. The urogenital tuberculosis takes the lead among all forms of extrapulmonary tuberculosis [10, 11, 12]. Genital tuberculosis (GT) makes 25% in the urogenital TB structure, within the extrapulmonary forms its share ranges from 1% in the US to 18% in India (in Russia - 12,5-16%), and it is increasingly often found in Europe [12-17]. Currently, GT causes female infertility in 10-27,8% of cases and it is considered as an extremely topical medical and social problem due to its development in women of reproductive age [16, 18-23]. Infertility is a typical GT symptom, which occurs in 10-85% of patients [15, 16, 22-25].

Questions related to GT diacrisis represent a very complex problem. Clinical signs of genital tuberculosis are nonspecific, bacteriological diacrisis methods are laborious and less informative because of little information related to bacterioexcretion; histological methods have low sensitivity, (including Mantoux test, and DST), and the information on the results of endoscopic examination of the pelvic organs in the case of infertility and GT is limited [12, 19, 21, 27, 28]. The application of molecular genetic methods (PCR) improved diacrisis of extrapulmonary tuberculosis [9, 21, 27, 28], however, the information regarding informativity of this method in the GT diacrisis is controversial, many authors noted lack of sensitivity and a significant number of false negative results [16, 27, 28, 30, 31]. Specific process is often detected in chronic phase flow, resulting in TPIF in 74,7-97% of clinical observations, which indicates poor prognosis for recovery of fertility [12, Kulshrestha V, Kriplani A, 11, Sharma J.B., 2015, 16, 23,

24, 32]. Examination of the humoral immunity, including tissue immunity, in patients with TPIF is a promising method for diagnosis of the ascending genital infection.

Research works in this field provided data for the study of anti-Chlamydia antibodies to *Chlamydia trachomatis* in the peritoneal fluid, follicular fluid in patients with TPIF, for diagnosing the ascending chlamydial infection, relevant in case of female infertility [7, 33, 34]. Earlier studies of the local specific humoral TB immunity in patients with TPIF showed quite high informativity of the method [35-37]. In this regard, the questions related to endoscopic evaluation of features and performance of the local humoral immunity for timely TPIF diagnosis associated with genital tuberculosis are highly relevant, for this will undoubtedly have a positive impact on the fertility recovery in these patients.

**Research objective:** Determination of significant endoscopic and immunological signs, typical for tuboperitoneal infertility associated with genital tuberculosis.

**Materials and methods:** This research implied a prospective study on the basis of gynecological department of NGEI "Clinical Hospital at the Omsk-Passenger station", JSC "Russian Railway" and PHCIOO "Clinical TB Dispensary No. 4" in Omsk, which included 44 patients aged between 23 and 39, who were under laparoscopic treatment (LT) related to infertility in 2011-2013. The ethics committee approved the study and informed consent was obtained from all patients.

The first stage implied evaluation of the clinical and endoscopic TPIF features associated with genital tuberculosis, and a comprehensive phthisiopelvic exam (n = 32). In this regard, two groups of patients were selected. Criteria for inclusion into the first main group were the following: reproductive age, the observation related to infertility for more than 2 years, laparoscopic TPIF confirmation, detected or old genital tuberculosis (after basic therapy completion). Criteria for inclusion into the comparison Group 1 were the following: reproductive age, the observation related to infertility for more than 2 years, laparoscopic TPIF confirmation and the lack of GT data. Sampling of peritoneal fluid was performed in patients of both groups.

In accordance with the set criteria, the first main group (n = 16) included women with detected (4) and clinically treated (12) GT; the first comparison group (n = 16) included patients with TPIF and the lack of data related to genital tuberculosis. The second stage implied evaluation of tissue specific humoral immunity according to the level of specific immunoglobulins of A, M and G classes relating to tuberculosis mycobacteria (TMB) in optical density units

(OD) by enzyme-linked immunoassay (ELISA) in the peritoneal fluid of 39 patients (88.6%, who were under LT). In

this regard, three study groups were selected.

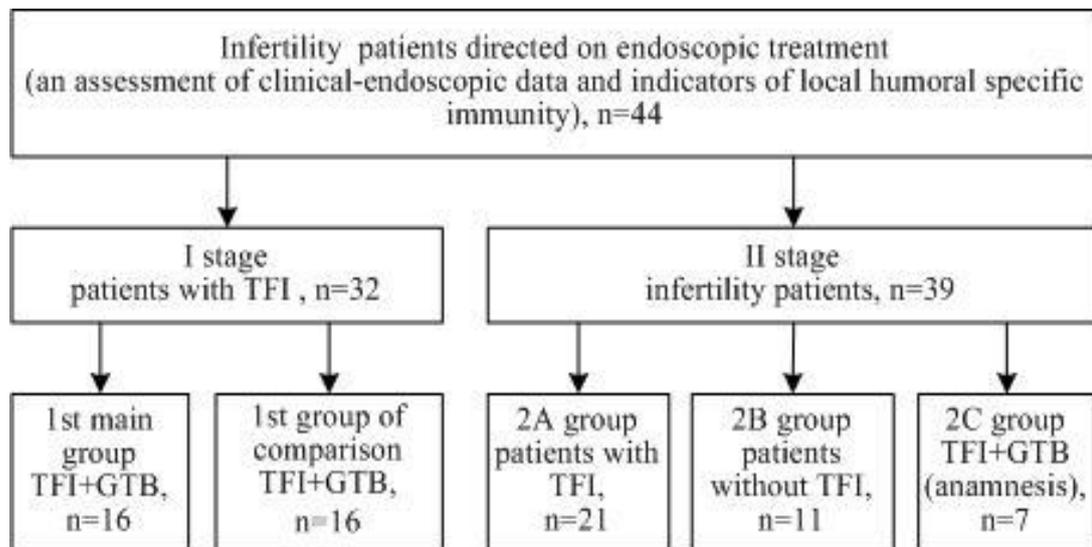
Criteria for inclusion into the Group 2A were the following: reproductive age, the duration of observation related to infertility for over 2 years, laparoscopic TPIF confirmation, peritoneal fluid (PF) sampling.

Criteria for inclusion into Group 2B were the following: infertility, not associated with the fallopian tubes involvement, according to laparoscopic data, PF sampling. Criteria for inclusion into Group 2C were the following:

previously detected genital tuberculosis on completion of the basic course of therapy, TPIF detection, according to the LT data, PF sampling. In accordance with these criteria, Group 2A included 21 patients with TPIF, Group 2B - 11

patients with infertility, not associated with the fallopian tubes involvement (endometriosis, tumor-like pelvic mass, polycystic ovarian syndrome, PCOS), Group 2B included 7 patients with TPIF and genital tuberculosis in past history

(Figure 1)



**Figure 1. Examination chart.**

(TFI = TPIF, GTB = GT)

The diacrisis "genital tuberculosis" was established by a commission (the order of the Health Ministry of the Russian Federation No. 109 dated 21.03.03 "On improvement of Antituberculous Measures in the Russian Federation") based on a comprehensive examination. Comprehensive examination of patients with infertility, along with clinical, included traditional methods of staged infertility diacrisis: ultrasound examination of the pelvic organs, metrosalpingography, laparoscopy with PF sampling, hysteroscopy, timed endometrial biopsy of fallopian tubes, histological examination. Diacrisis of genital tuberculosis required the application of the following traditional methods: bacterioscopic, bacteriological, laser fluorescence of plasma (LFP), tuberculin diacrisis, DST, trial

treatment. The authors used adjuvant methods: polymerase chain reaction (PCR) of menstrual blood, biopsies of internal genitalia, immunological examination with the assessment of the modified reaction of blast lymphocyte transformation with tuberculin stimulation (mRBLT with STD-L) by the stimulation index (SI), the definition of interferon -gamma (IFN- $\gamma$  with STD-L, pg / ml) in control samples of blood and after tuberculin induction in suboptimal dilution, as well as ELI with a view to determine levels of specific immunoglobulins to TMB in blood serum. The specific humoral tissue immunity was assessed by determining the level of specific immunoglobulins of IgA, Ig M and Ig G to M. tuberculosis in OD units by using ELISA in the peritoneal fluid obtained during laparoscopy (test system «Omega», UK). PF sampling was conducted before performing surgical operations on the pelvic organs: through the tip of the suction introduced through a side trocar, the supernatant fluid was subject to ELISA (test system «Omega», UK) [37]. Given the lack of recommendations of the test system manufacturer regarding PF examination, the authors studied intact and diluted fluid of 39 patients.

Statistical analysis was performed using STATISTICA-6 packages, biostatistics and MSEXCEL software. In all statistical analysis procedures, the critical value of p made 0.05 (ranking with regard to three levels of statistically obtained significant differences:  $p < 0,05$ ;  $p < 0,01$ ;  $p < 0,001$ ). The average sample values of quantitative characters are given in the text in the form of  $M \pm SE$ , where M - single sample mean, SE - standard error of the mean value. In distribution of values different from normal ones, the median was indicated ( $P_{0,5}$ ), 25 - percentile ( $P_{0,25}$ ) and the 75 - percentile ( $P_{0,75}$ ). In the analysis of contingency tables, the  $\chi^2$  values were estimated. Variance analysis with clustering was used to determine significant clinical and endoscopic signs of TPIF associated with genital tuberculosis.

## Results and Discussion

In studying the clinical and endoscopic features of the verified tuboperitoneal infertility at the first stage, among patients of the first main group ( $n = 16$ ), in 4/16 patients the GT diacrisis was first established, 12/16 women completed the basic TB therapy course (clinically treated GT). The average age of patients in the studied groups had no statistical difference ( $31,67 \pm 0,83$  and  $30,12 \pm 1,15$  years, respectively,  $p = 0.294$ ). The rate of primary and secondary infertility had no essential distinctions in patients of the studied groups ( $p > 0,05$ ). Primary infertility dominated in the first main group - 11 women (68,7%), against 7 (43,7%) patients in the first group of comparison ( $p = 0,192$ ). Long infertility (5 years and more) was equally often revealed in both groups ( $p > 0,05$ ). Thus, the average

duration of infertility in the first group made 6,4±1,05 years with the range of 2 - 16 years, in the first group of comparison - 4,8±0,7 years with the range of 1 - 12 years (p=0,215).

The authors paid attention to the high frequency of surgical interventions on the pelvic organs in the history of patients in both groups (8/16 and 6/16 in the first main group and in the first control group, p = 0.722). The number of operational cases in the first main group of patients made 11, exceeding 1.8 times the figure in the second comparison group (6 cases) (p = 0.156). The study therefore detected a threefold prevalence of operations on the fallopian tubes in the main group (reconstructive plastic surgery, tubectomy in regard to ectopic pregnancy): the total number of surgical interventions on the fallopian tubes made 9/16 cases, given the repeated operations on the fallopian tubes in every second patient, in the first comparison group - 2/16 ( $\chi^2 = 4,664$ , p = 0.026). Some authors [10, 23, 31]note specific feature of medical history of patients with genital tuberculosis - a long, sometimes lasting for years, inpatient and outpatient ineffective treatment of pelvic inflammatory disease (PID), including operational cases.

Laparoscopic examination detected a one- or two-way occlusion of the fallopian tubes in 14/16 patients of the comparison group, in the main group - in 10/16 women (p = 0,222) (Table 1). Bilateral complete occlusion was detected in 5/10 patients of the first main group and in 8/14 patients of the first group of comparison (p>0,05). Partial occlusion of the fallopian tubes was equally frequently detected in both groups (2 cases in each group). Uterine tubes patency was observed in 4/16 (25%) patients of the first main group upon unexpressed adhesive process. Different scholars believe that the uterine tubes retain patency under GT in 11.7 - 47% of cases [15, 21, 26, 29, 38].

**Table 1. Laparoscopic signs of TBIF, associated but not linked to genital tuberculosis, abs. (%).**

Laparoscopic findings	The first main group, n=16		The first group of comparison, n=16		Total n=32	
	Abs.	%	Abs.	%	Abs.	%
State of uterine tubes						
Full occlusion of the uterine tubes:						
- total	10	62,5	14	87,5	24	75,0
- proximal	8	50*	-	-	8	25,0
- distal	2	12,5	14	87,5*	16	50
Partial occlusion	2	12,5	2	12,5	4	12,5
Patent tubes	4	25,0	-	-	4	12,5

Specific signs (peritoneal eruptions, adhesions etc.)	9	56,2*	-	-	9	28,1
peritubal and/or periovarian adhesions						
Absence	2	12,5	-	-	2	6,5
1-2 degree	11	68,7	5	31,3	16	51,6
3-4 degree	3	18,7	11	68,7*	14	43,7
* Level $p < 0,05$ when comparing the first main group and the first group of comparison, <sup>2</sup>						

Proximal occlusion of uterine tubes was dominant in the first main group – 8/16 cases, whereas in the first group of comparison, proximal occlusion was not detected ( $\chi^2 = 8,167$ ,  $p = 0.004$ ). The frequency of distal tubal occlusion in patients of the first main group made 2/16, in the first group of comparison - 14/16 cases ( $p < 0,001$ ). In the first main group of patients, distal occlusion of the third-fourth stage (according to J. Doonez) was detected in 2/10 cases, in contrast to the first group of comparison, where prevalence of this type of occlusion was detected in 10/14 cases ( $\chi^2 = 4,286$ ,  $p = 0.038$ ).

Chlamydial infection in past history was detected among patients with occlusion in the first comparison group in 6/14 (42.8%) of cases (37.5% of patients in the comparison group), in contrast to the patients of the main group, wherein this infection was not detected ( $\chi^2 = 5,128$ ,  $p = 0.024$ ). Findings of this study are consistent with other research findings related to low risk of chlamydial infection in patients with pelvic inflammatory disease (PID), leading to the distal occlusion of the uterine tubes (20-44%), complicated by infertility of every fourth patient [34, 39, 40 M. A. Huaman, C. T. Fiske, 2015].

The frequency of adhesive process (according to J.F. Hulka et al.) had no significant differences (14/16 and 16/16 cases in the main group and in the comparison group), but its severity was statistically very different: stage 1-2 dominated in the first main group (11/16 cases), stage 3-4 was predominant in the first control group (11/16 cases) ( $\chi^2 = 6,222$ ,  $p = 0.013$ ) (Table 1).

Most (9/16) patients ( 56.2%) of the first main group during the pelvic exam were marked by the "rosary" type tortuosity of the uterine tubes, multiple grutum on pelvic peritoneum, serous cover of the uterus and tubes; patients of the comparison group had none of the mentioned macroscopic signs ( $\chi^2 = 9,894$ ,  $p = 0.002$ ). According to research findings, various degrees of peritubal and/or periovarian adhesions were detected in 32,6 - 80% of women with GT, in 33,3-68,8% - tubercles, hyperemia of the peritoneum [Sharma J.B., Sneha J., Singh U.B., 2015; 14, 18, 21, 26, 29, 38]. Other manifestation of adhesions – the Fitz-Hough-Kurtissyndrome, which had been initially described as a

chlamydial and gonorrhoeal infection, detected in 37,5-56% of patients with GT [Sharma J.B., Sneha J, Singh, 2015, Sharma 2007], was originally detected in one patient. According to the data provided by [14, 16, 21, 29, 30] described macroscopic signs that are pathognomonic for GT and are found in 12,6-60% of cases; therefore, they should be assessed during laparoscopy.

At the same time, the macroscopic picture changes depending on the phase of a specific process, laparoscopic picture may have no visible changes [21, 26, 27, 29, 38, Sharma J.B., Sneha J., Singh U.B., 2015, Sharma, J.B. 2015]. The second stage of this study implied evaluation of the local humoral immunity indicators through determining the level of immunoglobulins of A, M and G classes to Mycobacterium tuberculosis using ELISA method in the peritoneal fluid of 39 patients (mean age -  $30,25 \pm 0,96$  years) obtained during laparoscopy in 2011-2013. The examined groups were equivalent with regard to age parameters:  $30,38 \pm 0,92$  years – Group 2A,  $30,0 \pm 1,57$  years – Group 2B,  $30,42 \pm 0,75$  years - Group 2C ( $p > 0,05$ ). The mean values of Ig A, M and G levels to TMB both in the intact and diluted PF are shown in Table 2.

**Table 2. The level of specific immunoglobulins to tuberculosis mycobacteria in the peritoneal liquid of patients with infertility, OD (M±SE), units.**

Indicator	Group 2A, n=21	Group 2B, n=11	Group 2C, n=7	S 1-2	S 1-3	S 2-3
Intact PF						
Ig A	0,783±0,09	0,498±0,17	1,646±0,16	0,158	0,021	0,041
Ig M	2,550±0,16	1,253±0,53	3,500±0,46	0,020	0,087	0,164
Ig G	0,773±0,09	0,516±0,23	1,565±0,32	0,264	0,030	0,025
Diluted PF						
Ig A	0,114±0,02	0,079±0,01	0,194±0,1	0,233	0,262	0,245
Ig M	0,377±0,06	0,367±0,12	0,464±0,27	0,939	0,658	0,744
Ig G	0,188±0,02	0,131±0,02	0,280±0,07	0,137	0,128	0,067

Statistically significant differences in the Ig level in the comparison groups were obtained during the study of intact PF. The permanently high level of specific Ig A in the intact PF in patients from Group 2B with GT ( $1,646 \pm 0,16$  OD), along with high levels of Ig G ( $1,565 \pm 0,32$  OD) detected in the patients of this group, confirmed genital localization of the specific process and the slow pace of reduction in antibody levels after specific therapy (Table 2). Laparoscopic treatment of patients from this group was performed 2-7 months after completion of the basic TB

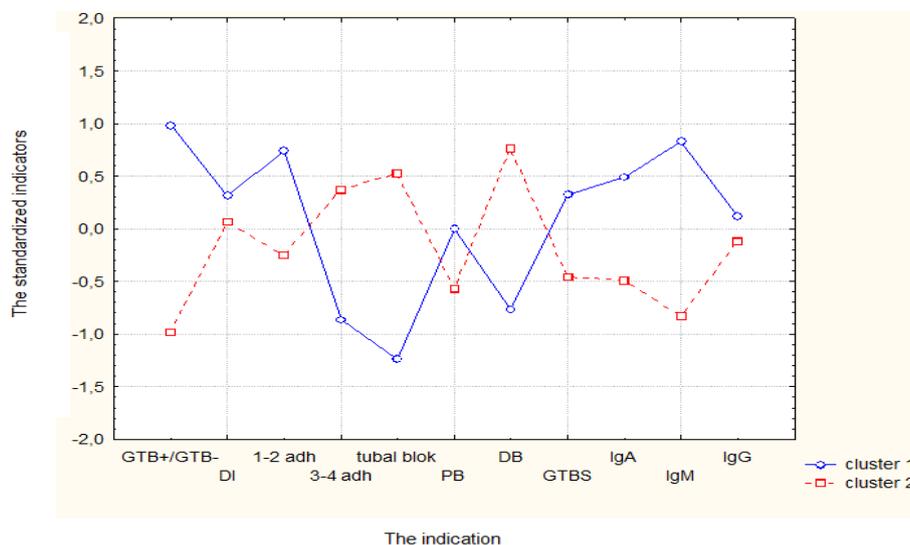
treatment course. The level of specific Ig M in the intact peritoneal fluid of patients with TPIF (Group 2A) made  $2,550 \pm 0,16$  OD, which exceeded the level of the corresponding indicator in the group of patients with infertility, not associated with uterine tube involvement (Group 2B) -  $1,253 \pm 0 53$  OD ( $P = 0.020$ ). Indicators reflecting the levels of Ig A and IgG, had no statistically significant differences in the studied groups. Taking into account the findings related to Ig M informativity to TMB in the intact peritoneal fluid of patients with TPIF (2A), the authors determined the Ig M level to M. tuberculosis in the intact PF among the patients of the first stage of the study (TPIF combined and without GT: the first main and the first comparison group) (Table 3).

**Table 3. Ig M level to M. tuberculosis in the intact PF among the patients of different groups, OD (M±SE).**

Groupsofcomparison	M±SE	P25-P75
The first main group TPIF+GT (n=16)	2,950±0,12	3,172-2,728
The first group of comparison TPIF+GT (n=16)	1,684±0,29	2,507-0,861

The IgM level is 1.75 times higher than the index value in the first control group ( $p = 0.031$ ). Keeping in mind the obtained findings, the authors established the significant IgM level for specific humoral immune response in the peritoneal fluid during GT diacrisis in women with TPIF. Given the IgM level to M. tuberculosis is less than 2.5 OD, active tuberculosis process was absent; 2.7 OD and higher indicated the active and specific TB process.

In order to identify significant endoscopic and immunological characteristics for early detection of tuboperitoneal infertility associated with genital tuberculosis, cluster analysis was carried out among patients with TPIF ( $n = 32$ ) (Figure 2).



**Figure 2 – Clinical-endoscopic and immunological signs in patients with tubal-peritoneal infertility.**

Notes: GTB + - genital tuberculosis; GTB-absence of genital tuberculosis; DI – infertility duration; 1-2 adh –1-2 degrees of adhesions; 3-4 adh - 3-4 degrees of adhesions; tubal block – the presence of uterine tube occlusion according to hydrotubation data; PB - proximal uterine tube occlusion; DB - distal uterine tube occlusion; GTBS – specific laparoscopic signs; Ig A – immunoglobulins of A class to TMB in the peritoneal fluid; Ig M – immunoglobulins of M class to TMB in the peritoneal fluid; Ig G – immunoglobulins of G class to TMB in the peritoneal fluid

According to the results of variance analysis by clustering method (STATISTICA-6), two clusters were determined. For the first cluster, the following signs were determined: the "genital tuberculosis" diacrisis, infertility duration exceeding 5 years, the prevalence of adhesive process of 1-2 stage, according to the laparoscopic data, along with rare signs of tube occlusion, with a predominance of proximal occlusion; the presence of "peritoneal signs" was macroscopically more often noted (eruptions on the peritoneum of the pelvic organs and the band adhesions of the uterine tubes), higher levels of immunoglobulins of A, M and G classes to TMB in the intact peritoneal fluid. For the 2nd cluster, the following signs were determined: the unconfirmed diacrisis of "genital tuberculosis", infertility duration up to 5 years, the prevalence of uterine appendages adhesions of 3-4 stage according to the laparoscopy, along with apparent signs of uterine tubes occlusion, with predominant distal occlusion; the presence of "peritoneal signs" was macroscopically rarely detected (multiple grutum on pelvic peritoneum and band adhesion of uterine tubes), lower levels of A, M and G immunoglobulins to TMB in the intact peritoneal fluid.

Thus, clustering gave the possibility to identify the main features typical for TPIF and GT, with the utmost diacritic importance. Tuboperitoneal infertility during GT, as compared with patients with TPIF, not associated with genital tuberculosis, is characterized (in decreasing order) by: the increased level of specific Ig M in the peritoneal fluid ( $F = 37,9$ ,  $p < 0,001$ ), non-typical distal occlusion of the uterine tubes, unlike the proximal ( $F = 21,0$ ,  $p < 0,001$ ) and partial occlusion of the uterine tubes, maintaining their patency ( $F = 15,9$ ,  $p < 0,01$ ) non-typical expressed adhesions of 3-4 stage (according to Hulka), which is more common during TPIF not linked to tuberculosis ( $F = 11,6$ ,  $p < 0,01$ ), as compared with the adhesion process of 1-2 stages ( $F = 5,09$ ,  $p < 0,05$ ); the increase in the level of specific Ig A in peritoneal fluid ( $F = 4,7$ ,  $p < 0,05$ ) matters for patients with clinically cured GT.

The use of laparoscopic method in the GT diacrisis is being discussed [14, 18, 21, 30, 38 Sharma JB, Sneha J, Singh, 2015]. According to some researchers, laparoscopy is an invasive, expensive, but non-specific method of GT diacrisis, although it is possible to identify specific characteristics of this disease and to obtain research samples [21,

26, 38]. Along with laparoscopy, hysteroscopy and other endoscopic procedures associated with operational risk may cause activation of infection in patients with GT [18, 30]. However, most authors believe that laparoscopy with mandatory histological, microbiological, PCR study of the obtained material is suitable in the final stage of GT diacrisis [15, 16, 26, 27, 29, Sharma JB, Sneha J, Singh, 2015, Sharma JB 2015]. Both Russian and foreign researchers consider that the data providing the possibility to suspect a disease are obtained through laparoscopy only in 26,3-59,7% of GT cases, thus only in 9-16% of cases specific nature of that process was verified [Geetika Jain, 2015, Sharma JB, Sneha J, Singh, 2015, Khanna A, Agrawal, 2011, Kulshrestha V, Kriplani A,2011, Vasilyev 2000, R.B. Thangappah, 2011]. The original results reflect the presented data – in 56,2% of cases TPIF signs were detected through laparoscopy.

The infertility problem caused by genital tuberculosis is actual and GT clinical signs along with assessment of informational content related to its standard diacrisis are being studied. However, the information regarding the application of new diagnostic technologies, including immunological inspection of women with infertility associated with GT is limited [E.N. Kolachevskaya, 1996; A.N. Oleynik, 2007, Sharma J.B., 2015; VenkannaBhanothu, Vemu Lakshmi, 2015; S.K. Mondal; T.K. Dutta, 2009, S.K. Mondal, 2013]. Some research papers dealing with the Chlamydia infection problem [Novikov, Klinyshkova, 2006; Gasparov, Klinyshkova 2005, Coppus S. F. P. J, 2011] also include the search of modern methods that could allow diagnosing salpingitis in case of infertility as well as studies related to the specific humoral immune response at the local level. ELISA studies became a priority in differential diagnosing of TB, especially its extrapulmonary forms [Starshinova A.A., Korneeva A.A., 2011, Andersen P., Doherty T., 2007; Lalvani A., 2007; Steingardt K., 2009]. In this respect, a number of researchers showed the role of cellular and humoral immunity systems in women, including women with infertility – in the presence of extrapulmonary TB, the immune response is developed by the mixed type, along with the increase in specific proliferation of T-lymphocytes and high level of antitubercular antibodies (ATAB) - the Ig M level and the ATAB level is increased in most patients with active GT, the Ig G level is increased in patients who were subject to clinical treatment of GT. [John C, Simren M, 2014; Venkanna B, Vemu L, 2015; Baykeev R.F., Nefedova L.N., 2012; Shalygin K. V., 2005; Omrani M., Ansari M.H., 2009].

The extrapulmonary tuberculosis is currently studied in terms of assessing the immunity factors at the local level – in biological liquids and infected tissues. Most studies show the existence of ATAB in the peritoneal fluid of women with GT [Guchetel E.V., Mokritskaya D.E., 2006; Gatongi D.K., Gitan G, 2005; Sharma J. B., Roy K., 2008].

Modern studies related to antitubercular antibodies in biological liquids of infected tissues include the ideas regarding the role of the pathological center causing apparent local immune response [Guchetel E.V., Mokritskaya D.E., 2006, Phthisiatrics 2010]. There is an opportunity to diagnose female GT through the tuberkulin test - segmentary Mantoux test by using anti-gene in volume of 0,1 and 0,01, which gives the possibility to determine deviation of the quantity of antitubercular antibodies from the norm in the obtained peritoneal fluid as well as the probability of hydrosalpinx [Guchetel E.V., Mokritskaya D.E., 2006]. It is possible to consider deficiency of this method, keeping in mind the fact that the study was carried out in TB dispensary as a diagnostics test (most patients with infertility were examined in general health care centers for a long time and did not go to the TB dispensary). In this case, general health care centers and gynecology departments of hospitals do not have the possibility to apply the said method. Another setback of the described method implies the fact that while receiving PF from the abdominal cavity, the diagnostic value of important macroscopic signs can be estimated only visually during laparoscopy. Besides, the assessment of total ATAB doesn't give the possibility to assess the levels of specific A, M and G immunoglobulins to MBT, which is very important in terms of defining phases of current and activity of specific processes [Phthisiatrics, 2010; Steingardt K., Dendukuri N., 2009 Omrani M., Ansari M.H., 2009].

The authors for the first time determined the levels of specific A, M and G immunoglobulins to MBT in the integral peritoneal fluid by using the enzyme-linked immunoassay with the view of studying the indicators of local specific humoral immunity. The integral peritoneal fluid was received at a laparoscopy in the first phase of a menstrual cycle. The level of specific Ig M ( $2,550 \pm 0,16$  OD) to MBT in patients with TPIF exceeded twofold the same level in the group of patients with infertility which was not associated with TPIF ( $1,253 \pm 0,53$  OD) ( $r=0,020$ ). This can serve as a marker of active tubercular process in the group of patients with TPIF.

Given the obtained results, the authors consider it effective and reasonable that medical diagnostic laparoscopy be conducted in patients with infertility at the examination stage in general health services, combined with determination of clinical, endoscopic and immunological characteristics of the specific tissue immunity typical for tuboperitoneal infertility associated with genital tuberculosis. This tactic would reduce the time of GT diacrisis and improve treatment outcomes in this group of patients.

## **Conclusions**

1. The patients with tuboperitoneal infertility affected by GT regardless of the stage related to a particular process (active, old) were characterized by long-lasting infertility (over 5 years), the prevalence of proximal tubal

occlusion, compared with infertile patients without genital tuberculosis, with predominance of distal occlusion, the expressed adhesion process of the uterine appendages and the increased rate of Chlamydial infection in past history.

2. Determination of specific TB tissue immunity in the intact peritoneal fluid is regarded as an informative diacritic method.
3. Taking into account the increased level of Ig M to M. tuberculosis in patients with TPIF as compared to this indicator in the group of patients with infertility, not associated with uterine involvement, women with laparoscopically verified TPIF can be considered as a risk group related to GT.
4. In patients with tubal infertility and active genital tuberculosis, the Ig M level to M. tuberculosis in the intact peritoneal fluid exceeded the relevant value in the group of patients with tuboperitoneal infertility, not associated with genital tuberculosis, which justifies the use of cluster analysis with a view to clarify the etiology of infertility.
5. Cluster analysis gave the possibility to identify the following main features of tuboperitoneal infertility associated with genital tuberculosis: the increase in specific Ig M level in the intact peritoneal fluid, the proximal tubal occlusion, partial occlusion of the uterine tubes and their patency preservation, the incomplete adhesions of 1-2 stage; in addition, the increase in the level of specific Ig A in the peritoneal fluid matters for patients with clinically cured GT.

### **Abbreviations**

**PID** – pelvic inflammatory disease

**GT** – genital tuberculosis

**ELISA** – enzyme-linked immunoassay

**MBT** – Mycobacterium tuberculosis

**OD** – optical density

**PF** – peritoneal fluid

**PCR** – polymerase chain reaction

**TPIF** – tuboperitoneal infertility factor

**Ig** – immunoglobulin

### **Competing interests**

The authors declare that they have no competing interests.

### Authors' contributions

The study was planned by T.V. Klinyshkova. The endoscopic treatment of patients was carried out by T.V. Klinyshkova, N.B. Frolova and Y.V. Kuzmenko. A.A. Yakovleva observed the patients with infertility associated with GT and was in charge of the text. T.V. Klinyshkova, A.A. Yakovleva and M.A. Plekhanova analysed the obtained results. All the authors have read and approved the final version of the manuscript.

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