UDC 616-022:616.983-08

SUSTAINABLE THERAPY OF UROGENITAL CHLAMYDIOsis

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There is presented the comparative characteristic of 2 methods of treatment of urogenital chlamydiosis in women of reproductive age: doxycycline monohydrate monotherapy and combination of doxycycline monohydrate with interferon inducer Meglumini acridonacetas. In 2 groups of comparison there was performed the analysis of indicators of neutrophils functional activity (phagocytic index, phagocytic number, NBT-test – spontaneous and induced) and also the rate of T-lymphocytes producing gamma interferon in the total pool of T-lymphocytes in peripheral blood and concentration of gamma interferon in the blood serum during treatment.

Key words: urogenital chlamydiosis, neutrophils functional activity, T-lymphocytes, gamma interferon, doxycycline, Meglumini acridonacetas.

Urogenital chlamydial infection is one of the most spread sexually transmitted infections [20, 21]. According the World Health Organization (WHO), in the world there are annually registered about 100 mln new cases [23, 24].

Chlamydial infection (CI) is widely spread among persons of reproductive age, while young age in women is one of the basic factors of infection risk. Due to cylindrical epithelium tropism, C. trachomatis effects urogenital organs and presents one of the important reasons of reproductive function disorder.

Inflammation by urogenital chlamydiosis (UGC) often lacks symptoms. The absence of timely diagnosis and, consequently, therapy leads to the development of complications in women – such as inflammatory diseases of pelvic organs (POID), extra-uterine pregnancy and tubal infertility. A special problem are the cases of CI persistence, when POID proceed without symptoms of inflammation [9, 23].

Basic factors of non-specific antimicrobial protection of organism are phagocytosis and complement system. Chlamydia can persist in phagocytes (neutrophils and macrophages). In patients with chronic CI there was registered the reduction of digestive function of peripheral blood neutrophils [2].

The influence of chlamydia suppresses not only phagocytic system, but also T-cell segment. Patients with urogenital chlamydiosis showed the decrease of total T-lymphocytes (CD3+) in peripheral blood with the disorder of their functional activity [7].

The activator of macrophages is γ-interferon (γ-IF), which is mainly produced by T-lymphocytes. Low concentration of γ-IF is the main condition for persistence development [4]. The factors of persistent chlamydial infection are also sub-therapeutic doses of antibiotics and treatment with drugs of low chlamydia activity [17].

It is known, that high doses of γ-IF inhibit growth of chlamydia. Low doses, on the contrary, induce the development of morphologically aberrant forms of inclusions [8].

Sufficient production of γ-IF leads to the suppression of C. trachomatis growth due to the induction of intracellular enzyme - 2,3-dioxygenase indolamine destroying tryptophan, which is necessary for chlamydia reproduction. As a result, this mechanism leads to the disorder of cell wall synthesis. Moreover, γ-IF boosts the activity of nitrogen oxide forming synthase in macrophages and epithelial cells, which results in releasing of nitrogen and bactericidal effect [12, 14].

For many years, clinicians have been widely using various combinations of etiotropic drugs with immunotropic agents in order to increase the efficiency of CI therapy [2, 3, 18, 19].

It is known, that γ-IF stimulates the functional activity of macrophages due to the intensification of digestive ability of phagocyting cells, complete process of phagocytosis and lytic decomposition of chlamydia. Consequently, the level of γ-IF is one of the key components determining the type of infectious process [4].

Interferon inducers are drugs causing the formation of endogenous interferon. They lead to the synthesis of the genuine interferon, which, in contradiction to most widely used recombinant IF, lacks antigenicity. Medications of this group are able to stimulate immune reactivity of organism increasing phagocytosis and antibody production [10].

According to immunologists, intervention into the immune system is possible only in case of correspondent indications, specifically, in case of immune deficiency. Thus, the prescription of such drugs is rational only after additional immunological tests. There is still no consensus on the rationality of combination of etiotropic drugs with immunotropic agents by urogenital chlamydiosis [1].

Research objective: to perform comparative analysis of the results of urogenital chlamydiosis treatment and dynamics of particular immuno-
logical indexes in two groups of patients: exposed to doxycycline monohydrate monotherapy (DM) and combination therapy with doxycycline monohydrate with interferon inducer Meglumini acridonacetas (MA).

Materials and methods
The study included 60 women with urogenital chlamydiosis. The diagnosis was based on clinical data confirmed by PCR method.

Other urogenital infections (UGI) were revealed by means of microscopic, cultural and molecular-biological (PCR) methods. Patients with trichomonas and gonococcal infection and also pregnant women were not included into the study.

The disease prescription constituted: up to 2 months – in 14 (23,3%) patients, from 2 to 6 months – in 19 (31,7%) patients, from 6 months to 1 year – in 16 (26,7%), over 1 year – in 11(18,3%) patients.

The age of patients varied within the range of 18-46 years, there prevailed the group of patients at the age of 21-30 years, the average age of patients constituted 25,4±7,2 years.

Depending on the therapy type, the women were divided into 2 groups. Patients of the first group (DM) received doxycycline monohydrate monotherapy (Unidox Solutab) in the dose 100 mg 2 times a day during 7 days. Patients of the second group (DM+MA) received cycloferon in the dose of 2 ml intravenously 10 times during the course together with doxycycline monohydrate in analogous daily doses.

Doxycycline monohydrate is a broad-spectrum antibiotic. It inhibits synthesis of proteins in the microbial cell breaking the connection of aminoacyl-t-RNA with 30S subunit of ribosomal membrane. It is recommended by the treatment of urogenital chlamydial infection as the drug of choice [5, 6, 22].

Meglumini acridonacetas (Cycloferon) – a 12,5% solution for injections) is a low-molecular-interferon inducer with the broad spectrum of biological activity (antiviral, immunomodulatory, anti-inflammatory, etc.). According to the medication instructions, the drug can be used by UGC treatment [10, 16].

The clinical-laboratory control of recovery was performed in 4 weeks after the end of treatment.

The blood sampling in patients for immunological examinations was conducted three times: before treatment, after the course of antibiotics and also upon the control of recovery in 4 weeks.

The phagocytic activity of neutrophils were determined according to their ability to grasp latex particles by counting the rate of active phagocytes (phagocytic index – PI) and average number of bacteria absorbed by one phagocyte (phagocytic number – PN). The metabolic activity of leucocytes was studied by NBT-test – spontaneous and induced [11, 15].

The rate of T-lymphocytes producing gamma interferon in the total pool of T-lymphocytes (CD-3) (TI-IF) was determined by means of flow cytometry with cyt fluorometer Beckman coulter cytomics FC 500 with the automatic hematomal analyzer Sysmex XT 2000i (Roche, Switzerland). The γ-IF concentration in blood serum was determined by means of EIA with the immunoenzymometric kit for quantitative determination of human γ-IF in blood serum (Bender Med Systems GmbH, Austria).

Analogous studies were performed in the control group – 30 practically healthy women of reproductive age (average age – 25,8 years).

The research was approved by the Ethics Committee of ISMU, all patients were previously informed about the examination, diagnosis, treatment and confirmed their participation in the study by voluntary written consent.

The statistical processing of obtained results was conducted by means of STATISTICA 6.1. The statistical analysis included Mann-Whitney test. Considering the small size of groups, the average means and standard deviation are presented only for better understanding of obtained results. Differences between compared values were considered statistically significant by p<0,05 [13].

Results and discussion
Monochlamydeous infection was registered in 18 (30%) patients, in 42 (70%) patients, C.trachomatis was combined with other infectious agents: with U. urealitimic - in 25 (41,7%), with M. genitalium - in 5 (8,3%), with M. hominis - in 18 (30%), with HPV - in 15 (25%), with Candida fungi - in 7 (11,7%), with Herpes simplex – in 4 (6,7%). Clue cells were observed in 10 (16,7%) patients.

The disease duration constituted: up to 2 months – in 14 (23,3%) patients, from 2 to 6 months – in 19 (31,7%), from 6 months to 1 year – in 16 (26,7%), over 1 year – in 11 (18,3%).

The anamnesis of 1 (1,7%) patient included cystic ovaries, of 2 (3,3%) – frequent cystitis, of 1 – chronic pyelonephritis. Among the examined women, 5 (8,3%) had irregular menstrual cycle, 4 (6,7%) complained about painful menstruations, 5 – about excessive menstruation.

The anamnesis of 36 (60%) women included pregnancy, of 35 (58,3%) – delivery, of 5 (8,3%) – miscarriage, in 1 patients there was previously registered missed miscarriage.

Out of 60 patients, 35 (58,3%) presented problems of vaginal discharge, 12 (20%) – of itching, 14 (23,3%) – discomfort in the area of external sex organs. Painful urination was registered in 2 (3,3%) women, lower abdominal pains – in 11 (18,3%), dyspareunia – in 3 (5%) patients.

The examination revealed signs of cervicitis in 56 (93,3%) patients. A typical symptom was cervical discharge, primarily mucoid – in 55 (91,7%).
In 1 (13.3%) patients there was observed purulent cervical discharge caused by concomitant infection with *M. genitalium* and aerobic potentially pathogenic microbiota - *Staphylococcus spp.*, *Streptococcus spp.* in high titer (>10^5 CFU/ml).

In four patients by the lack of clinical symptoms of inflammation, there was revealed *C. trachomatis* DNA in the cervical canal.

By microscopic examination of cervical canal discharge, the leukocytic reaction constituted: in 36 (60%) patients – up to 20 cells per field of view, in 17 (28.3%) – from 20 to 40, in 7 (11.7%) – 41-50.

Symptoms of urethritis (hyperemia, urethral sponge edema and mucous discharge) were revealed in 2 women, there was revealed *C. trachomatis* DNA in their urethral material and increase of leucocytes up to 25-30 per field of view.

The analysis of functional activity of peripheral blood neutrophils in comparison with the women with UGC showed the reduction of neutrophil ability to absorb foreign substances (PI), PN before treatment was also low. In the DM group, the spontaneous NBT-test was insignificantly increased, while in the group DM+MA, on the contrary, it had the tendency to decrease, the induced NBT in both groups before treatment was lowered. This indicates low absorbing and metabolic activity of the phagocytic system, i.e. low phagocytic response to the infection (Table 1).

### Table 1

| Groups                  | PI (%)       | PN            | spontaneous NBT (%) | induced NBT (%) |
|-------------------------|--------------|---------------|---------------------|-----------------|
| Control group (n=30)    | 64.87±7.29   | 5.30±1.83     | 37.3±6.27           | 51.0±6.26       |
| DM group before treatment (n=30) | 57.73±10.77 | 4.87±2.10     | 37.87±6.43          | 47.23±6.65      |
| DM group after treatment (n=30) | 57.63±9.91 | 4.97±2.09     | 39.96±7.71          | 49.47±8.51      |
| DM+MA group before treatment (n=30) | 54.87±12.62 | 5.20±2.14     | 35.03±5.97          | 43.30±6.15      |
| DM+MA group after treatment (n=30) | 53.27±12.44 | 5.23±1.70     | 35.13±6.11          | 43.27±6.83      |

Note: *p* – level of statistical significance by comparison of indexes before treatment with the control group; *p*<sub>1</sub> – level of statistical significance by comparison of indexes before treatment and after treatment.

After the treatment in the group of patients receiving doxycycline monohydrate monotherapy, the rate of neutrophils got into phagocytosis remained practically the on the same level (Table 1), other indexes of functional activity of neutrophils insignificantly increased: PN – by 1.02 times, spontaneous NBT – by 1.06 times, induced NBT – by 1.05 times.

In the group of patients receiving doxycycline monohydrate with Meglumini acridonacetate, the change of indexes of functional activity of neutrophils after the treatment was identical: PN, spontaneous and induced NBT-test remained practically on the previous level, while PI even slightly decreased (by 1.03 times).

In the general group of patients the rates of TI-IF in the common pool of T-I and the level of γ-IF in blood serum did not differ significantly from the analogous indexes in the group of healthy people. In this regard, patients were divided into 3 subgroups: 1 subgroup – the level of TI-IF and γ-IF is normal, 3 subgroup - the level of TI-IF and γ-IF is is decreased (Table 2).

The analysis of studied parameters in the group of patients receiving doxycycline monohydrate monotherapy showed, that in the 2<sup>nd</sup> subgroup of patients, both indexes after the treatment and upon control of recovery practically did not change. In the 1<sup>st</sup> subgroup of patients both indexes increased before the treatment and upon control of recovery practically did not change. In the 3<sup>rd</sup> subgroup of patients both indexes after the treatment and upon control of recovery remained practically on the same level (Table 2).

The analysis of studied parameters in the group of patients receiving doxycycline monohydrate with Meglumini acridonacetates showed, that in the 2<sup>nd</sup> subgroup, both indexes after the treatment and upon control of recovery practically did not change staying normal. In the 1<sup>st</sup> subgroup, there was registered slight increase of TI-IF rate and γ-IF level after the treatment and further re-
duction upon control of recovery, while in comparison with the control group, the studied indexes remained high. In the 3rd subgroup there was registered the increase of Tl-IF rate and γ-IF level after the treatment and upon control of recovery. The difference of the degree of index increase is the following: the rate of T-lymphocytes producing γ-IF grew by 1,96 times after the treatment and by 3,34 times in 4 weeks after the end of treatment. However, in spite of the positive dynamics in terms of Tl-IF, the level of γ-IF in blood serum increased by only 1,3 times after the treatment and by 1,5 times upon control of recovery (Table 3).

Table 2

| Indexes                              | Control (n=30) | General group of UGC patients (n=30) | Subgroup 1 (n=12) | Subgroup 2 (n=7) | Subgroup 3 (n=11) |
|--------------------------------------|----------------|-------------------------------------|-------------------|------------------|------------------|
| Tl-IF (%) before treatment           | 4,34±1,94      | 4,46±3,53 p=0,574                   | 8,03±2,43 p=0,01  | 3,32±1,51 p=0,278| 1,29±1,09 p=0,00003|
| Tl-IF (%) after treatment            |                |                                     | 4,51±0,88 p=0,248 | 3,33±1,32 p=0,949| 1,31±1,30 p=0,006|
| Tl-IF (%) upon control of recovery   |                |                                     | 4,21±0,88 p=0,003 | 3,47±1,12 p=0,848| 1,30±1,12 p=0,002|
| γ-IF (pg/ml) before treatment        | 2,67±0,47      | 2,81±0,89 p=0,631                   | 3,79±0,48 p=0,00001| 2,68±0,12 p=0,713| 1,98±0,39 p=0,0008|
| γ-IF (pg/ml) after treatment         |                |                                     | 2,73±0,53 p=0,007 | 2,62±0,36 p=0,749| 2,01±0,50 p=0,194|
| γ-IF (pg/ml) upon control of recovery|                |                                     | 2,85±0,56 p=0,001 | 2,61±0,32 p=0,701| 2,03±0,35 p=0,004|

Note: p - level of statistical significance by comparison of indexes before treatment with the control group; \( p_1 \) - level of statistical significance by comparison of indexes before treatment and after treatment; \( p_2 \) - level of statistical significance by comparison of indexes after treatment and upon control of recovery; \( p_3 \) - level of statistical significance by comparison of indexes before treatment and upon control of recovery.

By the clinical-laboratory control of recovery, the *C. trachomatis* elimination constituted: by doxycycline monohydrate monotherapy – 100%, by combined therapy – 96,7%. In 1 patient receiving combined therapy, upon control of recovery in 4 weeks after the end of therapy by the lack of clinical inflammatory symptoms there was revealed *C. trachomatis* DNA in the cervical canal by leukocytosis 15-20 cells per field of view. Upon the examination of patient in 2 weeks, the clinical-laboratory picture remained the same. It should be noted, that before the treatment, in patient, there was registered asymptomatic chlamydial infection of cervical canal by analogous leukocytosis. The level of γ-IF in blood serum was reduced to 1,34 pg/ml by the Tl-IF rate reduced to 0,98% in the common pool of T-lymphocytes. In 2 days after the end of treatment, these indexes slightly (and insignificantly) increased up to 1,38 and 1,0% respectively, remaining practically on the same level in the beginning of the 5th week after the treatment upon control of recovery.

After the treatment, the microscopy of urogenital discharge showed the absence of leukocytosis in the cervical canal, urethra and urine in 59 women out of 60.

The comparative analysis of the results of immunological study in the most problematic group of patients – with lowered parameters before the treatment – showed, that the addition of Meglumini acridonacetis to doxycycline monohydrate generally leads to the growth of % concentration of T-lymphocytes producing γ-IF and increase of its level in blood serum. However, the etiological and clinical efficiency of doxycycline monohydrate monotherapy in combination with the immunotropic drug in these groups of patients turned out to be nearly identical: by doxycycline monohydrate monotherapy – 100%, by combined therapy – 91,7% (in 11 out of 12 patients).
Table 3

| Table 3 | Tl-IF rate and γ-IF level in peripheral blood of UGC patients during combined treatment with doxycycline monohydrate with Meglumini acridonacetas |
|---|---|
| **Indexes** | **Control** | **General group of UGC patients** | **Subgroup 1** | **Subgroup 2** | **Subgroup 3** |
| | (n=30) | (n=30) | (n=12) | (n=7) | (n=11) |
| **Tl-IF (%)** | | | | | |
| before treatment | 4,34±1,94 | 4,80±4,45 p=0,751 | 7,84±5,02 p=0,034 p1=0,525 | 4,92±2,51 p=0,616 p2=0,753 | 1,07±0,65 p=0,00003 p3=0,009 |
| after treatment | 8,45±5,17 p1=0,525 | 4,76±1,0 p2=0,014 | 2,10±0,93 p3=0,0002 | |
| upon control of recovery | 5,63±2,01 p3=0,0453 | 5,54±0,77 p3=0,294 | 3,57±1,05 p3=0,0002 | |
| **γ-IF (pg/ml)** | | | | | |
| before treatment | 2,67±0,47 | 2,73±0,93 p=0,515 | 3,85±0,43 p=0,00002 p1=0,650 | 2,59±0,10 p=0,720 p2=0,002 | 1,88±0,40 p=0,00004 p3=0,004 |
| after treatment | 3,95±0,48 p1=0,002 | 2,76±0,17 p2=0,753 | 2,44±0,37 p3=0,013 | |
| upon control of recovery | 2,99±0,41 p3=0,001 | 2,79±0,15 p3=0,012 | 2,81±0,33 p3=0,00001 | |

Note: p – level of statistical significance by comparison of indexes before treatment with the control group; p1 – level of statistical significance by comparison of indexes before treatment and after treatment; p2 – level of statistical significance by comparison of indexes after treatment and upon control of recovery; p3 – level of statistical significance by comparison of indexes before treatment and upon control of recovery.

**Conclusion**

Consequently, the results of the current study allow to conclude, that the main chain in the treatment is the etiotropic therapy. In our research, the interferon inducer did not have the expected effective influence on the system of phagocytosis and cytokine segment and did not increase the etiological efficiency of treatment. Moreover, the implementation of this drug significantly increased the duration of therapy and unreasonably increased its price.

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