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CLINICAL CARE OF ACANTHAMOEBA KERATITIS PATIENTS

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✧ Recently, akanthamoeba keratitis (AK) is seen more and more often in ophthalmological practice. However, today there are no standard guidelines concerning diagnosis and treatment of patients with AK. In the article, the experience in care for such patients is presented. ***Purpose:*** to estimate the efficiency of diagnosis and treatment of patients with AK. ***Materials and methods.*** Case histories of patients, who received treatment for akanthamoeba keratitis in the Eye Microsurgery Department No 4, City Ophthalmologic Center of the City Hospital No 2, from 2011 to 2016, were analyzed. Under observation, there were 25 patients (26 eyes) with akanthamoeba keratitis aged from 18 to 77 years; there were 15 men and 10 women. Patients were observed during 1 year. Full ophthalmologic examination was conducted in all patients. Additional diagnostic methods included microbiological investigation of corneal scrapes and washings, culturing them on innutritious agar (with *E. coli* covering), confocal corneal microscopy (HRT 3 with cornea module, Heidelberg Retina Tomograph Rostock Cornea Module). A superficial punctate keratits (AK stage 2) was found in one patient. All other patients were divided into two groups. Stromal ring-shaped keratitis was diagnosed in patients of the first group (7 patients, AK stage 3). The 2nd group consisted of 17 patients with corneal ulcer (AK stage 4). All patients received medicamentous treatment. However patients of the 2nd group required different kinds of surgical treatment. ***Results.*** In AK diagnosis, corneal confocal microscopy is the most informative method. In patients with AK stages 2 and 3, there was an improvement in visual functions as a result of medicamentous therapy. As a result of treatment at the discharge from the hospital, the best corrected visual acuity was 0.5-1.0 for most patients. In the 2nd group patients, who were subjects to different types of surgical treatment visual functions stabilized. However non-compliance with recommendations led to disease recurrences with worse outcomes in four cases. ***Conclusion.*** It is possible to stop the inflammatory process preserving at the same time high visual functions only when patients are addressed in time, and when appropriate AK therapy is prescribed and patients are compliant with it for a long time.

✧ ***Keywords:*** Acanthamoeba keratitits; contact lenses; corneal confocal microscopy; cation-active antiseptics; corneal autoconjunctivaltenonoplasty.

ТАКТИКА ВЕДЕНИЯ ПАЦИЕНТОВ С АКАНТАМЁБНЫМ КЕРАТИТОМ

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✧ В последнее время акантамёбный кератит (АК) всё чаще встречается в практике врача-офтальмолога. Однако на сегодняшний день нет общепринятых рекомендаций, касающихся диагностики и лечения пациентов с АК. В статье представлен опыт ведения таких пациентов. ***Цель*** — оценить эффективность диагностики и лечения пациентов с акантамёбным кератитом. ***Материалы и методы.*** Были проанализированы истории болезни пациентов, проходивших лечение по поводу акантамёбного кератита в отделении микрохирургии глаза № 4 ГМПБ № 2 с 2011 по 2016 г. Под наблюдением находились 25 пациентов (26 глаз) с акантамёбным кератитом в возрасте от 18 до 77 лет — 15 мужчин и 10 женщин. Срок наблюдения составил 1 год. Всем пациентам было проведено полное офтальмологическое обследование. Дополнительная диагностика включала микробиологическое исследование соскобов и смывов с роговицы, посев соскобов и смывов с роговицы на непитательный агар (с покрытием *E. coli*), конфокальную микроскопию роговицы (HRT 3 с роговичным модулем, Heidelberg Retina Tomograph Rostock Cornea Module). Поверхностный точечный кератит был обнаружен у 1 пациента (2-я стадия АК). Остальные пациенты были разделены на две группы. У пациентов 1-й группы (7 чел.) диагностирован стромальный кольцевидный кератит (3-я стадия АК). Во 2-ю группу вошли 17 пациентов с язвой роговицы (4-я стадия АК). Все пациенты получали консервативную терапию, однако пациентам 2-й группы потребовалось проведение различных видов хирургического лечения. ***Результаты.*** Наиболее информативным методом диагностики АК является конфокальная микроскопия роговицы. На фоне консервативной терапии пациентам со 2-й и 3-й стадиями АК удалось улучшить зрительные функции. В результате лечения максимальная корригированная острота зрения (МКОЗ) большинства пациентов при выписке составила 0,5–1,0. У пациентов 2-й группы (4-я стадия АК), получивших различные виды хирургической помощи, стабилизировались зрительные функции. Однако несоблюдение рекомендаций, нарушение комплаентности привели к развитию рецидивов заболевания с более тяжёлыми исходами у четырёх пациентов. ***Выводы.*** Купировать воспалительный процесс, сохранив при этом высокие зрительные функции, возможно лишь при своевременном обращении пациентов, назначении и соблюдении терапии АК в течение длительного времени.

✧ ***Ключевые слова:*** акантамёбный кератит; контактные линзы; конфокальная микроскопия; катионные антисептики; аутоконъюнктивотенонопластика роговицы.

BACKGROUND

Acanthamoeba keratitis (AK) is a parasitic disease due to the corneal invasion by free-living amoebas. The first case of AK was recorded in South Texas (USA) in 1973 in a farmer who has washed his injured eye with tap water [2]. The pathogenesis of the disease includes parasite mediated cytolysis and phagocytosis of the corneal epithelium, with invasion and dissolution of the corneal stroma [4]. AK is rare but potentially severe and is one of the main causes of vision disability in working population. This is mainly due to the wide spread use of contact lenses (CLs). Globally, more than 140 million people wear CLs, with about 5 million of them living in Russia. Only 3% of all CL users comply with safety procedures for CL use [1]. In addition, AK diagnosis is usually delayed due to a lack of awareness about the disease, its atypical course, and a long latent period. In the early stages, due to the absence of specific etiologic signs, AK is often confused with herpetic keratitis, adenovirus keratoconjunctivitis or toxic-allergic reaction. This results in the treatment being ineffective or delayed. In addition, no officially registered medicines currently exist for the treatment of AK in the Russian Federation and in many countries around the world.

Our *aim* was to evaluate the effectiveness of diagnosis and treatment of patients with AK.

MATERIALS AND METHODS

We obtained our data from the results of examinations and treatment records of 25 patients (26 eyes) aged 18–77 (mean 34) years with established diagnoses of AK, who were hospitalized in the Department of Eye Microsurgery No 4 of the City Multi-Field Hospital No 2, between 2011 and 2016. Upon admission, all patients underwent a complex of diagnostic procedures, includ-

ing visometry, perimetry, tonometry according to Maklakov, biomicroscopy, ophthalmoscopy, OCT of cornea (OptoVue RTvue100), and B-scan whenever necessary. The diagnoses were verified with a microbiological study of scrapings and lavage from the cornea (staining with calcofluor white, a fluorescent stain to visualize amoebic cysts and fungi; Romanovsky-Giemsa; and Gram stains) followed by inoculation on non-nutritive agar (coated with *E. coli*). Results of the tests were evaluated in the clinical laboratory of the City Multi-Field Hospital No 2, at the P.N. Kashkin Scientific Research Institute of Medical Mycology. All patients underwent corneal confocal microscopy (HRT 3 with corneal module, Heidelberg Retina Tomograph Rostock Cornea Module). Superficial punctate keratitis was detected in one patient (AK stage 2). Remaining patients were divided into two groups. Group 1 (7 people) consisted of patients diagnosed with stromal ring keratitis (AK stage 3). Group 2 included 17 patients with corneal ulcers (AK stage 4). Patients with AK at stages 2 and 3 received a standard treatment regimen consisting of amoebicidal medications, antiseptics, nonsteroidal anti-inflammatory agents, cycloplegic, and IOP-lowering medications according to indications. Patients with stage 4 AK underwent various types of surgical treatment, including one enucleation.

RESULTS

All patients complained of severe pain, probably due to the introduction of trophozoites into the perineural space of the corneal stroma; pronounced corneal syndrome; and reduced visual acuity (Table 1). In a majority of patients, the disease developed after prolonged CL use and violation of the rules for lens care (Table 2). Factors contributing to the onset AK in CL users were corneal hypoxia, microdamage of the epithelium, disruption in the integrity of the tear film, and prolonged exposure to the pathogen under the CL.

Table 1

Best-corrected visual acuity in patients on admission

Таблица 1

Максимально корригированная острота зрения у пациентов при поступлении

Best-corrected visual acuity	Number of eyes
Hand motion near the face	6
0.001	1
0.01	5
0.02–0.08	6
≥0.1	8

In our practice, we often came across a fast-course form of AK. The clinical picture then includes a conjunctival redness, fan-shaped folds in the posterior limiting membrane diverging from the projection zone of infiltration, and decreased corneal sensitivity. Moreover, there may be keratic precipitates, and a hypopyon in the anterior chamber. AK is suspected by the presence of radial keratoneuritis. The latter is characterized by the presence of radial branched infiltrates located along the corneal nerves in the anterior stroma. A classical corneal ring-shaped infiltrate with raised edges was observed in seven patients (8 eyes) with different localizations: 36% in the optical zone, 24% in the paraoptical zone, 28% in the optical and paraoptical zones, and 4% in perilimbal zones. Progressive disease was accompanied by an increase in the size of necrotic zones in the stroma, with descemetocele formation, and perforation of the cornea. AK is characterized by the absence of neovascularization of the cornea, even in severe infections. The unilateral nature of the lesion was observed in 92% of cases, and bilateral lesions were seen in 8%, which agrees with published reports.

Lavage from the cornea was informative only in seven cases; in one of them, in addition to acanthamoeba, yeast fungi were found. Scrapings from the cornea were informative in four cases (Figure 1).

According to the corneal confocal microscopy results, acanthamoeba cysts in corneal epithelial layers up to the middle stroma in the form of bright rounded highly reflexive two-layer structures were found in all patients (Figures 2, 3). The method enabled AK differentiation from fungal keratitis and indicated the mixed nature of the infection (Figures 4, 5). In addition, the method revealed an important diagnostic point, namely keratoneuritis in the form of thickened nerve trunks and white lines along the nerves, due to the neurotropism of amoebas.

All patients received medical therapy according to the standard scheme. We used two biguanides

Table 2

The main risk factors for AK, based on our clinical data

Таблица 2

Основные факторы риска возникновения акантамёбного кератита на основании наших клинических данных

Risk factors	Number of patients
Violation of the maximum wear periods (over-wearing, sleeping with lenses) and care violations (washing lenses with tap water, bathing with lenses)	14
Injury of the cornea (industrial, domestic) and tap water washing	6
Rosacea keratitis exacerbation	1
Herpetic keratitis after washing with tap water	1
Wearing CLs during an episode of herpetic keratitis	1
Bathing in outdoor settings	1
Unclear	1

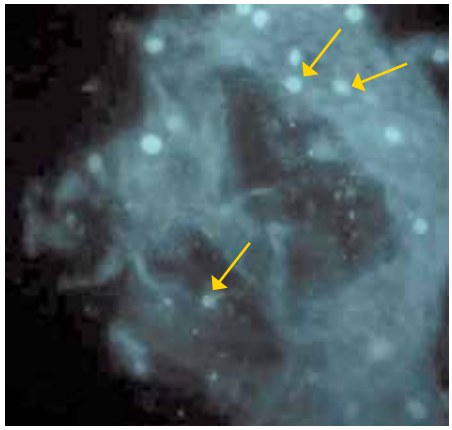

Fig. 1. Fluorescent microscopy of corneal scrapings. Calcofluor white fluorescent technique. Cysts and trophozoite form of acanthamoeba (indicated by arrows)

Рис. 1. Флюоресцентная микроскопия соскоба с роговицы. Окраска калькофлюором белым. Цисты и трофозоит акантамёб (указаны стрелками)

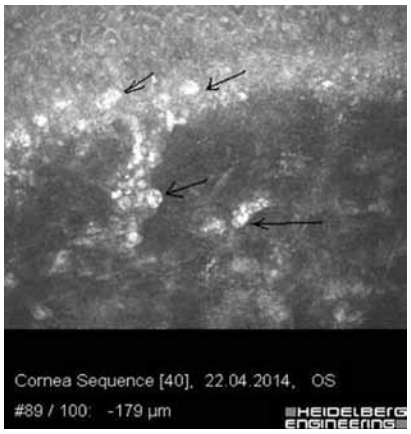

Fig. 2. Patient V. Acanthamoeba cysts in the basal epithelial layer (indicated by arrows). The mechanism of penetration of acanthamoeba cysts into the stroma through a defect of the epithelium

Рис. 2. Пациент В. Акантамёбные цисты в слое базального эпителия (указаны стрелками). Механизм проникновения акантамёбных цист в строму через дефект эпителия

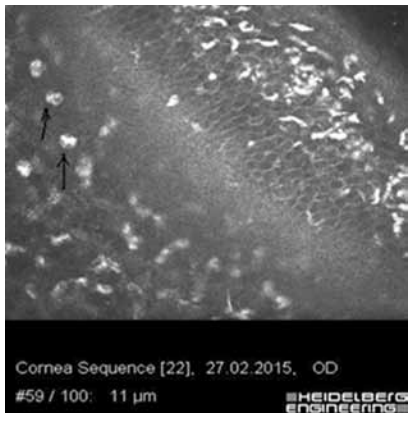

Fig. 3. Patient K. Acanthamoeba cysts (indicated by arrows) in the subepithelial layers of the stroma

Рис. 3. Пациент К. Акантамёбные цисты (указаны стрелками) в субэпителиальных слоях стромы

Fig. 4. Patient F. In the middle stroma layers in the infiltrate region are hyperreflective spindle-shaped particles visualized — *Candida* pseudofilaments (indicated by arrows)

Рис. 4. Пациент Ф. В слоях средней стромы в области инфильтрата визуализируются гиперрефлективные веретеновидные частицы — псевдофиламенты *Candida* (указаны стрелками)

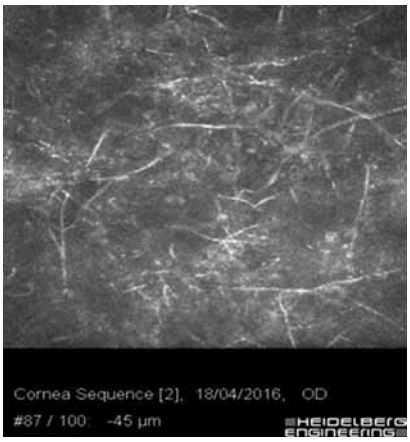

Fig. 5. Patient A. Numerous high-contrast fungi hyphae (*Fuzarium*) are visualized in the projection of the basal epithelium and subepithelial layers of the stromain the subepithelial layers of the stroma

Рис. 5. Пациент А. В проекции базального эпителия и субэпителиальных слоях стромы визуализируются многочисленные высококонтрастные гифы грибов (*Fuzarium*)

to treat AK: 0.02% chlorhexidine bigluconate solution (2 ml 0.05% chlorhexidine bigluconate solution, and 3 ml 0.9% sodium chloride solution or water for daily injection preparation) and 0.02% solution of polyhexamethylene biguanide (PGMB, for example, Baquacil). Both preparations showed clinical efficacy and can be used as initial monotherapy: 1 drop in the conjunctival cavity hourly (including night hours) for the first 3–5 days, followed by every 2 hours when awake, and every 4 hours at night for 2–4 weeks. The number of instillations is then gradually decreased to to 4–6 times a day for several months (from 3 to 6 and more). Among the diamedins, we used propamidine isethionate 0.1% (Brolene, UK), dibromopropamidine isethionate 0.15% (Brolene ointment), and hexamidine isethionate 0.1% (Desomedine, available in France and Belgium). Instillation was performed as 1 drop/hour in the conjunctival cavity (including night hours) for the first 24–48 hours, then 4 times a day for 2–4 months according to tolerability. Clinically, diamedins are well tolerated by ocular tissues, but prolonged treatment with desomedin 0.1% can lead to toxic keratopathy. The combination of biguanides and diamedins showed the best efficacy.

In four patients, AK was associated with a bacterial, fungal, or viral infection, which required additional prescription of antibacterial, antifungal, or antiviral drugs.

The administration of antifungal agents is possible only after the verification of the fungal species. In the Russian Federation, there are no officially registered medications for the treatment of fungal keratitis. For the treatment of filamentous fungi (mold), local antimycotic therapy with 0.15–0.3% amphotericin B solution was administered daily. The method of preparation of the solution was as follows: amphotericin B from the vial is diluted with 5.0 ml of 5% glucose solution, of which 0.2 ml aliquot is withdrawn with an insulin syringe and is added to 0.3 ml of 5% glucose solution. Thus, the necessary concentration of the solution for instillation into the eye is achieved. One drop of the resultant solution is instilled in the conjunctival cavity every hour for the first 48 hours (except during the night sleep). Then, it is administered 4 times a day on a long-term basis. In addition, a systemic antimycotic therapy is prescribed; the first-line treatment includes 400–600 mg voriconazole (Vifend) administered orally 2 times a day. With non-filamentous fungi (yeast), the use of 2% solution of diflucan for 10 days with a frequency of instillations up to 4 times a day gives a good effect (only treatment forms intended for intravenous administration are registered in the Russian Federation; according to foreign authors, the solution for intravenous administration could be used for instillations). Systemic therapy was not used.

Antibacterial agents used belonged to fluoroquinolones, aminoglycosides, or cephalosporins with instillations up to 6 times a day. At the beginning of treatment, 100 ml metronidazole intravenous infusion was administered for 2–3 times a day for 5–10 days. Antiviral drugs could be used (Cycloferon i/m injections; and Poludanum eye drops, with instillations up to 6–8 times a day).

The pathogenetic treatment included the use of medications of different groups, such as antiseptics (Okomistin 0.01% or Vitabact 0.05%, instillations up to 6 times a day), NSAIDs (Broxinac 0.09%, Nevanac 0.1%, Indocollyre 0.1 %, Acular 0.4%), and cycloplegic and IOP-lowering medications. Local use of glucocorticoids in instillations up to 2 times a day and/or injections is possible, but should be avoided during an acute infection. Lubricants should be used with caution during AK therapy, since they lead to formation of an additional film on the surface of the cornea. This hinders

the access of medications to the underlying layers of the cornea and promotes the dissemination of acanthamoeba, leading to corneal perforation. In five patients with the presence of an extensive de-epithelization area, soft bandage CLs were used, in addition to the medical therapy (0.02% chlorhexidine bigluconate solution), and this approach proved to be effective for treating them.

Surgical treatment was performed in patients of the Group 3 (AK stage 4) with ineffectiveness of medical therapy (due to increased thickness and loosening of the infiltrate, thinning of the cornea in the infiltrate zone, or presence of micro cracks of the posterior limiting membrane).

Auto conjunctival tenoplasty (ACTP) of the corneal defect was performed most often (in seven patients) with intraoperative scraping of the infiltrate (with the aim of removing the acanthamoeba and achieving the best engraftment of the autograft) and obligatory temporary blepharorrhaphy. This method seems most justified because of the better engraftment of the autograft to the cor-

Table 3

Clinical data of patients

Таблица 3

Клинические данные пациентов

Group	Patient's number	BCVA on admission	BCVA after treatment	BCVA by the end of the 1-year follow-up	Type of surgical procedure	Relapse
I	1	0.95	0.95	0.95	–	–
	1	0.2	0.2	0.6	–	–
	2	0.04	0.2	0.5	–	–
		0.02	0.1	0.5	–	–
	3	Hand motion near the face	0.08	0.5	–	–
	4	0.08	0.08	0.08	–	–
	5	0.1	0.9	0.9	–	–
	6	0.1	1.0	1.0	–	–
	7	0.01	0.9	0.9	–	–
II	1	Hand motion near the face	Blepharorrhaphy	0.5	ACTP	–
	2	0.001	Blepharorrhaphy	Hand motion near the face	ACTP	–
	3	Hand motion near the face	0.04	0.2	–	–
	4	Hand motion near the face	0	0	Enucleation	+
	5	0.4	0.4	0.02	–	+
	6	0.1	0.1	0.1	Diode-laser coagulation of the cornea + ACTP	–
	7	0.1	Blepharorrhaphy		ACTP	–
	8	Hand motion near the face	Blepharorrhaphy		Defect coverage with an amniotic membrane graft	–
	9	0.08	0.08 (cataract)	0.08 (cataract)	–	–
	10	0.02	0.02		–	–
	11	0.01	Blepharorrhaphy		2 ACTP, administration of auto-blood into the AC	–
	12	Hand motion near the face	Blepharorrhaphy	Hand motion near the face	ACTP	+
	13	0.01	Blepharorrhaphy	0.2	ACTP	–
	14	0.01	0.01	0.01	–	–
	15	0.01	Blepharorrhaphy	0.2	ACTP	–
	16	0.3	Blepharorrhaphy	0.08	Administration of cefuroxime into the AC	+
	17	0.08	0.08	0.08	–	–

Note: BCVA: best-corrected visual acuity; ACTP: auto conjunctival tenoplasty; AC: anterior chamber

nea. In one case with gross cicatricial changes in the conjunctiva (contraction of the conjunctiva and Tenon's capsule due to previous surgical interventions), amniotic membrane was used to cover the cornea. However, due to early rejection of the amniotic flap and development of corneal perforation, this method was ineffective. In a case with posterior limiting membrane defects in the form of micro cracks and pronounced edema of the corneal stroma, auto-blood was used (0.2 ml) for injection into the anterior chamber, after which the patient was placed face down. Closure of micro cracks and reduction of stromal edema became prevalent at the beginning of the third day after the procedure. In one patient with a small perilimbal dense infiltrate and an ulcer with depth of $^1/_3$ of the stroma, the corneal defect was coagulated along the entire area with a diode laser ($\lambda = 1.47$ μm) on the Lakhta-Milon device at the Military Medical Academy (laser department) with consequent auto conjunctival tenoplasty. The use of a medium IR-range diode laser has shown good efficacy, facilitating the sanitation and rapid healing of corneal ulcers [3]. In order to improve the optical properties of the cornea, a penetrating keratoplasty (PKP) is indicated 4–8 months after resolution of the acute process. The efficacy of PKP in the early stages of AK is controversial.

Patient examinations were performed daily (several times a day) until significant improvement was achieved, then every 1–3 weeks. Treatment lasted from 6–12 months to several years and included instillations of 0.02% chlorhexidine bigluconate solution (as the medication least toxic for the cornea among the amoebicidal agents) and medical examinations (standard ophthalmological examination, OCT of the cornea using the OptoVue RTvue100, corneal confocal microscopy using HRT 3 with corneal module, corneal lavage). The early unauthorized withdrawal of 0.02% chlorhexidine bigluconate solution resulted in AK relapses in four cases. In three of these cases, corneal melting occurred, which required additional coating of the cornea with an autograft; and in one case endophthalmitis developed, and the eye was enucleated.

DISCUSSION

The most informative method for AK diagnosing is the confocal microscopy of the cornea. As a result of medical therapy, patients with AK stages 2–3 managed to improve visual functions. The best-corrected visual acuity of the majority of patients ranged between 0.5 and 1.0. In patients with AK stage 4, who were subjects to various types of surgical care, it was possible to stabilize visual functions (Table 3). Non-compliance with recommendations and delayed medical care can lead to recurrences.

CONCLUSIONS

1. AK should be suspected in:
a) patients who use CLs by violating the safety rules for lens wear and care;
b) patients with mechanical corneal trauma;
c) patients with a chemical or thermal eye burn if contaminated with water or soil.
2. Early diagnosis of AK consists of careful evaluation of patient history and analysis of the clinical picture. The most accurate diagnostic method is the confocal microscopy of the cornea, which, in addition to detecting acanthamoeba cysts, helps differentiate the condition from fungal keratitis. Diagnostic methods such as scraping and lavage from the cornea are less informative.
3. Patients with AK require in-patient treatment. Combination treatment includes the use of two amoebicidal medications (0.02% chlorhexidine bigluconate solution and 0.1% solution of propamidine isethionate), antiseptics, NSAIDs, etc. Untimely withdrawal of therapy causes a relapse of the disease.
4. In the Russian Federation, there are no registered medications for AK treatment. Considering the severity of both the disease itself and its possible complications, it is necessary to use unregistered drugs and off-label medicines.
5. According to our data, early auto conjunctival tenoplasty with scraping of the affected area of the cornea prevents the development of corneal perforations and shortens the treatment duration. AK treatment requires long-term use of cationic antiseptics and careful follow-up.

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Research concept — Yu.S. Astakhov.

Collection and processing of materials — E.V. Skryabina, T.C. Varganova, V.P. Petukhov.

Analysis of the data obtained and writing of the text — Ya.S. Konenkova, K.V. Nokhrina, K.O. Dnestryanskaya.

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