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Contents
Содержание

PROBABILITY OF INFECTIOUS DISEASE IN HUMANS DURING EPIDEMIC

Karmishin AM[1] ✉, Borisevich IV[2], Skvortsova VI[2], Goryaev AA[1], Yudin SM[1]

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Popular SIR models and their modifications used to generate predictions about epidemics and, specifically, the COVID-19 pandemic, are inadequate. The aim of this study was to find the laws describing the probability of infection in a biological object. Using theoretical methods of research based on the probability theory, we constructed the laws describing the probability of infection in a human depending on the infective dose and considering the temporal characteristics of a given infection. The so-called generalized time-factor law, which factors in the time of onset and the duration of an infectious disease, was found to be the most general. Among its special cases are the law describing the probability of infection developing by some point in time *t*, depending on the infective dose, and the law that does not factor in the time of onset. The study produced a full list of quantitative characteristics of pathogen virulence. The laws described in the study help to solve practical tasks and should lie at the core of mathematical epidemiological modeling.

Keywords: infective dose, probability of infection, incubation period, duration of disease, quantitative characteristics of pathogen, laws of disease

Author contribution: All authors equally contributed to the methodology of the study, data acquisition, analysis and interpretation. All author participated in drafting the manuscript and editing its final version.

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ВЕРОЯТНОСТЬ ВОЗНИКНОВЕНИЯ ИНФЕКЦИОННОГО ЗАБОЛЕВАНИЯ ЧЕЛОВЕКА ПРИ ЭПИДЕМИИ

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Применяющиеся на практике для описания развития эпидемии, в том числе и эпидемии COVID-19, SIR-модели и их различные модификации оказались несостоятельными. Целью работы было обосновать законы, описывающие вероятность возникновения инфекционного заболевания биообъекта. С помощью теоретических методов исследования, основанных на теории вероятностей, обоснованы законы, описывающие вероятность возникновения инфекционного заболевания человека в зависимости от инфицирующей дозы с учетом временных характеристик реализации поражающего действия патогена. Показано, что наиболее общим является так называемый обобщенный факторно-временной закон заболевания, учитывающий время наступления и длительность заболевания. Из него следуют частные случаи: закон, описывающий вероятность заболевания к заданному моменту времени *t* и без учета времени наступления заболевания в зависимости от инфицирующей дозы патогена. Дано обоснование полного перечня количественных характеристик вирулентности патогена. Эти законы позволяют решать различные практически важные задачи и должны лежать в основе методов математического моделирования развития эпидемического процесса.

Ключевые слова: инфицирующая доза, вероятность заболевания, инкубационный период, длительность заболевания, количественные характеристики патогена, законы заболевания

Вклад авторов: все авторы внесли значимый вклад в разработку методики исследования, получение, анализ и интерпретацию данных. Участвовали в подготовке черновика рукописи и ее редактировании, а также в подготовке финального варианта статьи.

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The COVID-19 pandemic, which began in China in late November/early December 2019 [1], has raised the need for an adequate mathematical model that would accurately forecast epidemiological metrics, including the total number of cases and deaths, timeline, etc. Predictions generated by popular SIR models [2, 3] and their modifications turned out to be wrong because such models are based on false assumptions about how infection develops both in an individual and in the entire population. For example, such models predict the number of infected individuals at a given point in time from the number of contacts made by susceptibles and infectives, but not from the infective dose, which actually determines the probability of infection.

Similar to industrial accidents at hazardous facilities [4, 5], an epidemic should be described at 3 interrelated levels of generalization:

1) the low generalization level (with a focus on a host-pathogen interaction), which involves a) providing mathematical reasoning for the laws describing how a pathogen or a group of different pathogens establish an infection in a human or a non-human biological object and b) virulence assessment for each route of entry into the host;

2) the medium generalization level (with a focus on the transmission/spread of a studied infection in a population), which describes the infective dose received by a human or a non-human biological object via each route of transmission;

3) the high generalization level, which describes the integral spatiotemporal parameters of infection spread.

This article focuses on the first (low) generalization level, i.e. infection with one pathogen type.

The aim of this study was to find the laws describing the probability of infection in a human or a non-human biological object.

METHODS

The laws describing how infection is established in a human or a non-human biological object can be constructed theoretically or from experimental data. This article presents the results of theoretical research.

Importantly, the main quantitative characteristic of a pathogen that determines the probability of infection or death of a biological object is the infective dose D, as opposed to contact between a susceptible and infective individuals.

Similar to the concept of the toxic dose of toxic chemicals [6], an infective dose is the amount of pathogen (a biohazardous agent, BHA) entering the organism. This dose can be expressed in BHA mass units or special units like CFU (colony forming units), PFU (plaque forming units) and au (arbitrary units).

In order to find the laws describing how infection is established in a human or a non-human biological object, the following situations should be considered:

– exposure to different infective doses of one or a variety of pathogens, temporal characteristics of the infection not being accounted for;

– exposure to different infective doses of one or a variety of pathogens, time to onset of signs and symptoms being factored in;

– exposure to different infective doses of one or a variety of pathogens, time to onset *t* and duration τ (time to recovery) being factored in.

Obviously, the second situation is more general than the first, and the third situation is more general than the first two.

Graphs included in the article were constructed in Microsoft Office Excel 2013 (Microsoft; USA).

RESULTS

Using the toxicity of chemical agents or pharmaceutical drugs as an analogy [6], the simplest problem, in our case, can be set up as follows.

Let us assume that when a pathogen gains access to a given host type (e.g. an adult human) via a given route of entry, it is expected to produce a specific effect (a mild, moderate, severe or critical infectious disease). Because humans differ in their immune status, the infective dose needed to produce this effect will vary between the exposed hosts. Therefore, the amount of pathogen capable of producing a certain effect (evoking a certain response) can be considered a continuous random variable.

According to the probability theory, a random variable is best described by its distribution law; the distribution law of a continuous random variable can be described by:

– a uni- or multivariate probability density function;

– a probability distribution function of the considered random variable (integral function).

The probability density function $\varphi(\widetilde{D})$ of the random infective dose (ID) value *D* which elicits a certain response in a human or a non-human biological object is shown in Fig. 1.

By definition,

$$\varphi(\widetilde{D}) = \frac{dN}{NdD},$$

where dN is the number of objects for which the random ID value eliciting a certain response falls within a range between $\widetilde{D}$ and $\widetilde{D} + d\widetilde{D}$;

N is the total number of objects;

$\frac{dN}{N}$ is the proportion of objects for which the random ID value evoking a certain response ranges from $\widetilde{D}$ to $\widetilde{D} + d\widetilde{D}$ if *N* is relatively high, this proportion can be interpreted as the probability *dP* of the ID that evokes a certain response falling within the range between $\widetilde{D}$ and $\widetilde{D} + d\widetilde{D}$; so

$$\varphi(\widetilde{D}) = \frac{dN}{NdD} = \frac{dP}{dD}.$$

By definition, the distribution function (Fig. 2) describes the probability of the random ID value that elicits a certain response in a biological object being lower than D, i.e. $F(D) = P(\widetilde{D} < D)$.

Then, if a biological object is exposed to some infective dose *D* at $P = F(D)$, the random ID value capable of causing infection in this biological object will be lower than the applied dose; so, the harmful effects of the pathogen will not be below a given level at $P = F(D)$ (Fig. 3).

Similar to toxic chemicals leaking during industrial accidents [6–8], the following definition can be given:

The relationship between the probability of infection whose severity is not below a given level and the infective dose is called the hazard factor law (HFL).

In general, the integral representation of this law takes the form of:

$$P = \int_0^D \varphi(\widetilde{D})d\widetilde{D}. \quad (1)$$

This law is schematically shown in Fig. 3. Its specific representations are based on the data generated by experiments on animals. If an object is exposed to multiple hazards, the subintegral function from expression (1) can be represented by the normal or log-normal distribution, the Weibull or gamma distribution, or approximations by linear equations, the logistic curve, etc. [5].

It should be noted that when experimental data are processed, it is often impossible to favor one type of distribution

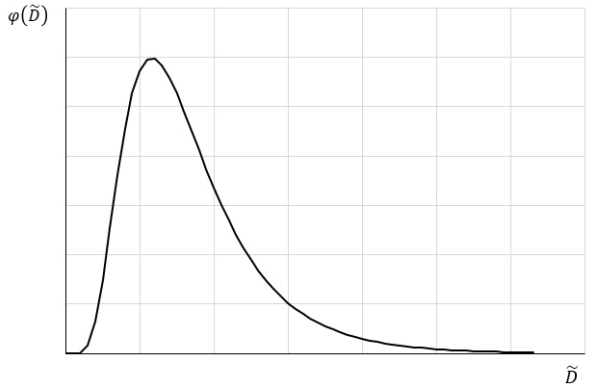

Fig. 1. The probability density function of the infective dose *D* eliciting a specific response in a human or a non-human biological object

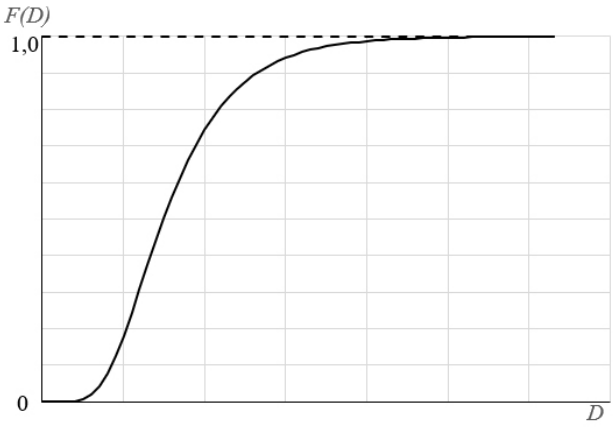

Fig. 2. The probability distribution function of the infective dose eliciting a certain response in a human or a non-human biological object

because the obtained experimental data conform to different types of distribution.

However, when more than one random variable is included in the equation (the infective dose, time to onset of symptoms, duration of the disease), i. e. we deal with a multivariate random variable, the situation with the distribution type clears up.

Let us consider exposure to one or a variety of pathogens and account for time to onset of mild, moderate, severe or critical symptoms.

According to experimental data, time between exposure to a given infective dose and onset of clinical symptoms of various severity (the incubation period), including death, is a random variable (conditional probability distribution of one random variable in the presence of another fixed variable). Typical time to onset characterized by mathematical expectations, modal or median times correlates with the actual exposure dose.

By analogy with some other studies [6–8], we conclude that the infective dose evoking a specific response and time to its onset are continuous correlated random variables. The probability density function φ ($\tilde{D}$, $\tilde{t}$) for such two-dimensional random variables is shown in Fig. 4. In practice, it is often required to calculate the probability of infection developing by a certain point in time t or the likelihood of death. This metric can be calculated using the formula [5–8]:

$$P = \int_0^D \int_0^t \varphi(\tilde{D},\tilde{t})\tilde{d}\tilde{D}d\tilde{t}. \qquad (2)$$

Expression (2) is a common integral representation of a solution to the problem of determining the probability of developing symptoms at or above the specified severity level by some point in time t depending on the infective dose; it is referred to as the hazard time-factor law (HTFL).

Thus, this law describes the probability of developing an infectious disease at or above the specified severity level by the point in time t depending on the actual infective dose (Fig. 5) [8].

In a special case, if time of onset from expression (2) approaches infinity, the expression takes the form of the hazard factor law (1). Therefore, at this stage of analysis we arrive at the conclusion about the form of subintegral functions from expressions (1) and (2).

The only known type of distribution for continuous random variables existing under the probability theory is the normal type. However, it cannot be used to solve the problem set in this paper because the normal distribution domain (–∞; ∞) does not coincide with the domain of random variables [0; ∞]. There are no laws of multivariate Weibull distributions, gamma distributions or the like that could, in a limiting or special case, produce a Weibull or gamma distribution or a Weibull-gamma distribution [5].

About 15 years ago, we were working on a mathematical model describing the combined effect of bioactive substances, such as pharmaceutical drugs and toxic chemicals, and discovered a multivariate log-normal distribution of continuous correlated random variables [6]. Now, in the case of death from infection, the bivariate probability density function from expression (2) can take the following form:

$$f(\tilde{D},\tilde{t}) = \frac{1}{2\pi\sqrt{1-r_{\ln D\ln t}^2}\,\sigma_{\ln t}\sigma_{\ln D}\tilde{D}\,\tilde{t}} e^{-\frac{1}{2(1-r_{\ln D\ln t}^2)}\left(\frac{\ln^2\frac{\tilde{D}}{LD_{50}}}{\sigma_{\ln D}^2} - 2r_{\ln D\ln t}\frac{\ln\frac{\tilde{D}}{LD_{50}}\ln\frac{\tilde{t}}{t_{50}}}{\sigma_{\ln D}\sigma_{\ln t}} + \frac{\ln^2\frac{\tilde{t}}{t_{50}}}{\sigma_{\ln t}^2}\right)}, \qquad (3)$$

where LD_{50}, t_{50}, $\sigma_{\ln D}$, $\sigma_{\ln t}$ and $r_{\ln D\ln t}$ are parameters of the equation.

If an object is exposed to a fixed amount of pathogen (infective dose), then time to onset of symptoms at or above the specified severity level (incubation time) is a continuous random

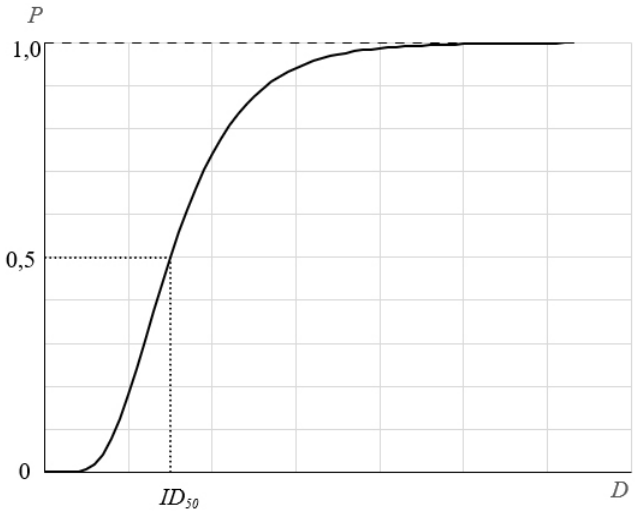

Fig. 3. The hazard factor law

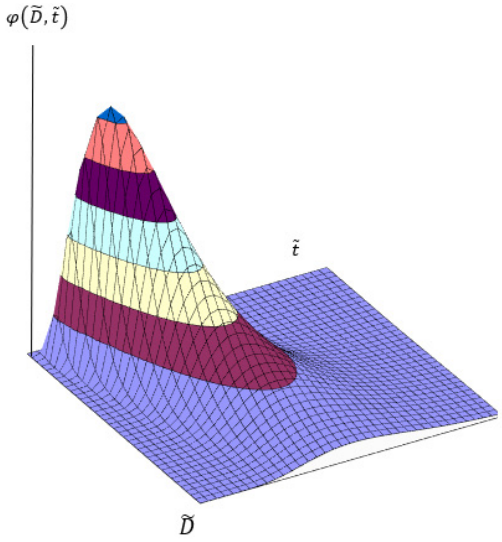

Fig. 4. The probability density function for random values of the infective dose ID causing a specific effect and time of onset.

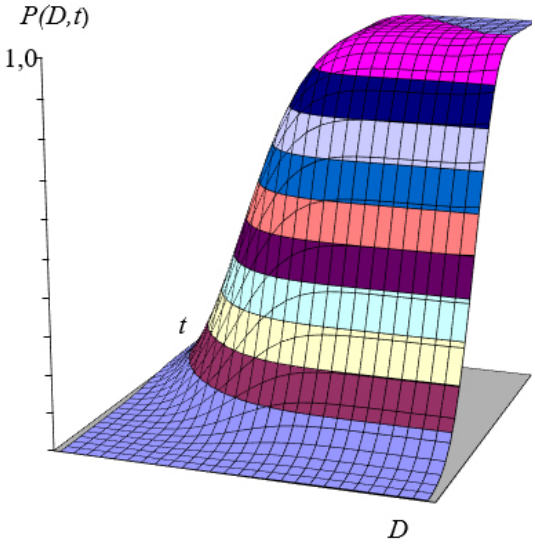

Fig. 5. The hazard time-factor law

variable characterized by log-normal distribution [5–8]. The conditional probability distribution of random time to onset (for symptoms at or above the specified severity level) describing the probability $P(t)$ of this random time being shorter than time t will take the following integral form:

$$P(t) = 0,5\left[1 + erf\left(\sqrt{k^*_{lnt}}\ln\frac{t}{t^*_{50}}\right)\right], \quad (4)$$

where t^*_{50} is median time to onset of symptoms at or above the specified severity level developing in response to a specific infective dose, expressed in days;

k^*_{lnt} is a parameter of the equation.

Parameters t^*_{50} and k^*_{lnt} are defined using quantitative characteristics of pathogen virulence and the actual infective dose as shown below [6, 8]:

$$\ln t^*_{50} = \ln t_{50} + r_{\ln D\ln t}\frac{\sqrt{k_{\ln D}}}{\sqrt{k_{\ln t}}}\ln\frac{D}{D_{50}}, \qquad \sqrt{k^*_{lnt}} = \frac{\sqrt{k_{lnt}}}{\sqrt{1-r^2_{lnD\,lnt}}}; \qquad \sqrt{k} = \frac{1}{\sqrt{2}\sigma}.$$

According to experimental data, the amount of pathogen that causes a disease in a human biological object, disease incubation time and duration (time from onset of clinical symptoms to recovery) are continuous random variables.

In 2007, it was demonstrated that the probability of the harmful effect (which, in our case, is infection) that is not less than a given severity by the time t and for a duration τ (that not less than a given one) can be defined as follows [8]:

$$P = \int_0^{Dt}\int_0^{\infty}\int_{\tau} f(\tilde{D},\tilde{t},\tilde{\tau})d\tilde{D}d\tilde{t}\,d\tilde{\tau}\,, \quad (6)$$

where $f(\tilde{D},\tilde{t},\tilde{\tau})$ 3-variate log-normal distribution density of continuous correlated random values: the infective dose $\tilde{D}$ capable of causing infection at or above the specified severity level, time of onset $\tilde{t}$ and duration of infection $\tilde{\tau}$, characterized by 9 parameters, which, in the case of a pathogen, are quantitative characteristics of pathogen virulence

$$f(\tilde{D},\tilde{t},\tilde{\tau}) = \frac{1}{(2\pi)^{1,5}\sqrt{\Delta_1}\tilde{D}\tilde{t}\tilde{\tau}\sigma_{\ln D}\sigma_{lnt}\sigma_{lnt}}e^{-\frac{1}{2\Delta_1}[A+B]},$$

where $\Delta_1 = 1 - r^2_{lnDlnt} - r^2_{lnDlnt} - r^2_{lntlnt} + 2r_{lnDlnt}r_{lnDlnt}r_{lntlnt}$

$$A = \frac{\left(1-r^2_{lntlnt}\right)ln^2\frac{\tilde{D}}{ID_{50}}}{\sigma^2_{lnD}} + \frac{\left(1-r^2_{lnDlnt}\right)ln^2\frac{\tilde{t}}{t_{50}}}{\sigma^2_{lnt}} + \frac{\left(1-r^2_{lnDlnt}\right)ln^2\frac{\tilde{\tau}}{\tau_{50}}}{\sigma^2_{lnt}};$$

$$B = -2\frac{\left(r_{lnDlnt} - r_{lnDlnt}r_{lntlnt}\right)ln\frac{\tilde{D}}{ID_{50}}ln\frac{\tilde{t}}{t_{50}}}{\sigma_{lnD}\sigma_{lnt}} - 2\frac{\left(r_{lnDlnt} - r_{lnDlnt}r_{lntlnt}\right)ln\frac{\tilde{D}}{ID_{50}}ln\frac{\tilde{\tau}}{\tau_{50}}}{\sigma_{lnD}\sigma_{lnt}} - 2\frac{\left(r_{lntlnt} - r_{lnDlnt}r_{lnDlnt}\right)ln\frac{\tilde{t}}{t_{50}}ln\frac{\tilde{\tau}}{\tau_{50}}}{\sigma_{lnt}\sigma_{lnt}}$$

This probability is referred to as generalized HTFL [8].

If duration of an infectious disease is not included in the equation, as is the case with deaths from infection, then, assuming that (6) $\tau = 0$, we will arrive at HTFL (2).

If the generalized HTFL does not account for the timeline of infection, then, assuming that $t = \infty$ and $\tau = 0$, we will arrive at HFL (1):

$$P = \int_0^D f(\tilde{D})d\tilde{D} = 0,5\left[1 + erf\left(\frac{1}{\sqrt{2}\sigma_{lnD}}ln\frac{D}{D_{50}}\right)\right] = 0,5\left[1 + erf\left(\sqrt{k_{lnD}}\,lnT\right)\right], \quad (7)$$

because

$$f(\tilde{D}) = \frac{1}{\sqrt{2\pi}\sigma_{lnD}\tilde{D}}e^{-\frac{ln^2\frac{\tilde{D}}{ID_{50}}}{2\sigma^2_{lnD}}}, \qquad \sqrt{k} = \frac{1}{\sqrt{2}\sigma}$$

where *erf(u)* is the integral of error function

$$erf(u) = \frac{2}{\sqrt{\pi}}\int_0^u e^{\frac{t^2}{2}}dt.$$

The list of quantitative characteristics of pathogen virulence in the case of exposure to one pathogen, their probability and physical interpretation are provided in Table.

Thus, the problem formulated at the beginning of this study is now completely theoretically solved.

Virulence depends on the species and strain of the studied pathogen, the route of entry (inhalation, ingestion, through mucous membranes) and the type of the biological object exposed to the pathogen (adults, children, the elderly, individuals with chronic conditions). Virulence should be experimentally assessed at the lab using model objects. The obtained results are then expected to be translated to humans.

Methods used to determine quantitative characteristics of virulence have been subjected to critical analysis. Among such methods are Kärber's method [9], *Finney's* probit analysis [10] and *Bliss's* probit analysis [11]. Using the method of moments, maximal likelihood estimation and the method of least squares, researchers designed ways to measure 9 toxicological (virological) parameters of bioactive agents (pathogens) based on primary data from laboratory studies on model objects [8].

There is another important issue that needs to be discussed. The laws covered by this study are referred to as conditional static (deterministic) laws. In real life, the infective pathogen dose is a stochastic variable due to a number of subjective and objective reasons [8]. At the same time, quantitative characteristics of virulence are population parameters, i.e. deterministic variables. On the other hand, given the methods for their determination, they are estimates of the general population parameters and, therefore, are continuous random variables (this is a fundamental property of estimates).

Therefore, the studied probabilities of infection are functions of continuous random variables and are stochastic themselves. This raises the question of accounting for the random nature of variables in the laws described above. This problem can be discussed and solved under the stochastic theory of infection, the emergent, independent field of research [8, 12–17].

The literature offers a wealth of data on the incubation period: its minimum t_{min}, maximum t_{max} and sometimes average duration [18, 19]. To a first approximation, such data provide an insight into the temporal characteristics of virulence. Assuming that the minimum and maximum duration of the incubation period reported by the literature are in agreement with the 0.95 probability of random incubation period duration falling within this range, the following quantitative characteristics can be calculated:

– the median duration of the incubation period

$$It_{50} = \sqrt{t_{min}t_{max}};$$

– the mean squared error σ_{lnt}

$$\sigma_{lnt} = 0,25ln\frac{t_{max}}{t_{min}}.$$

However, more accurate estimates can be obtained in special experiments on model objects, followed by their translation to humans [8].

DISCUSSION

Our theoretical research allowed us to find the laws describing the probability of infection after exposure to one pathogen type, depending on the infective dose and considering the temporal characteristics of a given infection. The correctness of these laws was confirmed by dimensional analysis and their correct behavior in limiting and special cases.

An important practical implication of this theoretical research is the complete list of quantitative characteristics of virulence. It is important to know 9 or 5 quantitative characteristics of virulence, for reversible and irreversible effects, respectively.

Table. The full list of quantitative characteristics of pathogen virulence in the case of exposure to one pathogen

Characteristic	Units	Interpretation	
		Probability (statistical)	Physical
ID_{50}, LD_{50}	mg (au, PFU, CFU)	Median infective and lethal doses (exposure doses) causing an infectious disease of given severity or death	Infective (exposure) doses for which the probability of infection or death is 0.5
ICT_{50}, LCT_{50}	mg•min•L^{-1} (au•min•L^{-1}, БОЕ•min•L^{-1})		
It_{50}, Lt_{50}	hours, days.	Median time to infection of given severity following exposure to the median infective dose	Period after which all infected persons exposed to the median infective dose will develop clinical symptoms or die at 0.5 probability
IT_{50},		Median time of random duration of an infectious disease following exposure to median ID	Period during which the infected individual exposed to the median infective dose will recover at 0.5 probability
σ_{lnD}	—	Standard deviation (SD) of the natural logarithm of a random ID value causing infection or death	Characterizes the homogeneity (heterogeneity) of a given population in terms of its susceptibility to infection
σ_{lnt}	—	SD of the natural logarithm of random time to onset of infection or death	Characterizes the homogeneity of a given population in terms of time to onset of clinical signs and symptoms
σ_{lnT}	—	SD of the natural logarithm of random infection duration	Characterizes the homogeneity (heterogeneity) of a given population in terms of disease duration
r_{lnDlnt}	—	Correlation coefficient of the natural logarithms of – random infective dose causing infection or death and time of onset; – random infective dose causing infection and the duration of the infectious disease; – random time of onset and the duration of the infectious disease	Characterizes the relationship between complex processes underlying the development of harmful effects of a bioactive compound on a biological object over time $-1 \le r_{lnDlnt} \le 0$; $0 \le r_{lnDlnT} \le 1$; $-1 \le r_{lntlnT} \le 0$;

Today, these quantitative characteristics are almost unknown, which is a serious setback for accurate epidemic modeling.

At present, the probability of establishing an infection is described based on the number of contacts between susceptibles and infectives [2, 3, 20, 21], which is wrong in principle.

CONCLUSION

We have constructed the hazard factor law, the hazard time-factor law and the generalized time-factor laws describing the probability of infection in humans and non-human biological objects (like agricultural animals) following exposure to one as opposed to a variety of pathogens. These laws help in solving practical tasks and should lie at the core of mathematical epidemiological modeling.

In order to successfully solve practical epidemiological tasks, further research should focus on identifying all quantitative characteristics of pathogens for every route of entry into the body and the obtained data should be compiled into a comprehensive database.

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CONCEPT OF MEDICAL PSYCHOPHYSIOLOGICAL EXAMINATION OF PERSONNEL OF NUCLEAR FACILITIES

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Ensuring safety of the facilities employing radiation and nuclear hazardous technologies is a priority task for medical organizations serving these facilities. To perform safely at their jobs, it is important for the personnel of nuclear facilities (NF) to have their central nervous systems functioning flawlessly. Certain categories of nuclear industry workers are required to undergo compulsory annual medical examinations (ME) and psychophysiological examinations. This study aimed to develop a concept of psychophysiological examination of NF personnel allowing to assess the central nervous system's functional status. The study involved three groups of nuclear corporation employees (male) counting 720, 364 and 24 people aged from 46 ± 5.3 to 49 ± 6.1 years. The report describes the suggested concept of psychophysiological examination of the specified category of workers, discusses goals, objectives and the procedure of such an examination at all stages of compulsory ME, covers the developed hardware and software sets. The proposed methodological approach is evaluated through consideration of the results of psychophysiological examination of the specified category of workers.

Keywords: workers, nuclear facilities, psychophysiological examination, concept, functional state, central nervous system

Author contribution: FS Torubarov, ZF Zvereva — data processing and article authoring. All authors participated in the discussion of the results.

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КОНЦЕПЦИЯ ПСИХОФИЗИОЛОГИЧЕСКОГО ОБСЛЕДОВАНИЯ ПЕРСОНАЛА ОБЪЕКТОВ ИСПОЛЬЗОВАНИЯ АТОМНОЙ ЭНЕРГИИ В МЕДИЦИНСКИХ ОРГАНИЗАЦИЯХ

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Обеспечение безопасности объектов, использующих радиационно и ядерно опасные технологии, является приоритетной задачей медицинских организаций, обслуживающих эти предприятия. Для безопасной реализации работниками объектов использования атомной энергии (ОИАЭ) профессиональной деятельности важно высокое функциональное состояние их центральной нервной системы. Отдельные категории сотрудников атомной отрасли обязаны проходить обязательные ежегодные медицинские осмотры (МО) и психофизиологические обследования. Целью исследования было разработать концепции психофизиологического осмотра персонала ОИАЭ для оценки функционального состояния центральной нервной системы. В исследовании участвовали три группы работников (мужчины) атомной корпорации численностью 720, 364 и 24 человека в возрасте от 46 ± 5,3 до 49 ± 6,1 года. Предложена концепция психофизиологического обследования указанной категории работников, обсуждаются цели, задачи, порядок проведения психофизиологического обследования на всех этапах обязательных МО, разработаны аппаратно-программные комплексы. Для оценки предлагаемого методического подхода рассмотрены результаты психофизиологического обследования указанной категории работников.

Ключевые слова: работники, объекты использования атомной энергии, психофизиологическое обследование, концепция, функциональное состояние, центральная нервная система

Вклад авторов: Ф. С. Торубаров, З. Ф. Зверева — обработка данных и написание текста статьи. Все авторы участвовали в обсуждении результатов.

Соблюдение этических стандартов: исследование одобрено этическим комитетом ГНЦ ФМБЦ им. А. И. Бурназяна (протокол № 32с от 31 октября 2018 г.); все процедуры, выполненные в исследовании с участием людей, соответствуют требованиям институционального и/или национального комитета по исследовательской этике и Хельсинкской декларации 1964 г. и ее последующим изменениям.

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Ensuring safety of the facilities employing radiation and nuclear hazardous technologies is a priority task for the relevant services and medical organizations serving such facilities. Despite technological advancements and widespread automation, human factor still plays a significant role. To mitigate the possible adverse impact thereof, certain categories of specialists permitted to work in the field of atomic energy are required to undergo medical examinations (ME) and psychophysiological examinations (PPE) in medical organizations.

Currently, under normal operating conditions, the staff of nuclear facilities cannot be exposed to ionizing radiation in doses exceeding the established standards. Professionally, such specialists perform under the burden of high responsibility, significant mental stress, need to be able to quickly perceive information, process it and respond. To successfully discharge these duties and work in the conditions described, they need their central nervous system (CNS) to have a high functional state (FS).

According to the current concepts, FS of CNS determines the general functional state of the body [1]. Nervous system is considered to be the physiological basis of regulation mechanisms. From the psychophysiological viewpoint, FS of CNS is conditioned by the level of activation of and interaction between modulating — specific and nonspecific — structural-functional formations (SFF) of the brain [2, 3]. In case of nuclear facility employees, process safety and efficiency directly depends on the FS of their CNS. PPE allows assessing FS of CNS.

The purpose of PPE is to identify persons with psychophysiological contraindications for employment at NF. The goals of PPE include assessment of the functional activity of SFF of CNS and conclusion about FS of the employee's CNS [4, 5].

As of 2019, there are 26 psychophysiological examination laboratories (PPEL) operating under medical organizations serving nuclear facilities in Russia. This fact necessitates development and implementation of common methodological approaches and methods, as well as a single PPE protocol applicable at all stages of medical monitoring of the personnel's condition [4, 5].

This study aimed to develop nuclear industry personnel PPE concepts and evaluate the suggested methodological approach.

METHODS

The study involved three groups of nuclear corporation employees that underwent PPE in 2015–2017 as part of routine medical checkups. The inclusion criteria required the participants to not have any contraindications for working at a nuclear facility.

Group 1: 720 employees of ten nuclear power plants (NPP), mean age 49 ± 6.1 years; inclusion criterion — underwent PPE as part of routine medical checkup.

Group 2: 364 NPP operators, mean age 46 ± 5.3 years; inclusion criterion — underwent PPE as part of pre-shift medical examination.

Group 3: 24 people, mean age 48 ± 6.3 years; inclusion criterion — underwent PPE as part of evaluation of the results of rehabilitation and health improvement courses (RHIC) in a hospital.

PPE In the context of routine medical checkups of NPP employees, as well as those that underwent RHIC, relied on the PFS-Kontrol hardware and software set (H&S) [6]. The subject of evaluation were the results of application of the following tests/methods.

– psychodiagnostic techniques: MMPI methodology; Sixteen Personality Factor Questionnaire, 16PF; Raven's Matrices; subjective control level (SCL);

– psychophysiological techniques (visual-motor tests): simple visual-motor reaction test, SVMRT; complex visual-motor reaction test, CVMRT, reaction to moving object (RMO)

– physiological technique: heart rate variability (HRV).

Prognoz H&S was used in the context of pre-shift examinations [7].

The statistical differences were assessed with the help of the χ^2 test, the level of significance was set at $p < 0.05$.

RESULTS

The search for a common approach to assessment of the FS of CNS based on the employee's PPE results yielded a psychophysiological examination concept (Fig. 1).

Three structural and functional formations in the brain were selected as those allowing assessment of FS of CNS relying on the PPE results. Psychodiagnostic techniques (MMPI, 16PF, Raven's Matrices, SCL) allowed identifying the "cortex" SFF, psychophysiological techniques (SVMRT, CVMRT, RMO) — the "cortical-subcortical interaction" SFF, physiological techniques (HRV) — the "cardiovascular system central regulation" SFF [6, 8, 9].

The functional activity (FA) of the SFF could be high, medium and low, all within the limits of acceptable values. It could also go beyond those limits. The SFF FS indicators allowed making a final conclusion about FS of CNS.

All stages of medical monitoring routines should include PPE, but the purpose attached to each stage is unique. Figure 2 shows the medical monitoring diagram.

During the preliminary ME, the main task is to identify psychophysiological contraindications for work. PPE results are included in the preliminary ME's general report; if there are psychophysiological contraindications, the candidate is not hired. It should be noted that it is advisable to accumulate indicators registered with each PPE test and the general conclusion drawn thereof in a special database.

For persons hired, further medical monitoring routines are shaped by the results of preliminary ME and PPE.

In the context of regular ME, PPE solves two tasks:

1) identify persons with unacceptable values of indicators of functional activity of CNS SFF, who are suspended from work for an in-depth medical examination to make a decision on the possibility of continuing the employment;

2) identify persons with low but permissible values of indicators of functional activity of CNS SFF, who are added to the risk group and sent to RHIC.

As a rule, regular medical examinations take place once a year, and on the daily basis, the employee's FS of CNS is controlled with pre-shift ME and PPE.

Pre-shift ME allow identifying persons in a disabled state, including those intoxicated with alcoholic, narcotic or other toxic substances or exhibiting residual effects thereof. Pre-shift psychophysiological control uncovers functional disorders of CNS that may significantly hamper professional reliability of an employee.

Thus, the goal of pre-shift PPE is to identify workers whose FS of CNS prevents them from working the given shift. The time allocated for PPE as part of the pre-shift control routine is limited. Therefore, the important technical requirements for PPE of this stage are efficiency, personalized character, exhaustive descriptiveness.

At the RHIC stage, PPE aims to objectively assess the FS of CNS before and after the procedures.

It is mandatory for medical organizations conducting PPE to develop and deploy a special database that summarizes the results of examinations at all stages.

A PPE database enables timely medical, organizational and managerial decisions made with the aim to improve radiation and nuclear safety of the nuclear industry plants and facilities.

From our point of view, in the context of laboratory ME, PPE should not only apply a set of methods and techniques common to all sych examinations, but also employ H&S sets that meet a unified list of requirements. Such an approach would allow comparing PPE results obtained at different (all) laboratories.

PFS-Kontrol H&S set enables full-scale PPE as part of preliminary, regular and RHIC-related ME, with the output being a medical report on the FS of CNS delivered without any delay.

Prognoz H&S set enables PPE as part of pre-shift control. The psychophysiological methods used by this H&S set are designed to assess visual and auditory sensory systems, as well as optical-motor reactions. The results of application of each method translate into a systemic function organization stability indicator (SFOSI), which describes CNS as a single functional system.

Prognoz H&S set employs an innovative admission control method, which allows determining whether an NF operator may be admitted to work. The method relies on the indicators reflecting normal state of each operator (personalized

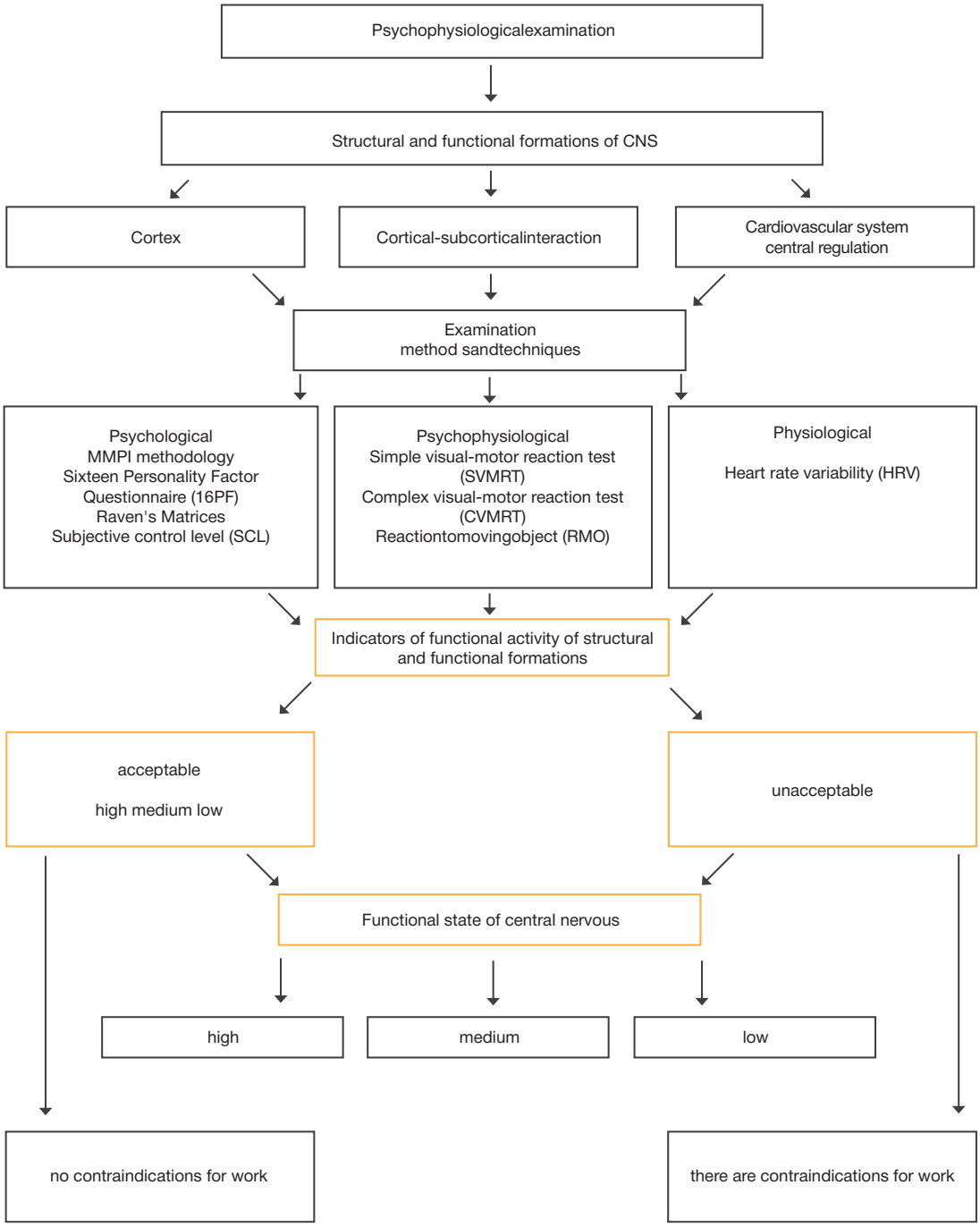

Fig. 1. Concept of psychophysiological examination

approach), the values of which accumulate and are shaped into "norms" for the given operator automatically after 20th pre-shift checkup, provided the operator had no health complaints and was always admitted to work during the corresponding period. Personal norms factor in psychophysiological characteristics and their daily fluctuations. As the personal norm data are accumulated, it is automatically recalculated every month.

Examination of Group 1: assessment of the results of PPE as part of regular ME

Tables 1 and 2 show the results of PPE performed in the context of regular ME of 720 NPP employees. The examination made use of PFS-Kontrol H&S set.

The FS of CNS in the majority of examined individuals was medium (56.4%) and high (28.5%); only 15.3% had it at the low level. The differences between groups were significant: χ^2, $p < 0.05$.

To determine the contribution of each SFF into fluctuations of FS of CNS, we analyzed the SFF indicators peculiar to high, medium and low functional activity (Table 2).

The dominating (50.6%) SFF in cases of high FA was "Cortex". The number of "Cortical-subcortical interaction" SFF was significantly less (24.8%), and that of "Cardiovascular system central regulation" even less (10.3%) (χ^2, $p < 0.05$).

In cases of medium FA, the dominating SFF indicators were "Cortical-subcortical interaction" and "Cardiovascular system central regulation" (61.9% and 59.9%, respectively). The "Cortex" SFF was slightly less (47.7%), however, the differences with the number of "Cortical-subcortical interaction" and "Cardiovascular system central regulation" SFF were significant (χ^2, $p < 0.05$).

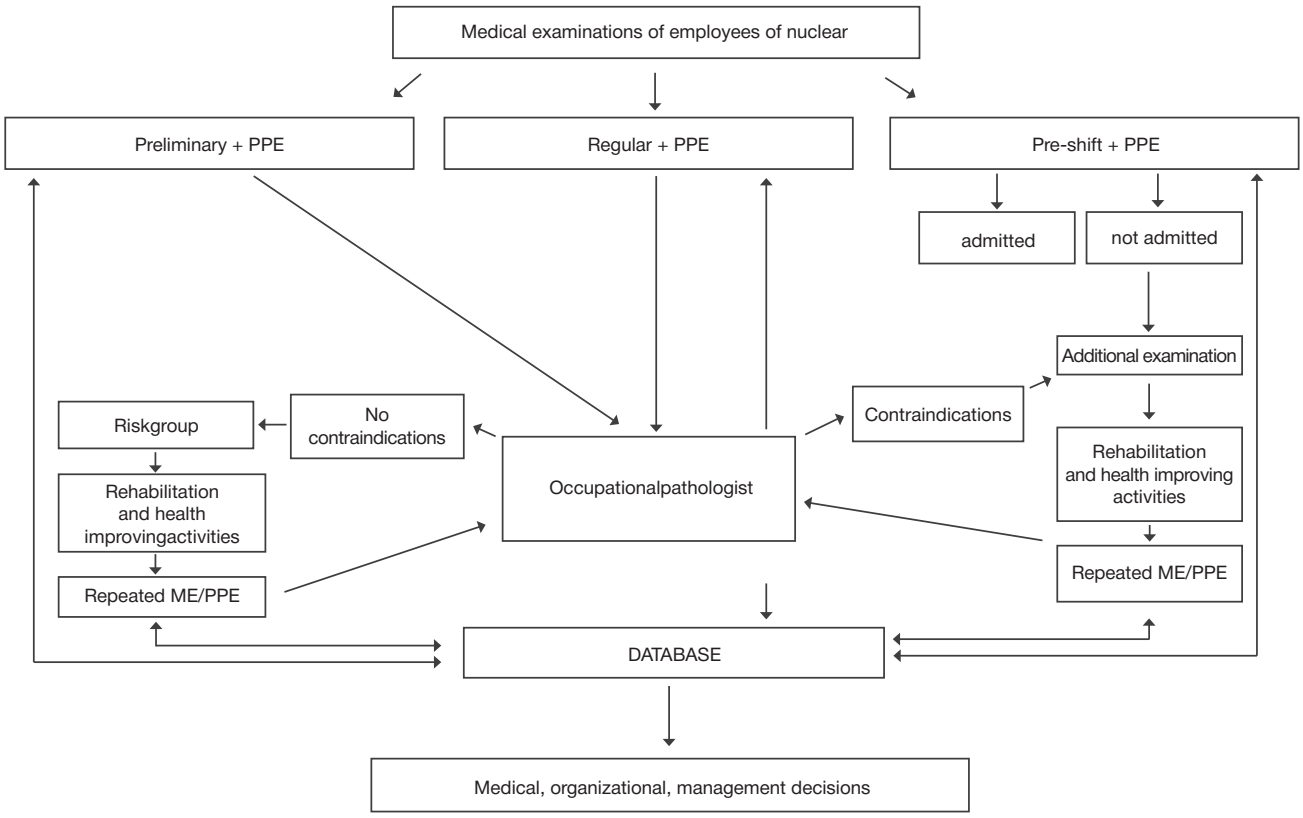

Fig. 2. Medical monitoring system for employees of nuclear facilities

At high FA, the dominating indicators were those of "Cardiovascular system central regulation" (relative to the indicators of the "Cortical-subcortical interaction" and "Cortex" SFF) — 29.9% versus 12.3% and 3.2% (the differences between the groups are significant: χ^2, $p < 0.05$).

Based on the data presented, it can be assumed that when SFF function at a high level, the SFF influencing FS of CNS most is the "Cortex" SFF. When the FA is medium, the most influential as the "Cortical-subcortical interaction" and "Cardiovascular system central regulation" SFF. In cases of low FA, the FA of CNS is mostly shaped by the "Cardiovascular system central regulation" SFF.

Examination of Group 2: assessment of the results of pre-shift examinations

At Kursk NPP, Prognoz-enabled PPE has been part of the pre-shift checkup since 2010. Every year, 260–400 people undergo such examinations. Overall, Prognoz H&S set is used in over 70,000 pre-shift examinations a year. Every day, one or two persons are not admitted to work because of the low FS of CNS. Within a year, the figure is 80. After examination by a paramedic, 75–80% of them receive a conditional admission to the shift with notification of the shift manager. About 15% are not admitted and sent to the workshop therapist for additional examination.

The results of PPE are the basis for the report (Table 3) that contains all the indicators and admission data.

As the report above shows, operator 2 had the current SFOSI value significantly exceeding his personal norm, which was the reason for him not being allowed to work.

Examination of Group 3: PPE before and after RHIC

The pre- and post-RHIC PPE was carried out in the psychophysiological laboratory of the Center for Occupational Pathology of the State Scientific Center of A. I. Burnazyan Federal Medical and Biological Center.

Before RHIC, all patients had the mean time of sensorimotor reactions slightly increased, although within the permissible value range. The integral indicator of visual-motor reaction tests (SVMRT, CVMRT) determined by the mean time of sensorimotor reactions and the number of precise reactions, was below normal in all of them (Table 4).

RHIC improved the visual-motor test indicators significantly, which confirms improvement of the FS of CNS.

DISCUSSION

The presented concept of psychophysiological examination of NF personnel corresponds to the existing concepts of adaptation, the basis for which is the theory of functional

Table 1. FS of CNS registered in Group 1 (n = 720)

FS of CNS	Examinedindividuals	
	abs.	%
High	205	28.5
Medium	406	56.4*
Low	110	15.3*

Note: * — significant differences with the group showing high indicator value at ($p < 0.5$), as established by the χ^2 test

Table 2. SFF CNS indicators at high, medium and low functional activity, Group 1 (n = 720)

SFF CNS	Functionalactivity					
	High		Medium		Low	
	abs.	%	abs.	%	abs.	%
"Cortex"	365	50,6	340	47,2	15	3,2
"Cortical-subcorticalinteraction"	179	24,8*	446	61,9*	95	13,2*
"Cardiovascularsystemcentralregulation"	74	10,3*•	431*	59,9*	215	29,9*•

Note: * — significant differences with SFF "Cortex" at ($p < 0.5$), as shown by the χ^2 test; * • — significant differences with SFF "Cortical-subcortical interaction" at ($p < 0.5$), according to the χ^2 test

Table 3. Automated PPE reportexample

#	Name	Shop	Position	SFOSI indicators				Admitted/ Notadmitted
				Mean	RMS (σ)	Personal admission threshold (mean + 2 σ)	Current SFOSI value	
1	**********	«--------«	«--------«	1145	682	2505	1953	Admission
2	**********	«--------«	«--------«	3241	1498	6237	7320	Non-admission

Table 4. Integral indicator of visual-motor reaction tests, RHIC patients (n = 24)

Integral indicator of visual-motor reaction tests. Indicator values in %					
Before RHIC		After RHIC			
Abovenorm		Norm		Without changes	
abs.	%	abs.	%	abs.	%
24	100	21	87,5	3	12,5

systems [3]. These concepts state that adaptation, through structural and functional changes, leads to development of a system functioning to support the body's activities.

Adaptation is a multilevel process. The levels of adaptation are interrelated, have a direct impact on each other and determine the integral characteristic of the general level of functioning of all systems of the body, or the functional state of a person [1].

The functional state of a person is considered as a process reflecting the interaction of levels of adaptation. This is the integral indicator of psychophysiological adaptation. The existing concepts have the level of psychophysiological adaptation determined by the structural and functional formations of CNS, which, combined, shape its FS [1, 2]. Determination of the FS of CNS and the functional activity of its structural and functional formations is an important objective pursued by PPE of NF personnel.

Psychophysiological examination based on the presented concept allows determining FS of CNS, level of psychophysiological adaptation, assess the state of individual structural and functional formations of CNS ("Cortex", "Cortical-subcortical interaction", "Cardiovascular system central regulation"). Considered cumulatively, this information allows prescribing, if necessary, targeted and personalized rehabilitation and health-improving courses, and evaluate the results thereof afterwards. Data on the "Cortical-subcortical interaction" SFF enables PPE during pre-shift checkup and allows quick and accurate evaluation of the FS of CNS of the examined persons.

CONCLUSION

Introduction of a common methodological approach to PPE and a unified H&S set to the NF employee medical care system significantly expands its diagnostic and preventive capabilities, enabling early detection of functional disorders of CNS, psychophysiological contraindications for work, timely interventions with RHIC and objective assessment of the results thereof. This helps to reduce the risk of human error accidents and extends professional longevity of NF personnel.

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EPIDEMIOLOGICAL CHARACTERISTICS OF COMMUNITY-ACQUIRED PNEUMONIA DURING THE COVID-19 EPIDEMIC IN THE RUSSIAN FEDERATION

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COVID-19 belongs to the group of acute respiratory infections and it is often complicated with pneumonia. This study aimed to investigate manifestations of community-acquired pneumonia (CAP) epidemic process during the COVID-19 epidemic in the Russian Federation. We analyzed the official statistical data reporting the incidence of CAP in the Russian Federation in 2013–2020 and incidence of COVID-19 as registered in March–July 2020. The mean average annual CAP incidence rate that we calculated and the 2020 CAP incidence prediction allowed assessing the relationship between CAP and COVID-19. It is shown that the long-term dynamics of the incidence of CAP in the Russian Federation is characterized by a pronounced upward trend with an average annual growth rate of 6.4%. The share of adult population among the CAP cases is the largest; on average, it is 64.7% (95% CI [63.1; 66.3]). In 2020, against the background of SARS-CoV-2 circulation, the discrepancy between the actual incidence of CAP and the predicted figures reached and exceeded 558% (in July 2020). As the COVID-19 epidemic developed, the incidence of CAP was registered to increase. There was established a direct and significant correlation between the incidence of CAP and COVID-19 (r_{xy} = 0.932; p <0.01).

Keywords: community–acquired pneumonia, epidemiological characteristics, COVID-19, SARS-CoV-2, correlation, coronavirus, coronavirus disease

Author contribution: all authors significantly contributed to the research methodology design, data collection, analysis and interpretation. All authors participated in the manuscript drafting and editing processes and preparation of the final version of the article.

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ПРОЯВЛЕНИЯ ЭПИДЕМИЧЕСКОГО ПРОЦЕССА ВНЕБОЛЬНИЧНЫХ ПНЕВМОНИЙ В ПЕРИОД ЭПИДЕМИИ COVID-19 НА ТЕРРИТОРИИ РОССИЙСКОЙ ФЕДЕРАЦИИ

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COVID-19 относится к группе острых респираторных инфекций и зачастую осложняется развитием пневмоний. Целью исследования было изучить проявления эпидемического процесса внебольничных пневмоний (ВП) в период эпидемии COVID-19 на территории Российской Федерации. Проводили анализ официальных статистических форм по заболеваемости ВП в РФ за 2013–2020 гг. и данных о заболеваемости COVID-19 за март–июль 2020 г. Рассчитывали среднегодовые показатели заболеваемости ВП, прогностические уровни на 2020 г. и оценивали взаимосвязь заболеваемости ВП и COVID-19. Показано, что многолетнюю динамику заболеваемости ВП населения РФ характеризует выраженная тенденция к повышению со среднегодовым темпом прироста 6,4%. Наибольшую долю среди заболевших ВП составляет взрослое население в среднем 64,7% (95% ДИ [63,1; 66,3]). В 2020 г. на фоне циркуляции SARS-CoV-2 расхождение фактической заболеваемости ВП от прогнозируемой достигало более 558% (июль 2020 г.). В условиях развития эпидемии COVID-19 было отмечено повышение заболеваемости ВП. Установлена прямая статистически значимая корреляционная связь между заболеваемостью ВП и COVID-19 (r_{xy} = 0,932; p < 0,01).

Ключевые слова: внебольничная пневмония, эпидемиологическая характеристика, COVID-19, SARS-CoV-2, корреляция, коронавирус, коронавирусная инфекция

Вклад авторов: все авторы внесли значимый вклад в разработку методики исследования, получение, анализ и интерпретацию данных. Участвовали в подготовке черновика рукописи и ее редактировании, а также в подготовке финального варианта статьи.

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In December 2019, an outbreak of a new respiratory infection was registered in the Chinese city of Wuhan. This infection was accompanied with an increase in the number of patients with pneumonia of unknown etiology. In a relatively short time, the outbreak became a pandemic. The patients exhibited symptoms of an upper respiratory tract infection: sore throat and rhinorrhea, as well as fever, cough, myalgia, shortness of breath, and signs of pneumonia visible on the chest x-ray pictures. Subsequently, it was established that the causative agent of this infection is a new coronavirus, dubbed SARS-CoV-2. The disease that followed was named COVID-19 [1].

As part of a large-scale study conducted in China, the researchers analyzed data describing the course of the disease in 1099 patients with laboratory-confirmed COVID-19 diagnosis. It was established that the majority of hospitalized patients (91.1%) were diagnosed with pneumonia [2].

In Russia, the first cases of COVID-19 were diagnosed in February 2020. The infected were citizens of the PRC. By early July, the number cases registered and reported has grown to over 650,000 [3–5].

Every year, there are 1.5 million community-acquired pneumonia (CAP) cases registered in Russia, which translates into approximately 390 cases per 100,000 people.

The average mortality rate is up to 5% of the number of cases [6].

According to epidemiological studies carried out in a number of foreign countries, the incidence rate of CAP varies depending on age, reaching the minimum in young and middle-aged populations (1–11.6 cases per 1000 people). In children under 17, the incidence of CAP ranges from 2 to 15 cases per 1000 people in different years. The group most susceptible to CAP is comprised of the elderly people, over 70 years of age: annually 25–44 cases per 1000 people [7, 8].

The situation with CAP incidence in USA is also alarming. There are 5–6 million CAP cases registered there annually, with 1.5 million of them requiring inpatient treatment [9, 10].

In recent years, the number of deaths from pneumonia has increased. According to the American Thoracic Society, for 18–20% of the total number of CAP patients the disease ends in death [11].

The outcome of CAP depends on a number of various risk factors, which, when exposed to, increase the likelihood of death. Of great importance are the patient's age, clinical form and severity of the disease, comorbidities [12].

According to the research data, young and middle-aged patients with mild and moderate clinical forms of CAP and without concomitant pathologies recover well; for these age groups, the mortality rate is 1–3% [13].

In elderly patients that endure CAP in its severe form and have upper respiratory tract comorbidities, cancers, cardiological diseases, alcoholism in the background, the mortality rate rises up to 15–58% [14].

At the same time, it has been shown that CAP becomes more common when influenza and acute respiratory viral infections (ARVI) are on the rise, and the highest mortality from CAP is recorded 1–2 months after the peaks of influenza and ARVI epidemics [15].

Since COVID-19 is also an acute respiratory infection, it will be relevant to study epidemic features of CAP during the COVID-19 epidemic.

This study aimed to investigate manifestations of community-acquired pneumonia (CAP) epidemic process during the COVID-19 epidemic in the Russian Federation.

METHODS

The long-term dynamics of CAP incidence in the Russian Federation was analyzed in the context of a descriptive retrospective epidemiological study relying on the data collected with the Federal Statistical Observation Form #2 "Information on Infectious and Parasitic Diseases" (hereinafter — Form #2) in 2013–2018.

Inside a year, the CAP incidence dynamics analysis and the calculation of the seasonal incidence level in Russia relied on the data collected with Form #1 in 2013–2019.

The 2019 incidence rate analysis made use of the data collected with the Federal Statistical Observation Form #1 "Information on Infectious and Parasitic Diseases (monthly)" that covered January–December 2019 (hereinafter — Form #1). We established the yearly CAP incidence level and calculated the prognostic incidence rate for the coming period.

To analyze the incidence of COVID-19, we relied on the official information on the number of cases registered in the Russian Federation [16]. Form #1 data for January–July 2020 allowed assessing the incidence of CAP against the background of the COVID-19 epidemic.

To assess the differences in relative indicators, we calculated the 95% CI ($m \pm 2.45 \times SEM$, where m is the mean incidence over the period, SEM is the standard error of the mean). The differences were considered statistically significant at $p < 0.05$. The least square method enabled calculation of the long-term CAP incidence dynamics, which was assessed by the average annual increase/decrease rate. We compared the value obtained with the gradation suggested by V.D. Belyakov [17]. To assess the relationship between the incidence of CAP and COVID-19, we established the Pearson correlation coefficient (r_{xy}). The relationship was considered statistically significant at $p < 0.05$, with Chaddock's scale used to identify the strength of the relationship.

Microsoft Excel 2013 (Microsoft; USA) application was used to process and analyze the data obtained.

RESULTS

The analysis of structure of infectious and parasitic disease cases registered in 2013–2019 revealed that in Russia, acute infections of the upper respiratory tract of multiple and unspecified localization (ICD-10 code: J06) (ARI) are the most common diseases, with their share averaging at 90.7% within the period. The share of CAP is 1.7%, and influenza accounts for 0.6% of cases (Fig. 1).

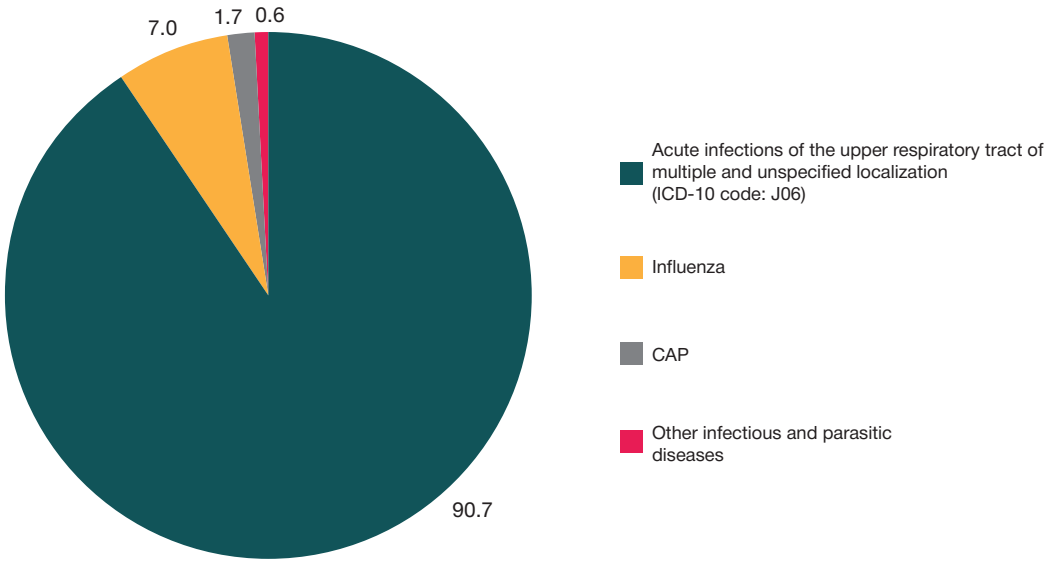

Fig. 1. Structure of infectious and parasitic diseases in the Russian Federation, mean, in %, years 2013–2019

Table 1. CAP incidence rates in the Russian Federation, years 2013–2019

Observation year	Total cases (abs. terms)	Incidence per 100 thousand people	Including children under 17		Including children under 14	
			Total cases (abs. terms)	Incidence per 100 thousand people	Total cases (abs. terms)	Incidence per 100 thousand people
2013	557,346	389.2	190,720	713.9	176,146	782.4
2014	509,765	349.5	182,014	660.6	172,278	734.4
2015	492,458	337.1	165,155	588.5	157,159	653.1
2016	612,012	418	197,594	688.8	187,549	759.7
2017	604,771	412.3	215,980	737.3	201,443	797.3
2018	721,987	491.7	270,495	908.4	247,113	962.2
2019	760,074	517.2	291,064	976.8	265,861	1034.6
Long-time average annual	608,345 (95% CI [514,432; 702,257])	416.4 (95% CI [353.9; 479])	216,146 (95% CI [172,511; 259,781])	753.4 (95% CI [624.9; 882.1])	201,078 (95% CI [163,503; 238,654])	817.7 (95% CI [694; 941.3])

The upward trend with the annual growth rate (AGR) of 6.4% was characteristic for CAP incidence in Russia in 2013–2019. Every year, there are 492–760 thousand new cases of the disease registered among the overall country's population, including 165–291 thousand cases in children under 17.

Children under 14 and up to 17, inclusively, have also exhibited a pronounced CAP incidence upward trend, with the AGR of 6.8% and 5.9%, respectively.

The 7-year analysis of CAP incidence in the Russian Federation showed that, on average, 608345 cases of CAP are registered annually among the adult population (the incidence rate is 416.4 per 100 thousand population), of which 216146 cases are in children under 17 years of age inclusively (753.4 per 100 thousand), including 201,078 cases (817.7 per 100 thousand of the population) in children under 14 years of age (Table 1).

In general, the CAP incidence in 2019 was 5.3% greater than in 2018. For children, the trend is the same, with the growth at 7.6%.

In the Russian Federation, within the period analyzed 64.7% (95% CI [63.1; 66.3]) of the CAP cases were registered among adults, 2.4% — among children aged 15–17 (95% CI [1.9; 2.9]) and 32.9% — in children under 14 inclusively (95% CI [31.8; 34.1]).

Analysis of the long-term dynamics of CAP incidence reveals a steady growth of the level thereof (approximation confidence factor R^2 = 0.72), with additional 26.5 cases per 100 thousand people registered every year. These indicators considered, the estimated 2020 CAP incidence rate in the overall population is 522.6 cases per 100 thousand people (95% CI [388.2; 657.1]) (Fig. 2).

Analyzing the yearly CAP incidence data of 2013–2019, we established seasonal character of the disease: about 70% of all the cases registered annually belong to autumn and winter periods.

The level of year-round and seasonal incidence of CAP in the overall population of the Russian Federation within the investigated period is 39.2 and 43.8 cases per 100 thousand people, respectively (Fig. 3). The obtained indicators allow determining when the incidence starts and ends rising, as well as to establish favorable and unfavorable periods.

Thus, in 2019, within the periods from January to April and from October to December population of the Russian Federation contracted CAP at a greater scale than on average throughout the year. As for the seasonal incidence levels, they are exceeded in January–March and October–December periods.

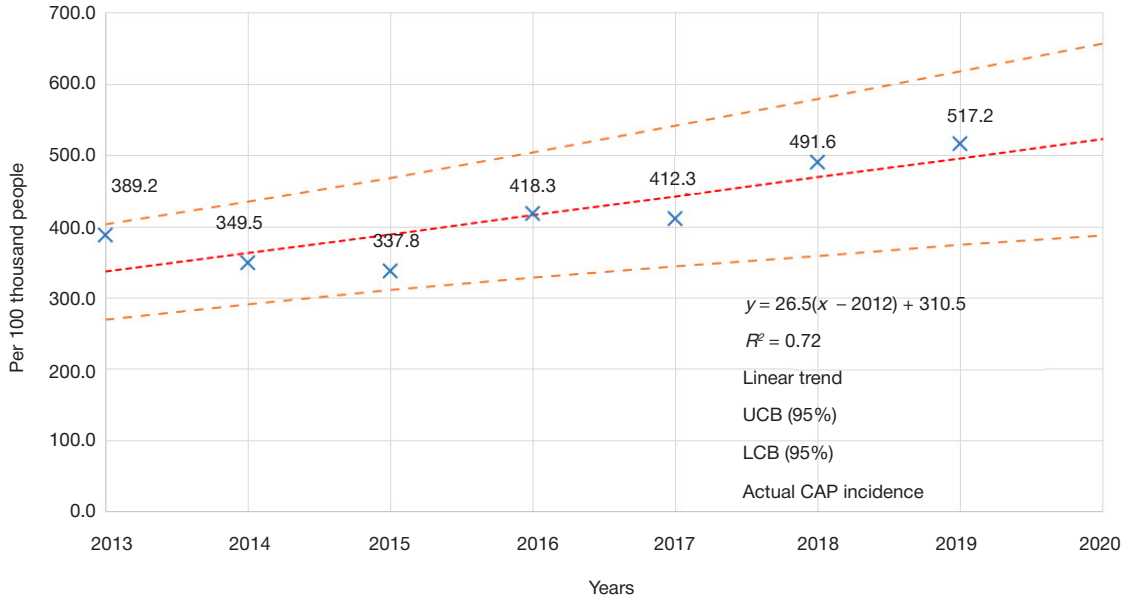

Fig. 2. Long-term dynamics of the incidence of CAP in the total population of the Russian Federation, per 100 thousand people, years 2013–2019

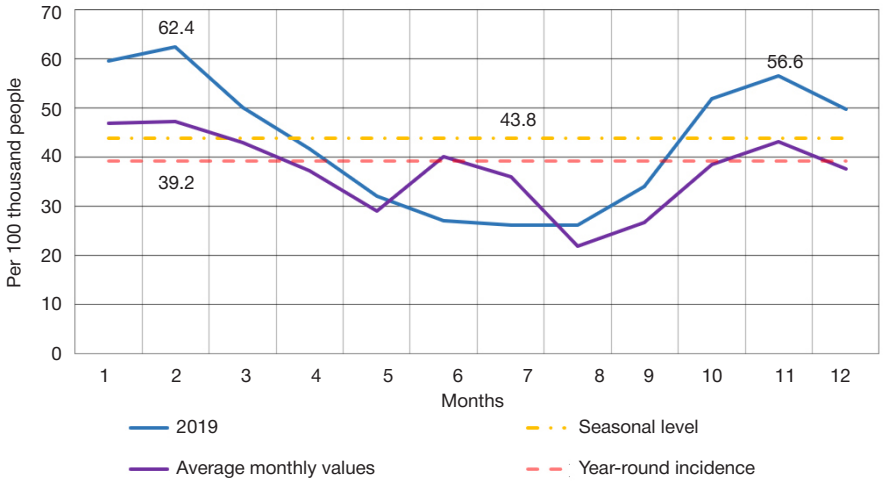

Fig. 3. Intra-annual dynamics of the incidence of CAP in the total population of the Russian Federation, per 100 thousand people

The analysis of seasonal manifestations revealed a more pronounced epidemiological stress peculiar to the first half of 2019 compared to the second part of the year.

In 2019, CAP incidence peaked in February, when it amounted to 62.4 per 100 thousand people, exceeding the year-round and seasonal levels by 71% and 42.5%, respectively.

Based on the actual CAP incidence recorded in 2013–2019, we calculated the prognostic level of monthly incidence for 2020. The lowest 2020 CAP incidence level in Russia (overall population) is forecast for July and August, with the figures being 20.7 and 21 cases per 100 thousand people, respectively. The peak is expected in in January, February and November of the year, with 46.2 and 40.0 cases per 100 thousand people, respectively.

The 2020 incidence forecast should approach the average rate recorded in the 2013–2019 period; the expected match value is 89.9% (± 9.6%; $p < 0.05$).

At the same time, against the background of SARS-CoV-2, 2020 saw a statistically significant ($p < 0.05$) discrepancy between the actual CAP incidence and the predicted level: in February, the gap reached 27.9%, and in July it has grown to 558.5% (Fig. 4).

With the epidemic spread of the new coronavirus infection (COVID-19) in Russia, from the scientific and practical viewpoints it is particularly interesting to study the results of the analysis comparing January–July 2020 CAP incidence data to the figures recorded during January–July 2019, when the COVID-19 epidemic was on the rise.

Within the period from January to July 2020, the incidence of CAP in the population of the Russian Federation increased by 125.2% compared to the same period of 2019, and reached 673.9 cases per 100 thousand people (Table 2).

Considered on the level of Federal Districts (FD), the greatest CAP incidence growth during the period analyzed (January-July), compared to the same period of the previous year, was registered in the Central FD (+282.4%) and the North Caucasian FD (+254%). In absolute terms, it is 278089 and 41203 cases, respectively.

At the same time, in the Far Eastern FD the CAP incidence dropped insignificantly by –4.1%.

From March to May 2020, the incidence of COVID-19 on the territory of the Russian Federation was growing steadily. In January and February, there were no COVID-19 cases registered. The most significant increase was recorded in May 2020, when the incidence grew 2.7 times compared to April 2020 (from 75.7 cases to 203.6 cases per 100 thousand people, respectively).

It should be noted that the analysis of relationship between CAP and COVID-19 incidence in the population of the Russian Federation within the period from January to July 2020 allowed us to establish a direct, very high and statistically significant link between these indicators (Pearson's coefficient $r_{xy} = 0.932$; $t = 5.731$; $p < 0,01$).

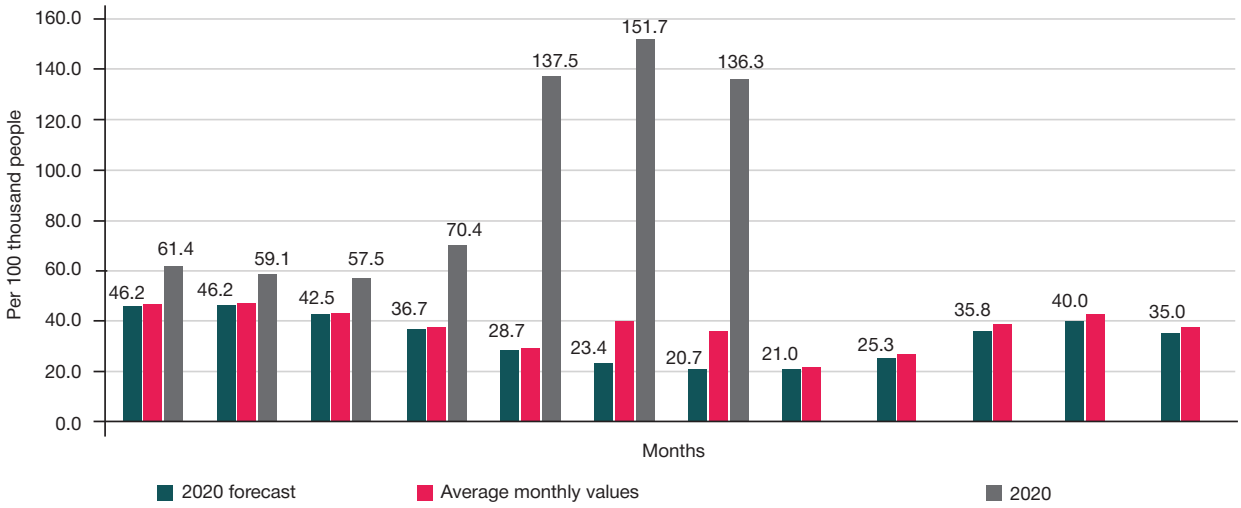

Fig. 4. Predicted and actual intra-annual incidence of CAP in the population of the Russian Federation, per 100 thousand people

Table 2. CAP incidence in the population of the Russian Federation by federal districts, per 100 thousand people, January–July 2019 and 2020, with COVID-19 epidemic in the background

Territory	CAP in 2020	CAP in 2019	Increase/decrease	COVID-19
Russian Federation	673.9	299.2	125.2%	574.4
Central FD	956.4	250.1	282.4%	1026.9
Northwestern FD	666.5	262.4	154.0%	554.8
Southern FD	355.8	221	61.0%	254.3
North Caucasian FD	582.2	164.5	254.0%	387.5
Volga FD	669.1	333.9	100.4%	349.1
Ural RD	583.7	406.2	43.7%	539.8
Siberian FD	488.2	327	49.3%	427.5
Far Eastern FD	622.5	649.1	–4.1%	473.7

DISCUSSION

The results of the study allowed investigating manifestations of the CAP epidemic process before and during the COVID-19 epidemic. Given that COVID-19 is a new infectious disease, most studies cover clinical manifestations of this infection [18, 19].

CONCLUSION

1. The long-term dynamics of CAP incidence in Russia shows a pronounced upward trend, which is seen in both the overall population (AGR = 6.4%) and among children (AGR = 6.8%). 2. Within the period analyzed, the majority of cases were adults (on average, 64.7% of the registered CAP cases). 3. In the period from 2013 to 2019, the year-round CAP incidence rate in the overall population of Russia is 39.2 cases per 100 thousand people, and the seasonal level is 43.8 cases per 100 thousand people. 4. In 2020, against the background of SARS-CoV-2 circulation, the discrepancy between the actual incidence of CAP and the predicted incidence value reached over 558% (July 2020), which indicates an increase in the incidence of CAP during the COVID-19 epidemic. 5. A direct, statistically significant correlation between the incidence of CAP and COVID-19 reveals the relationship between the development of the epidemic process of these infections.

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EVALUATION OF ANTITUMOR ACTIVITY OF SOME 4-AMINOPIPERIDINE DERIVATIVES — LOW MOLECULAR WEIGHT HSP70 INHIBITORS — ON TRANSPLANTABLE MOUSE TUMORS

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Low molecular weight compounds targeting chaperone proteins Hsp90 and Hsp70 have opened up a new avenue in the therapy of neoplasms. In 2020, we tested 3 Hsp70 inhibitors from the class of 4-aminopiperidine derivatives for their antitumor activity on *in vivo* models. The list of the tested compounds included N-(2-chlorobenzyl)-N-ethyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (compound 1), 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino)methyl) benzonitrile (compound 2) and N-(2,6- dichlorobenzyl)-1-(1-(2-(ethylthio)pyrimidin-4-yl)piperidin-4-yl)-N-methylmethaneamine (compound 3). The aim of this study was to compare the efficacy of 4-aminopiperidine derivatives *in vivo* using the models of transplantable murine L1210 lymphocytic leukemia and B16 melanoma. Compounds 2 and 3 used in combination with cyclophosphamide exhibited high cytotoxic activity ($p = 0.05$) against L1210 leukemia (an 80-82% increase in survival time) and B16 melanoma (98-99.7% tumor growth delay). For L1210 lymphocytic leukemia, compounds 2 and 3 used in combination with cyclophosphamide fell into the low (+) therapeutic potential category. For B16 melanoma, compounds 1, 2 and 3 used in combination with cyclophosphamide fell into either low (+) or moderate (++) therapeutic potential categories. On the whole, the tested doses of the compounds used in combination with cyclophosphamide hold promise for the therapy of L1210 leukemia and B16 melanoma in mouse models. Our findings confirm the potential of low molecular weight Hsp70 inhibitors for combination chemotherapy against cancer.

Keywords: heat shock proteins, Hsp70 inhibitors, transplantable tumor, L1210 leukemia, B16 melanoma

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Author contribution: Aldobaev VN planned the experiment, summarized its results and wrote this manuscript; Mikhina LV carried out the experiment in animal models; Present MA synthesized the tested compounds.

Compliance with ethical standards: the study was approved by the Ethics Committee of the Research Centre for Toxicology and Hygienic Regulation of Biopreparations (Protocol № 695 dated November 12, 2019). Housing conditions met the requirements of Sanitary Regulations 2.2.1.3218-14 (*Sanitary and Epidemiological Requirements for Design, Equipment and Maintenance of Vivarium Facilities*) and the guidelines provided in the *Guide for Care and Use of Laboratory Animals* (ILAR publication, 1996, National Academy Press, USA).

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ОЦЕНКА ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ РЯДА ПРОИЗВОДНЫХ 4-АМИНОПИПЕРИДИНА, НИЗКОМОЛЕКУЛЯРНЫХ ИНГИБИТОРОВ HSP70, НА ПЕРЕВИВАЕМЫХ ОПУХОЛЯХ МЫШЕЙ

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Применение низкомолекулярных агентов, мишенью которых являются молекулярные шапероны Hsp90 и Hsp70, стало основой для целого направления в терапии новообразований. В 2020 г. была проведена сравнительная оценка противоопухолевой активности на моделях *in vivo* трех производных 4-аминопиперидина ингибиторов Hsp70: N-(2-хлоробензил)-N-этил-1-(2-(метилтио)пиримидин-4-ил)пиперидин-4-амина (№ 1); 4-((метил(1-(2-(метилтио)пиримидин-4-ил)пиперидин-4-ил)амино)метил) бензонитрила (№ 2); N-(2,6-дихлорбензил)-1-(1-(2-(этилтио)пиримидин-4-ил)пиперидин-4-ил)-N-метилметанамина (№ 3). Целью работы было провести сравнительные испытания эффективности производных 4-аминопиперидина *in vivo* на перевиваемых опухолях мышей. Противоопухолевую активность исследуемых веществ изучали на моделях лимфоидной лейкемии L1210 и меланомы B16. Субстанции № 2 и 3 продемонстрировали высокую статистически значимую ($p = 0,05$) активность в случае комбинированной терапии с циклофосфамидом для лейкоза L1210 (увеличение продолжительности жизни — 80–82%) и для меланомы B16 (торможение роста опухоли — 98–99,7%). В случае L1210 вещества № 2 и 3 в комбинации с цитостатиком попали в низшую категорию перспективности «+» для модельных лейкозов животных. В случае B16 вещества № 1–3 в комбинации с цитостатиком попадали либо в низшую категорию перспективности «+», либо в категорию «++» для модельных солидных опухолей животных. Испытанные дозировки субстанций продемонстрировали обещающие результаты лечения в комбинации с циклофосфамидом на перевиваемых опухолях лимфоидной лейкемии L1210 и меланомы B16 мышей. Полученные эффекты подтверждают перспективность применения низкомолекулярных ингибиторов Hsp70 в составе комбинированной химиотерапии в онкологии.

Ключевые слова: белки теплового шока, ингибиторы Hsp70, перевиваемая опухоль, лейкемия L1210, меланома B16

Финансирование: государственное задание ФМБА № 22.001.18.800.

Вклад авторов: В. Н. Алдобаев — написание статьи, обобщение результатов, общее планирование работ; Л. В. Михина — обеспечение экспериментов на животных-опухоленосителях, отработка моделей; М. А. Презент — синтез субстанций для сравнительных испытаний.

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Low molecular weight compounds targeting molecular chaperones like Hsp90 and Hsp70 have opened up a new avenue in the therapy of neoplasms. Heat shock proteins Hsp90 and Hsp70 are overexpressed in many tumors, which explains selective accumulation of Hsp90 inhibitors in tumor tissue [1]. Inhibited expression and/or reduced functional activity of heat shock proteins result in the accumulation of damaged, partially denatured, functionally altered proteins in the cell. It is hypothesized that Hsp90 and Hsp70 might enhance the anticancer effect of cytotoxic drugs and help to overcome drug resistance when used in combination with chemotherapy agents. Some recent publications [2–4] discuss the synthesis of Hsp70 inhibitors, the small molecules designed by means of molecular docking. An article [4] describes the synthesis of 67 candidate Hsp70 inhibitors from the class of 4-aminopiperidine derivatives [4], whose activity was tested on cell cultures in vitro. The article provides information on the kinetic rate constants for each compound measured by surface plasmon resonance and evaluates the inhibitory effect of the synthesized compounds on Hsp70 ATPase activity. Another publication [5] describes an alternative technique for the synthesis of some of the 4-aminopiperidine derivatives from [4], including 1-(2-alkylthiopyrimidin-4-yl)piperidin-4-N-alkyl,N-hetaryl/aryl amines. The proposed technique allowed us to obtain larger combinatorial libraries for further screening tests on cell cultures and the subsequent optimization of candidate cytotoxic drugs.

In 2018–2019, our team synthesized a collection of 4-aminopiperidine derivatives, which partially overlapped with the collection described in [4] and performed an *in vitro* screening test of their cytotoxic activity on cell cultures. Three 4-aminopiperidine derivatives were selected for further in vivo tests on animal tumor models. The antitumor activity of the synthesized compounds was studied on transplantable mouse lymphocytic leukemia (L1210) and solid melanoma tumors (B16). Cyclophosphamide was used as a positive control following recommendations in [8] and as a treatment against induced cancers.

METHODS

Laboratory animals

The tumors were maintained in female C_{57}Bl/6 and DBA/2 mice. The specific activity of the synthesized compounds was assessed *in vivo* on inoculated female hybrid BDF1 mice (C_{57}Bl/6 × DBA/2). At the beginning of the study, the mice weighted 20 to 30 g. The animals were purchased from the Research Center for Biomedical Technology, FMBA (the branch of Stolbovaya breeding nursery, Moscow region). The animals were housed in a conventional room in the absence of other animal species.

The mice were kept in 26 × 17 × 12 cm^3 polycarbonate cages (Tecniplast; Italy) at 6 animals per cage. The cages were fitted with stainless-steel wire lids and in-built feed hoppers, a stainless-steel divider to separate the food zone, and label holders. The cages were arranged on stainless steel racks (Tecniplast; Italy).

Wood chips were used as a bedding (Laboratorkorm; Russia); the bedding was 5–10 mm thick.

The animals were allowed ad libitum access to a standard chow diet (Kombikorm PK-120 for laboratory rats, mice and hamsters by Laboratorkorm; Russia). The food was supplied via a food hopper.

The animals also had ad libitum access to filtered tap water supplied in standard drinking bottles with steel caps and sipper tubes. Water quality satisfied Sanitary Regulations 2.1.4.1074-01 (updated on April 2, 2018). Filtering was necessary to avoid water contamination that could have affected the results of the study.

The mice were housed under artificial 12 : 12 light-dark conditions in a controlled environment at 20–26 °C and 30–70% air humidity. The temperature and humidity in the room were maintained by an automated climate control system. The air exchange rate was 15 air changes per hour. Before the experiment, the animals were quarantined for 14 days.

Maintenance of transplantable tumors

The antitumor activity of the synthesized compounds was studied *in vivo* using the transplantable murine lymphocytic leukemia (L1210) and solid B16 melanoma models.

Cancer cell lines were provided by Blokhin Cancer Research Center (Russia), where they had been cryopreserved in 1 ml ampoules and stored in liquid nitrogen. The cells were shipped in liquid nitrogen. Protocols used to recover the cryopreserved cells were the same for both cancer cell lines. Briefly, the ampoules were retrieved from liquid nitrogen and left in an incubator at 37 °C for 30 min. After that, 0.5 ml of the cell suspension was administered to each animal. Lymphocytic leukemia L1210 was maintained in DBA/2 mice, B16 melanoma was maintained in C_{57}Bl/6 mice. The neoplasms were maintained by inoculation. To maintain L1210 lymphocytic leukemia, intact mice were inoculated intraperitoneally with 0.3 ml of L1210 ascitic fluid derived from hosts on days 5 or 6 and diluted with normal saline 1 : 60. To keep B16 melanoma viable, intact mice were subcutaneously inoculated with 0.5 ml of B16 melanoma preparation derived from hosts on day 16–20 (1 g of the tumor was homogenized in 10 ml of normal saline).

Treatment

Lymphocytic leukemia and solid melanoma cells were transplanted to female BDF1 mice (C_{57}Bl/6 × DBA/2) using the same protocol as for tumor maintenance. For inoculations, we used cells that had undergone at least 2 passages in mice after thawing. Therapy against L1210 lymphocytic leukemia was initiated 24 h after inoculation; therapy against B16 melanoma was initiated 48 h after inoculation [6]. As part of the experiment, we determined effective cyclophosphamide doses and regimens against the induced murine cancers. The choice of cyclophosphamide as a positive control for the L1210 model was dictated by the results of our previous study [7]. With the melanoma model, the choice of cyclophosphamide was based on our practical experience. Mice inoculated with L1210 cells received IM injections of 50 mg/kg cyclophosphamide twice, 24 h and 72 h after inoculation. This treatment regimen allowed us to prolong survival by an average of 31–43%, as compared with the negative control group (NC). For mice inoculated with L1210 lymphocytic leukemia cells, survival times ranged from 2 to 3 weeks.

Mice inoculated with B16 melanoma cells received 3 IM injections of 80 mg/kg cyclophosphamide on days 2, 5 and 9 after inoculation. This regimen resulted in 62–100% tumor growth delay during the observation period (1 month) and prolonged survival by 10–23%, as compared with the NC group. For mice inoculated with B16 melanoma cells, survival times ranged from 4 to 5 weeks.

For the experiment, 4-aminopiperidine derivatives were formulated as water-soluble hydrochlorides. Mice with lymphocytic leukemia received daily injections of

Table 1. Primary tests of antitumor activity in the murine L1210 model

Group	Number of animals in the group	Treatment	Dosage, mg/kg	Total number of injections
1	6	No treatment (NC)	–	–
2	6	Cyclophosphamide (PC)	50	2
3	6	Compound 1 + Cyclophosphamide	200 50	7 2
4	6	Compound 1	200	7
5	6	Compound 2 + Cyclophosphamide	150 50	7 2
6	6	Compound 2	150	7
7	6	Compound 3 + Cyclophosphamide	250 50	7
8	6	Compound 3	250	7

Note: NC — negative control; PC — positive control.

4-aminopiperidine derivatives for 7 days; the first injection was administered the day after inoculation. Mice with B16 melanoma received daily intraperitoneal injections of the synthesized compounds for 10 days; the first injection was administered 48 h after inoculation. The formulations were prepared in a laminar flow cabinet using a ready-to-use sterile normal saline solution.

Statistical analysis

The efficacy of treatment was evaluated relative to the outcomes in the NC group (inoculated mice, no treatment received). We compared the increase in the survival time, tumor growth delay and a related T/C parameter [6]. The increase in survival time and tumor growth delay were calculated from the average tumor volume at a specific point in time after inoculation (for B16 melanoma) and the survival time within the experiment (for both cancers). Differences between the studied parameters were measured using the approach of a function or several random variables [7]. Mean squared deviations (MSD) were calculated for the tumor volume at a specific point in time after inoculation (for B16 melanoma) and survival time within the observation period (both cancers). Based on mean values, MSDs and sample sizes (the number of animals in the groups), mathematical expectations (ME), 95% CI for tumor growth delay (TGD) and survival time increase (STI) were calculated using a code written in *Mathematica 9* [7].

RESULTS

Efficacy of synthesized 4-aminopiperidine derivatives in L1210 lymphocytic leukemia model

Prior to evaluating the efficacy of N-(2-chlorobenzyl)-N-ethyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (compound 1), 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino)methyl) benzonitrile (compound 2) and N-(2,6-dichlorobenzyl)-1-(1-(2-(ethylthio)pyrimidin-4-yl)piperidin-4-yl)-N-methylmethaneamine (compound 3), we conducted a series of preliminary experiments to determine their maximum tolerated dose (MTD, single intraperitoneal injection) for BDF1 (C_{57}Bl/6 × DBA/2) hybrid mice. After the injection, the animals were closely monitored and their weight was measured daily for 7 days. Based on clinical observations and weight dynamics, MTDs for compounds 1, 2 and 3 were 250 mg/kg, 200 mg/kg and 300 mg/kg, respectively. At these doses, the tested compounds increased the heart rate, induced rapid breathing and provoked clonic or tonic seizures in most experimental animals. These symptoms resolved within 10–15 min after the

Table 2. Antitumor activity of 3 synthesized compounds administered intraperitoneally to mice with transplantable L1210

Group	Treatment	STI, %		T/C	
		I*	II	I	II
1	No treatment (NC)	–		–	
2	CPA** (PC)	31	43	131	143
		37 ***		137	
3	Compound 1 + CPA	60	71	160	171
		65,5		166	
4	Compound 1	10	17	110	117
		13,5		114	
5	Compound 2 + CPA	63	80	163	180
		71,5		172	
6	Compound 2	5	11	105	111
		8,0		108	
7	Compound 3 + CPA	72	82	172	182
		77,0		177	
8	Compound 3	0	5	100	105
		2,5		103	

Note: * — experiment number; ** — cyclophosphamide; *** — mean value.

Table 3. Mathematical expectations (ME) for STI and their 95% CI

Group	Treatment	I* ME for STI, % [CI] %	II ME for STI, % [CI] %
1	No treatment (NC)	–	–
2	CPA ** (PC)	32 [6; 66]	43 [29; 60]
3	Compound 1 + CPA	62 [23; 110]	71 [48; 98]
4	Compound 1	11 [–17; 46]	17 [–4; 40]
5	Compound 2 + CPA	64 [34; 104]	80 [62; 100]
6	Compound 2	6 [–21; 40]	11 [–7; 30]
7	Compound 3 + CPA	74 [37;121]	82 [61; 106]
8	Compound 3	5 [–25; 41]	11 [–16; 40]

Note: * — experiment number; ** — cyclophosphamide.

injection. During the first 2–3 days after the injection, half of the animals lost 2-8% of their weight. By the end of the observation period, all animals had gained weight.

Considering the obtained MTDs and the guidelines on primary antitumor activity testing [8], we selected the following dosing regimen for the animals inoculated with L1210 cells: compound 1 — 200 mg/kg, compound 2 — 150 mg/kg, compound 3 — 250 mg/kg. The compounds were administered intraperitoneally, daily, for 7 days. The first injection was administered 24 h after inoculation. The animals were divided into 8 groups (6 animals per group except for the NC group, which consisted of 8 animals). The NC group did not receive any treatment. The positive control (PC) group was treated with cyclophosphamide (Table 1).

The results generated by a series of 2 experiments conducted on the L1210 model are provided in Table 2. Ranges and mean values for STI and T/C were used as repeatability indicators.

As seen from Table 2, a relatively high increase in the survival time was achieved only when the tested doses of compounds 1, 2 and 3 were used in combination with cyclophosphamide.

For L1210 lymphocytic leukemia, compounds 2 and 3 used in combination with the cytotoxic drug fall into the low therapeutic potential category (designated as + ; T/C ≥ 175%) [6].

A potentiating effect was demonstrated by compounds 1, 2 and 3 used in combination with cyclophosphamide.

Table 3 shows mathematical expectations for STI and their 95% CI in the L1210 model.

Considering CI shown in Table 3, it can be concluded that differences in STI were significant ($p = 0.05$) between groups 2 and 5 and between groups 2 and 7 in experiment II.

Efficacy of synthesized 4-aminopiperidine derivatives in B16 melanoma model

Based on the results of antitumor activity tests conducted on the L1210 leukemia model, we selected the following dosing regimen for the B16 melanoma model: compound 1 — 200 mg/kg, compound 2 — 150 mg/kg, compound 3 — 250 mg/kg. The compounds were administered intraperitoneally, daily, over the course of 10 days. The first injection was administered 48 h after inoculation. The animals were divided into 8 groups (6 animals per group except for the NC group, which consisted of 8 animals). The NC group did not receive any treatment. The PC group was treated with cyclophosphamide (Table 4).

The results generated by a series of 2 experiments conducted on the B16 melanoma model are provided in Table 5. Ranges (STI and TGD) and mean values (TGD) were used as repeatability indicators.

As seen from Table 5, relatively high TGD levels were achieved only when the tested doses of compounds 1, 2 and 3 were used in combination with cyclophosphamide.

For solid B16 melanoma, compounds 1, 2 and 3 used in combination with the cytotoxic drug fall into the low therapeutic potential category (designated as +; TGD < 51–80%) or the

Table 4. Primary antitumor activity tests conducted on the B16 melanoma model

Group	Number of animals in the group	Treatment	Dose, mg/kg	Total number of injections
1	8	No treatment (NC)	–	–
2	6	Cyclophosphamide (PC)	80	3
3	6	Compound 1 + Cyclophosphamide	200 80	10 3
4	6	Compound 1	200	10
5	6	Compound 2 + Cyclophosphamide	150 80	10 3
6	6	Compound 2	150	10
7	6	Compound 3 + Cyclophosphamide	250 80	10 3
8	6	Compound 3	250	10

Table 5. Antitumor activity of 3 synthesized compounds administered intraperitoneally to mice with transplantable B16 melanoma measured in a series of 2 experiments

Group	Treatment	TGD, %								STI, %	
		Day 13		Day 21		Day 28		Day 33			
		I*	II	I	II	I	II	I	II	I	II
1	No treatment (NC)	–		–		–		–		–	
2	CPA* (PC)	77	87	62	83	62	78	31	65	18	23
		82***		72.5		70		48			
3	Compound 1 + CPA	71	93	67	86	52	76	40	66	–	
		82		76.5		64		53			
4	Compound 1	–		–		2	11	5	12	–	
		–		–		8.5		8.5			
5	Compound 2 + CPA	89	100	75	98	69	82	46	55	24	30
		94.5		86.5		75.5		50.5			
6	Compound 2	–		–		17	22	8	17	–	
		–		–		19.5		12.5			
7	Compound 3 + CPA	82	100	67	94	56	86	47	89	–	
		91		80.5		71		68			
8	Compound 3	13	21	–		15	28	12	18	–	
		17		–		21.5		15			

Note: * — experiment number; ** — cyclophosphamide; *** — mean value.

Table 6. Mathematical expectations (ME) for TGD, STI and their 95% CIs

Group	Treatment	ME for TGD, % [CI] %					ME for STI, % [CI] %
		Day 13		Day 21	Day 28	Day 33	
1	No treatment (NC)	–		–	–	–	–
2	CPA* (PC)	I*	82 [23; 99]	57 [–51; 115]	59 [16; 83]	24 [–51; 70]	22 [–24; 86]
		II	85 [55; 95]	80 [47; 94]	76 [50; 91]	63 [29; 82]	29 [–23; 112]
3	Compound 1 + CPA	I	78 [–41; 148]	62 [–14; 92]	49 [–12; 83]	35 [–46; 88]	–
		II	92 [75; 96]	84 [53; 99]	72 [38; 98]	64 [–23; 89]	–
4	Compound 1	I	–	–	–5 [–157; 96]	–3 [–122; 72]	–
		II	–	–	–3 [–97; 58]	5 [–82; 54]	–
5	Compound 2 + CPA	I	92 [68; 95]	71 [6; 104]	67 [16; 102]	40 [–19; 77]	28 [–13; 89]
		II	99 [95; 104]	98 [94; 99]	79 [57; 95]	51 [–3; 88]	19 [–10; 115]
6	Compound 2	I	–	–	11 [–108; 87]	0 [–110; 68]	–
		II	–	–	9 [–75; 65]	10 [–77; 63]	–
7	Compound 3 + CPA	I	86 [43; 96]	62 [–20;99]	55 [4; 84]	42 [–17; 77]	–
		II	99,7 [95; 104]	93 [82;97]	84 [67; 96]	88 [78; 94]	–
8	Compound 3	I	32 [–189; 95]	–	9 [–113; 86]	4 [–98; 66]	–
		II	7 [–183; 83]	–	16 [–72; 78]	11 [–66; 54]	–

Note: * — experiment number; ** — cyclophosphamide.

moderate therapeutic potential category (designated as ++; TGD < 81–90%) on days 13, 21 and 28 after inoculation [6].

In combination with cyclophosphamide, compounds 2 and 3 demonstrated the potentiating effect with respect to TGD on day 13; compounds 1, 2 and 3, on day 21; compound 3, on day 33; the additive effect was demonstrated by compound 1 on day 33, compound 2 on days 28 and 33 and compound 3 on day 28.

A significant increase in the average survival time was observed only in the groups undergoing therapy with cyclophosphamide or cyclophosphamide + compound 2.

This, along with the comparable efficacy of compounds 1 and 3 demonstrated on the B16 melanoma model (Table 4) and the lymphocytic leukemia model (Table 2), allowed us to single out compound 2 as the most promising candidate for further dose optimization and development of effective treatment regimens.

Mathematical expectations for TGD, STI and their 95% CIs are shown in Table 6.

Considering CI shown in Table 6, it can be concluded that differences in TGD were significant ($p = 0.05$) between groups 2 and 5 and between groups 2 and 7 on day 13; between groups 2 and 5 on day 21 in experiment II. In all other cases of combination therapy, TGD trended towards significance.

Based on the increase in TGD and survival time achieved by applying the combination regimen vs monotherapy with cyclophosphamide (see Tables 3 and 6), compound 2 was singled out as the most promising candidate for further research.

DISCUSSION

Hsp70 inhibitors are traditionally classified by their mechanism of action and structure. As a rule, Hsp70 inhibitors bind to the nucleotide-binding domain and block the interaction of other factors with the nucleotide-binding and substrate-binding Hsp70 domains [9–11]; these agents also inhibit Hsp70 ATPase activity (the mechanisms are not specified) [12–15], selectively suppress GRP78 [16–18], interact with the EEVD Hsp70 domain [19], disrupt the interaction between Hsp70 and BAG3 [20–22], etc. At the same time, Hsp inhibitors can be grouped into the following classes by their chemical structure: ATP analogues (Ver-155008) [9, 23], dihydropyridines (MAL3-101, DMT3132, NSC 630668-R/1) [14, 15, 24], flavonoids (epigallocatechin-3-gallate, quercetin) [25, 26], imidazoles (Apoptozole, Az-TPP-O3) [27, 28], phenylethylsulfonamides (Pifithrin-μ) [29, 30], rhodocyanines and their derivatives (YM-1, MKT-077, JG-98) [21, 31, 32], methylene blue [33] and some other compounds.

The compounds described in this article belong to the class of Hsp70 APTase activity inhibitors, but due to their chemical structure they have opened up a new avenue in the study of non-specific low molecular weight Hsp70 inhibitors. This study is a logical continuation of the study [4], which modeled chemical structures showing affinity for the ATP binding site of the Hsp70 molecule, described conditions for the synthesis of 4-aminopiperidine derivatives (potential Hsp70 inhibitors), analyzed their kinetic rate constants by means of surface plasmon resonance and demonstrated the inhibition of Hsp70 ATPase activity using a colorimetric test. In addition, the authors of the study screened the original collection of the synthesized compounds for their activity against 16 cancer cell lines and 2 human fibroblast cell lines. The most toxic compounds demonstrated LC_{50} in the range from 0.7 to 2.0 μM. In the acute toxicity test, one of the compounds orally administered to model mice was found to have LD_{50} of 870 mg/kg.

Summing up, the aim of the study was achieved: we successfully evaluated the Hsp70-inhibiting potential of 4-aminopiperidine derivatives using murine models of transplantable L1210 lymphocytic leukemia and solid B16 melanoma.

CONCLUSION

As expected, our preliminary experiments showed that high doses of the synthesized 4-aminopiperidine derivatives used in combination with cyclophosphamide hold promise as chemotherapeutic drugs.

It was shown that therapy with compounds 2 and 3 resulted in significant differences in treatment efficacy ($p = 0.05$) between the groups that received combination therapy and monotherapy with cyclophosphamide. Specifically, combination therapy resulted in longer survival times in the groups with transplantable L1210 leukemia and in significant tumor growth delay on days 13 and 21 after inoculation in the groups with B16 melanoma.

Based on the obtained data, the most active compound 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino) methyl) benzonitrile formulated as a hydrochloride was selected for further optimization of dosing regimens and administration routes.

The strength of the sytotoxic effect observed in this study confirms the promise of low molecular weight Hsp inhibitors for combination therapy of cancer.

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ASSESSMENT OF HEALTH RISK BY WIND CHILL FACTOR IN THE KRASNOYARSK KRAI

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Wind affects functional state and health of human beings. Physical activity mitigates the risk of hypothermia, but not the discomfort felt in cold winds. Moreover, there appears a risk of body cooling and frostbite. This study aimed to assess the risk to health of a human being associated with the wind chill factor index in the various climatic zones of a Russian region. The calculation relied on the mean monthly daily temperature and wind speed values, minimum temperature and maximum wind values registered in the subarctic and continental climate zones during the two climatological normals determination observation periods, 1961-1990 (second period) and 1991–2020 (third period). In the third period, a significant decrease in wind strength was registered in the subarctic (8 months) and temperate continental (9 months) climates. The mean monthly temperatures increased in April by 3.5 °C (p = 0.006), April–June by 4.05 °C (p = 0.001) and 3.9 °C (p = 0.001). The maximum wind in the subarctic climate did not change, in the temperate continental zone it decreased within 9 months; the minimum temperature increased in 4 and 1 months. In the subarctic zone, the mean temperature and wind values made the ambient conditions uncomfortable for 6 months (versus 7), with one characterized as "extremely cold"; the cold exposure risk decreased during the "very cold" period; in the temperate climate zone, the potentially uncomfortable conditions period lasted for 4 months (versus 6). With wind at the maximum and temperature at the minimum, in the subarctic climate, the weather remained severe for 8 months a year in each of the determination periods ("uncomfortable, chilly" — 2 months, "cold, skin surface hypothermia" — 1 month, "extremely cold, possible hypothermia of the exposed parts of the body in 10 minutes" — 5 months); in the temperate continental climate zone, it was severe for 5 months of each year ("uncomfortable, chilly" — 2 months, "cold, skin surface hypothermia" — 3 month).

Keywords: wind chill factor; subarctic, continental climate; Krasnoyarsk Krai, health risk

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ОЦЕНКА РИСКА ЗДОРОВЬЮ ПО ВЕТРО-ХОЛОДОВОМУ ИНДЕКСУ НА ТЕРРИТОРИИ КРАСНОЯРСКОГО КРАЯ

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Ветер влияет на функциональное состояние, здоровье человека. При холодном ветре активность смягчает риск гипотермии, но не дискомфорт, возникает угроза охлаждения организма и обморожений. Целью работы было оценить риск здоровью человека, возникающий при проживании в различных климатических зонах региона России по ветро-холодовому индексу. Расчет проводили по среднемесячным значениям суточной температуры и скорости ветра, минимальной температуры и максимального ветра в субарктическом и континентальном климате в периодах определения климатических норм: 1961–1990 гг. (второй период) и 1991–2020 гг. (третий период). В третьем периоде установлено достоверное снижение силы ветра в субарктическом (8 месяцев) и умеренном континентальном (9 месяцев) климате. Среднемесячные температуры увеличивались в апреле на 3,5 °С (p = 0,006), апреле–июне на 4,05 °С (p = 0,001) и 3,9 °С (p = 0,001). Максимальный ветер в субарктическом климате не изменился, в умеренном снизился в течение 9 месяцев; минимальная температура возросла в течение 4 и 1 месяца. По средним значениям температуры и ветра в субарктическом поясе 6 месяцев (против 7) возникали дискомфортные ощущения, в том числе 1 месяц как «чрезвычайно холодно»; уменьшился риск холодового воздействия за счет «очень холодно»; в умеренном поясе риск дискомфортных ощущений был зарегистрирован 4 месяца (против 6). При максимальном ветре и минимальной температуре жесткость погоды в субарктическом климате в каждом периоде сохранялась 8 месяцев в году («дискомфорт, прохлада» — 2, «холодно, переохлаждение поверхности кожи» — 1, «чрезвычайно холодно, обнаженные части тела могут переохладиться за 10 мин» — 5); в умеренном 5 месяцев («дискомфорт, прохлада» — 2, «очень холодно, переохлаждение поверхности кожи» — 3).

Ключевые слова: ветро-холодовой индекс, субарктический климат, континентальный климат, Красноярский край, риск здоровью

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Being outdoors and in the open, a person is directly exposed to weather conditions, which affect, first of all, his/her thermal status. There is a number of weather-related factors that shape our perception of how warm we are: temperature, air movement speed, humidity, pressure, atmosphere's electrical status, radiation temperature, etc. The wind does not change the ambient temperature, but it does draw heat off the body of a human being. Cold wind alters how we perceive ambient temperature: the faster heat is drawn off the body, the colder it feels [1, 2].

Wind can have both sanogenic and negative effects on all aspects of life and health of a human being. The conditions that largely shape our feeling of comfort when outdoors in the open largely depend on wind; it redistributes moisture above

Table 1. Wind speed values in the subarctic climate, m/s

Observation	Wind speed by months of the year, M ± *m*											
	1	2	3	4	5	6	7	8	9	10	11	12
Average monthly daily speed												
1	5.2 ± 0.4	5.6 ± 05	5.8 ± 0.3	5.7 ± 0.3	5.0 ± 0.3	5.0 ± 0.2	4.4 ± 0.2	4.3 ± 0.2	4.5 ± 0.4	5.1 ± 0.3	5.5 ± 0.4	6.1 ± 0.2
2	5.0 ± 0.3	4.6 ± 0.4	4.8 ± 0.4	4.8 ± 0.2	4.2 ± 0.1	4.2 ± 0.1	3.6 ± 0.05	3.6 ± 0.1	3.6 ± 0.2	4.3 ± 0.3	4.4 ± 0.3	5.4 ± 0.2
p	0.68	0.09	0.71	0.018	0.14	0.001	0.001	0.015	0.02	0.048	0.041	0.05
Average monthly maximum speed												
1	15.5 ± 0.6	13.9 ± 0.7	14.3 ± 1.0	14.5 ± 0.6	12.3 ± 0.9	12.8 ± 0.6	12.1 ± 0.5	10.5 ± 0.4	13.5 ± 1.1	14.3 ± 0.9	14.2 ± 0.8	16.2 ± 0.7
2	16.3 ± 0.8	14.1 ± 0.7	16.9 ± 1.0	14.0 ± 0.9	12.8 ± 0.5	12.9 ± 0.9	10.9 ± 1.0	11.2 ± 0.9	12.2 ± 1.4	13.6 ± 1.0	14.4 ± 0.9	15.4 ± 0.6
p	0.41	0.83	0.14	0.14	0.64	0.92	0.278	0.252	0.469	0.61	0.86	0.4

the planet's surface, equalizes the temperature, purifies the air. Strong wind puts pressure on the surface tissues of the body, causes fatigue, headaches, anxiety, insomnia, hinders breathing, aggravates shortness of breath, and has a depressing effect on the human psyche. Such wind also contributes to dust formation and carries it over considerable distances. When it lifts droplets of water from the surface of seas and lakes into the air, strong wind plays its role in the spreading of infectious diseases [3]. Wind should be considered decisive factor in the transmission of a wide range of diseases and not just airborne, since it can modulate the dynamics of various spreaders and pathogens [4–7].

With wind's contribution, weather has direct or indirect effect on the functional state of a human being, shapes the overall health status in a population, conditions regional peculiarities of the diseases influenced by climatic factors [8–11]. Among the numerous bioclimatic indicators, the "cold stress" indices are distinguished as factors limiting the duration of a person's stay in the open in winter [12, 13]. Some researchers give preference to the Siple's wind chill factor (WCF) in assessment of influence of the cold [14, 15]. Determining the WCF allows identification of the possible health risks associated with low temperatures and cold winds [16–19].

This study aimed to assess the risk to health of a human being associated with the wind chill factor index in the various climatic zones of Krasnoyarsk Krai.

METHODS

Retrospectively, based on the long-term observation data and through the lens of the Siple's wind chill factor [20] (WCF), we assessed the risks the cold peculiar to subarctic and temperate continental climate zones of the Krasnoyarsk Krai presents to a human being upon exposure. The cold stress risk scale was as follows: from –10 to –24 °C — uncomfortable, chilly; from –25 to –34 °C — very cold, skin surface hypothermia; from –35 to –59 °C — extremely cold, possible hypothermia of exposed parts of the body in 10 minutes, and –60 °C down — extremely cold, possible hypothermia of exposed parts of the body in 2 minutes [21].

The World Meteorological Organization (1962) adopted 30-year periods for determination of climatological normals: the first from 1931 to 1960, the second from 1961 to 1990, and the third from 1991 to 2020. Our study relied on the data covering the last two of such periods, 1961–1990 (second period) and 1991–2020 (third period) [22, 23]. We assessed the average monthly daily temperature and wind speed values in the open as registered during the last 10 years of each determination period, 1981–1990 and 2010–2019, respectively. We also assessed the monthly average minimum temperature and maximum wind speed indicators. Meteorological information was obtained from the Central Siberian Department for Hydrometeorology and Environmental Monitoring, located in Norilsk (Taimyr Branch) and Krasnoyarsk (Experimental Field). Wind strength was assessed in points on the Beaufort scale [24].

The data was statistically processed with Statistica 6.0 (StatSoft; USA); we determined mean values and standard errors (M ± *m*), applied Student's *t*-test. The differences were considered significant at $p < 0.05$.

RESULTS

Assessing the average monthly wind speeds in the subarctic climate, we noticed that in the second climatological normals determination period (1961–1990) its strength was put at 3 points (from weak to moderate) for 7 months a year (May – October and January): from 4.3 ± 0.2 to 5.1 ± 0.2 m/s (Table 1). In the remaining 5 months, it was given 4 points (moderate): from 5.5 ± 0.4 to 6.1 ± 0.2 m/s. In the third period (1991–2020), for 11 months a year (except December) the wind strength's

Table 2. Wind speed values in the temperate continental climate, m/s

Observation	Wind speed by months of the year, M ± *m*											
	1	2	3	4	5	6	7	8	9	10	11	12
Average monthly daily speed												
2	2.4 ± 0.1	2.3 ± 0.2	2.8 ± 0.3	2.9 ± 0.2	2.7 ± 0.2	2.2 ± 0.2	1.7 ± 0.07	1.7 ± 0.05	1.9±0.1	2.6±0.2	3.0 ± 0.2	2.7± 0.2
3	1.8 ± 0.2	1.9 ± 0.08	2.1 ± 0.1	2.3 ± 0.1	2.1 ± 0.07	1.6 ± 0.07	1.4 ± 0.05	1.5 ± 0.09	1.7±0.1	2.0 ± 0.16	2.0 ± 0.13	1.9 ± 005
p	0.04	0.045	0.017	0.014	0.003	0.009	0.04	0.038	0.87	0.029	0.001	0.001
Average monthly maximum speed												
2	9.8 ± 0.4	8.6 ± 0.3	8.4 ± 0.4	8.3 ± 0.4	8.1 ± 0.4	6.8 ± 0.4	6.1 ± 0.3	5.4 ± 0.3	7.0 ± 0.4	8.1 ± 0.3	10.1 ± 0.1	10.0 ± 0.4
3	7.3 ± 0.6	7.3 ± 0.2	6.5 ± 0.2	6.7 ± 0.3	6.9 ± 0.7	5.1 ± 0.4	4.8 ± 0.2	5.3 ± 0.5	6.0 ± 0.4	6.9 ± 0.4	7.0 ± 0.3	7.9 ± 0.2
p	0.002	0.002	0.001	0.008	0.15	0.007	0.002	0.226	0.1	0.01	0.001	0.001

Table 3. Ambient temperature values in the subarctic climate, °C

Observation	Ambient temperature by months of the year, M ± *m*											
	1	2	3	4	5	6	7	8	9	10	11	12
Average monthly daily temperature												
2	–23.8 ± 2.2	–21.8 ± 1.7	–16.3 ± 1.0	–10.8 ± 1.6	0.2 ± 0.3	11.4 ± 1.9	19.9 ± 1.2	15.6 ± 0.6	7.6 ± 0.8	–4.6 ± 0.6	–18.0 ± 2.1	–20.5 ± 1.7
3	–23.3 ± 1.5	–21.5 ± 1.7	–16.5 ± 1.7	–7.3 ± 0.9	1.6 ± 1.0	15.3 ± 1.0	19.9 ± 0.9	15.2 ± 0.8	8.2 ± 0.9	–3.6 ± 0.9	–17.8 ± 1.2	–18.0 ± 1.4
p	0.83	0.905	0.119	0.006	0.31	0.062	0.962	0.707	0.649	0.464	0.926	0.29
Average monthly minimum temperature												
2	–30.2 ± 2.1	–29.0 ± 1.2	–26.2 ± 1.2	–19.2 ± 1.5	–7.8 ± 0.5	3.3 ± 0.8	10.3 ± 0.7	7.8 ± 0.7	1.0 ± 0.5	–11.5 ± 1.2	–24.9 ± 1.7	–27.8 ± 1.4
3	–30.5 ± 1.6	–30.5 ± 1.7	–20.4 ± 2.0	–13.5 ± 0.9	–5.4 ± 0.8	6.5 ± 0.6	11.2 ± 0.6	7.8 ± 0.6	2.2 ± 0.6	–8.9 ± 1.2	–24.8 ± 1.1	–25.3 ± 1.5
p	0.92	0.86	0.014	0.005	0.024	0.007	0.36	0.98	0.366	0.146	0.92	0.24

score was 3 points: from 3.6 ± 0.1 to 5.4 ± 0.2 m/s. With the exception of winter months, i.e. for 8 months in a year, the speed of air movement was registered decreasing significantly. The average monthly maximum wind speed values did not change. The lowest values were recorded in August (10.5 ± 0.4 and 11.2 ± 0.9 m/s; $p = 0.252$), the highest in December (16.2 ± 0.7 and 15.4 ± 0.6 m/s; $p = 0.4$) and January (15.5 ± 0.6 and 16.3 ± 0.8 m/s; $p = 0.41$). In July and September, they were 1.2–1.3 m/s less, but the difference with the values recorded in the previous determination period were insignificant: 10.9 ± 1.0 versus 12.1 ± 0.5 m/s ($p = 0.278$) and 12.2 ± 1.4 m/s versus 13.5 ± 1.1 m/s ($p = 0.469$).

In the temperate continental climate, the wind was given 2 points (weak wind) in all months of the year: from 1.7 ± 0.07 m/s to 3.0 ± 0.2 m/s (Table 2). In Junes and Julys of the third period it scored 1 point (very weak): from 1.4 ± 0.05 to 1.5 ± 0.09 m/s. With the exception of one month (September), there was a significant decrease in wind strength. Nine months of the third period (except for May, August and September) saw a significant decrease in the maximum wins speed. This decrease ranged from 1.0 m/s in February and June ($p = 0.002$) to 3.1 m/s in November ($p = 0.001$) and 2.5 m/s in January ($p = 0.002$).

In the summer months of the second climatological normals determination period, the average daily air temperature in the subarctic climate ranged from 11.4 ± 1.9 to 19.9 ± 1.2 °C, in the winter months — from –20.5 ± 1.7 to –23.8 ± 2.2 °C (Table 3). In the third period, compared to the data registered in the previous periods, it grew by 3.5 °C in April, which is a significant increase (–7.3 ± 0.9 versus –10.8 ± 1.6 °C; $p = 0.006$). In June, the temperature increased by 3.9 °C, but this value did not differ significantly from the previous one (15.3 ± 1.0 versus 11.4 ± 1.9 °C; $p = 0.062$). Besides, in the course of 4 months, March through June, the average monthly minimum temperature was registered increasing: from –20.4 ± 2.0 to –26.2 ± 1.2 ($p = 0.014$) and from 6.5 ± 0.6 up to 3.3 ± 0.8 °C ($p = 0.007$).

In April and June of the third determination period the average monthly daily air temperature was registered to grow by 4.05 °C (4.7 ± 0.5 versus 0.65 ± 0.7 °C; $p = 0.001$) and by 3.9 °C (18.2 ± 0.5 versus 14.3 ± 0.5 °C; $p = 0.001$) in April and June, respectively (Table 4). The average monthly minimum temperature difference was recognized as significant only in April (–0.3 ± 0.4 versus –3.5 ± 0.8 °C; $p = 0.002$).

Average 5-month WCF values registered in the subarctic climate in the second determination period and 6-month values of the third period show that for human beings, the ambient conditions did not grow uncomfortable (Fig. 1). In the second period, 2 months a year (October and April) saw the conditions assessed as "uncomfortable", the 4 months of November, December, February and March as "very cold", and January as "extremely cold". In the third period, the durations of unfavorable weather conditions changed: the "uncomfortable" period has grown one month (October) shorter, the "very cold" period did not include March anymore, which was marked as uncomfortable, and the period of "extremely cold" conditions remained as it was.

In the temperate continental climate zone, only 6 months (October through March) of the second period years the weather was labeled "uncomfortable", while the third period had only 4 such months (December through February) (Fig. 2).

Maximum wind, combined with minimum temperature, made the conditions less comfortable: the WCF values increased and the weather was perceived more negatively (Table 5). At the same time, for 8 months of each determination period the severity of weather did not change in the subarctic belt. In October and May, WCF made the conditions registered as "uncomfortable, chilly", in April as "very cold, skin surface hypothermia", in November–March as "extremely cold, possible hypothermia of exposed parts of the body in 10 minutes".

In the temperate continental climate, 6 months of the 2nd period had the weather perceived negatively, with 3 of them registering the conditions as "uncomfortable, chilly" and 3 as "very cold, skin surface hypothermia." November could also present conditions described as "uncomfortable." In the climatological normals determination period, the number of months registering weather described as "severe" dropped to 5, with 2 months categorized as "uncomfortable, chilly" and

Table 4. Ambient temperature values in the temperate continental climate, °C

Observation	Air temperature by months of the year, M ± *m*											
	1	2	3	4	5	6	7	8	9	10	11	12
Average monthly daily temperature												
2	–15.3 ± 0.9	–15.3 ± 1.7	–5.6 ± 1.3	0.65 ± 0.7	9.8 ± 0.7	14.3 ± 0.5	18.1 ± 0.5	15.7 ± 0.3	9.2 ± 0.3	1.7 ± 0.8	–7.7 ± 1.3	–13.7 ± 1.4
3	–17.3 ± 1.6	–13.9 ± 1.4	–3.9 ± 0.9	4.7 ± 0.5	9.4 ± 0.5	18.2 ± 0.5	18.8 ± 0.3	16.5 ± 0.3	9.4 ± 0.5	2.4 ± 0.9	–7.5 ± 0.9	–12.6 ± 2.0
p	0.354	0.536	0.261	0.001	0.629	0.001	0.241	0.113	0.869	0.592	0.92	0.676
Average monthly minimum temperature												
2	–19.3 ± 1.0	–18.2 ± 1.3	–9.8 ± 1.2	–3.5 ± 0.8	4.4 ± 0.5	9.4 ± 0.3	12.7 ± 0.3	10.7 ± 0.3	4.6 ± 0.3	–2.3 ± 0.7	–10.6 ± 0.7	–16.2 ± 1.2
3	–20.9 ± 1.5	–20.8 ± 1.3	–8.8 ± 0.8	–0.3 ± 0.4	4.0 ± 0.4	11.7 ± 0.3	13.6 ± 0.3	11.5 ± 0.3	4.7 ± 0.5	–1.1 ± 0.9	–13.5 ± 1.2	–16.0 ± 0.2
p	0.39	0.85	0.39	0.002	0.51	0.39	0.055	0.054	0.65	0.297	0.79	0.933

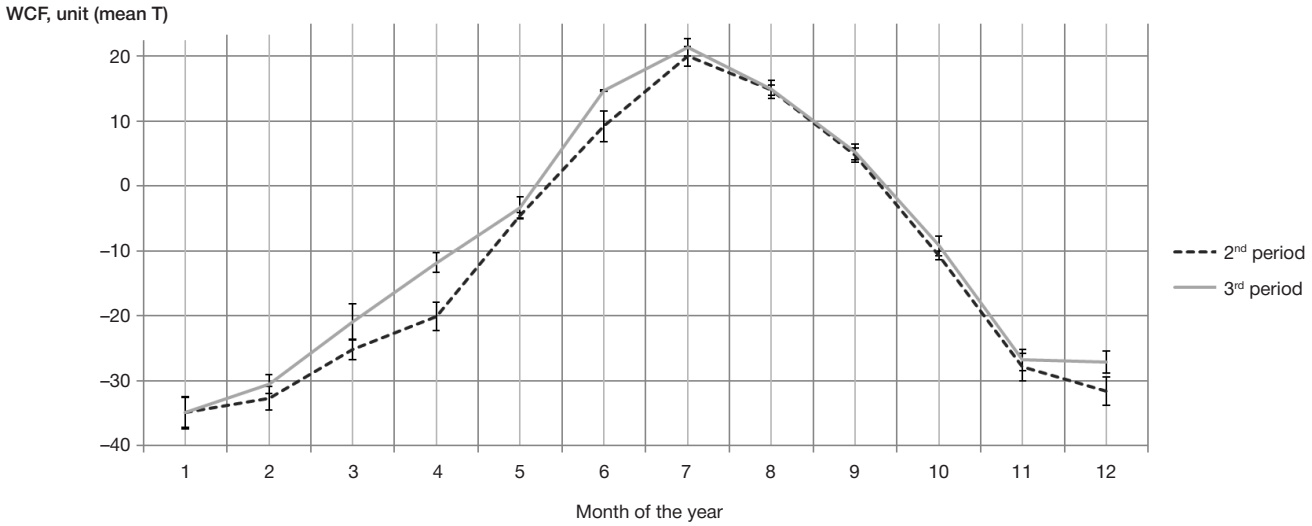

Fig. 1. Wind chill factor curve, average monthly daily ambient temperature and wind speed, subarctic climate, M ± *m*, units

3 falling into the category of "very cold, skin surface hypothermia." It should be noted that in January the WCF value practically reached the "extremely cold, possible hypothermia of exposed parts of the body in 10 minutes" level.

DISCUSSION

Wind speed is taken into account when determining the conditions applied to work performed outdoors and in unheated rooms, since personal protective equipment is limited in its capacity to prevent onset of hypothermia in harsh climatic conditions (zones "special", IV, III) mainly due to its inefficiency in keeping feet and hands warm and also because of cooling of face and respiratory organs [18, 25, 26]. WCF value is determined for the purposes of preserving health of hikers and athletes practicing winter sports, especially in the northern latitudes of Russia. It is known that the type of activity practiced can mitigate the risk of hypothermia, but it has no effect on the level of discomfort caused by exposure to a cold wind, which poses a risk of body cooling down and frostbite [27, 28]. WCF is also used when establishing the level of comfort in a specific region from the point of view of weather and climatic conditions therein [29]. High wind speeds make walks in the open unsafe without special winter clothing [30]. The severity of a winter correlates with wind strength closely [30, 31].

WCF corresponds to the air temperature in an open area that, with wind blowing at 4.2 km/h, would have the body cooling down same as it would in the actual ambient conditions [21]. The factor describes the degree of cooling caused by the wind as the air temperature equivalent, the temperature same as that provoking body cooling in the absence of wind, in shade and discounting perspiration. It is not a temperature value but an index that helps relate the cooling effect of wind to air temperature in calm conditions. Wind does not cool an object exposed to it below the temperature of the surrounding air. The faster it blows, the faster such object's temperature will drop to the level of ambient temperature [32]. The weighted average skin temperature of 33 °C is considered in the body cooling rate calculation. In the absence of any wind and relative humidity at 100%, only the ambient temperature conditions how warm a person feels. If the temperature remains the same but wind picks up and humidity drops, the person's body starts losing heat faster and that person feels as if the temperature was going down. This effect is reversed when wind calms and humidity grows up [33].

Through the considered periods, we discovered that the wind strength decreased in both subarctic and temperate continental climates. These findings are in line with the results reported by other researchers, who also noted that wind speed becomes less dependent on the climatic zone considered [11].

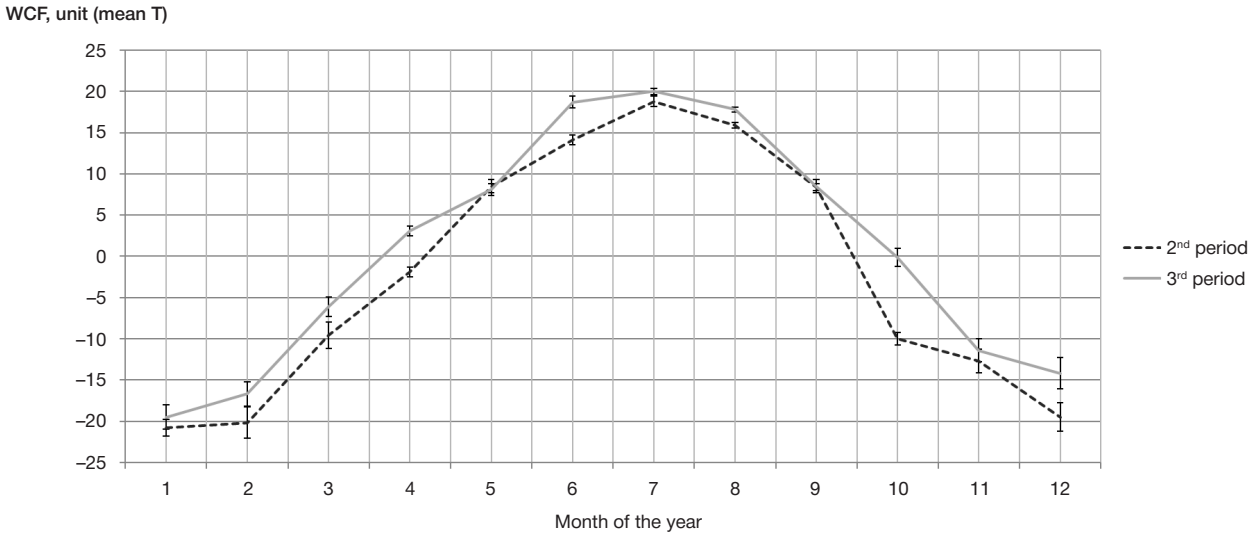

Fig. 2. Wind chill factor curve, average monthly daily ambient temperature and wind speed, temperate continental climate, M ± *m*, units

Table 5. WCF values peculiar to the combination of minimum temperature and maximum wind strength in different climatic zones, °C

№ п/п	Determination period	Month of the year, M ± *m*							
		1	2	3	4	5	10	11	12
1	Subarctic climate								
2nd period		–50.1 ± 2.8	–37.9 ± 9.9	–44.0 ± 1.2	–33.9 ± 2.0	–18.2 ± 0.6	–20.9 ± 3.3	–42.1 ± 2.4	–42.7 ± 1.9
3rd period		–50.8 ± 2.2	–48.1 ± 2.2	–36.9 ± 2.6	–26.4 ± 1.2	–15.2 ± 1.0	–20.1 ± 1.5	–41.9 ± 1.3	–43.2 ± 2.1
p		0.845	0.321	0.002	0.004	0.02	0.829	0.38	0.195
2	Temperate continental climate								
2nd period		–32.5 ± 1.4	–30.3 ± 1.8	–19.2 ± 1.5	–11.0 ± 1.0	–0.7 ± 0.6	–9.3 ± 0.9	–21.2 ± 1.7	–28.5 ± 1.6
3rd period		33.9 ± 2.0	–29.0 ± 1.8	–16.6 ± 1.2	–6.1 ± 0.5	–0.6 ± 0.6	–7.2 ± 1.0	–20.1 ± 1.2	–27.1 ± 2.7
p		0.577	0.621	0.152	0.001	0.953	0.142	0.61	0.66

The researchers have also registered growth of the summer temperature levels. We have also witnessed a significant temperature increase in both climates, but in the subarctic zone such increase was registered only in April, and in the temperate continental zone — in April and June. Decreased wind strength and increased air temperature made the cold stress risk lighter in both the duration of the risk periods and the severity of its manifestations. In the last decade of the third climatological normals determination period (1990–2020), the risk of cold exposure has decreased, which is probably reflected in the health status of the population in each climatic zone of the region.

The assessment of influence of weather and climatic conditions, temperature and wind strength in particular, has shown that these indicators play an important part in determining the health risk levels peculiar not only to the subarctic zone, but also to the temperate climate zone. These findings are confirmed by the results reported by other researchers [8, 9, 16–18, 33]. In the context of our study, we established WCF value for the months of May through September and found no health risks arising therefrom, while other studies (e.g., at high air temperatures) note the positive effect wind strength has on a person's perception of own health status by imbuing the feeling of comfort [29, 30]. In contrast to the studies published by other researchers, we evaluated the role of WCF in conjunction with the extreme values of meteorological factors, i.e. minimum temperature and maximum wind, which allows stating longer duration of seasons when ambient conditions affect human beings negatively.

In addition, we selected WCF based on the priority it takes in the process of assessment of ambient conditions from the point of view of safety of work in the open.

CONCLUSION

Determination of WCF allows identifying health risk factors and their parameters. At low temperatures, weather-dependent sensations are aggravated by both strong winds and high humidity, which necessitates an extended study to assess the risks peculiar to cold habitats. The results obtained show that the living environment improves, but the underlying changes may have consequences that should be investigated. This methodology can be used to assess public health risks in other climatic zones of the country.

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MODERN METHODS FOR ANALYSIS OF CHANGES TO EPIGENETIC LANDSCAPE CAUSED BY EXPOSURE TO ENVIRONMENTAL POLLUTANTS

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The diagnosis and treatment of diseases caused by the exposure of human epigenome to environmental pollutants are hampered by epigenomic plasticity, instability and nonlinear cumulative effects of existing transcriptional regulatory pathways. DNA methylation, histone acetylation and histone methylation are the best studied epigenetic modifications. There are simple methods for assessing genome-wide DNA methylation; however, it is essential to study the epigenetic landscape in detail in order to uncover the mechanisms underlying pollutant-associated effects on the organism. This prompts researchers to employ whole-genome sequencing and analyze vast arrays of sequencing data that can be compiled into extensive databases of human and animal epigenomes. Drugs developed to counter epigenetic disorders neutralize their symptoms and either affect epigenetic modifications across the entire genome or regulate the activity of enzymes that play a critical role in such disorders. Promise is held by targeted genome editing methods supported by modern technologies that are undergoing preclinical trials. This review discusses the potential of modern science in the diagnosis and treatment of diseases caused by environmental pollutants.

Keywords: face transplant, microsurgery, facial flap, composite flap

Author contribution: Zanyatkin IA systematized literature data and wrote the manuscript; Titova AG provided additional literature for the review and edited the manuscript; Bayov AV edited the manuscript.

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АКТУАЛЬНЫЕ МЕТОДЫ АНАЛИЗА ИЗМЕНЕНИЙ ЭПИГЕНЕТИЧЕСКОГО ЛАНДШАФТА ОРГАНИЗМА, ВЫЗВАННЫХ ВОЗДЕЙСТВИЕМ ЗАГРЯЗНИТЕЛЕЙ ОКРУЖАЮЩЕЙ СРЕДЫ

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Диагностика и лечение заболеваний, вызванных воздействием поллютантов на эпигеном человека, затруднены пластичностью и нестабильностью эпигенома, наличием нескольких путей регуляции транскрипции с нелинейной суммацией эффектов. Наиболее исследованные пути — метилирование ДНК, ацетилирование и метилирование гистонов. Доступны простые способы оценки уровня глобального метилирования ДНК, однако для определения механизмов воздействия загрязнителя на организм необходимо изучать эпигенетический ландшафт в деталях. Это заставляет ученых применять методы полногеномного секвенирования и обрабатывать огромные массивы результатов, что привело к появлению нескольких баз данных эпигенома человека и животных. Препараты для лечения эпигенетических нарушений сосредоточены на симптоматическом лечении и действуют на глобальное редактирование эпигенома или на регуляцию активности ферментов, играющих критическую роль в нарушении. Более перспективны методы селективного эпигеномного редактирования, основанные на абсолютно новых технологиях, находящихся на стадии лабораторных исследований. Представлен обзор современных возможностей науки в области диагностики и лечения заболеваний, вызванных воздействием поллютантов на эпигеном человека.

Ключевые слова: эпигенетические сигнатуры, метилирование, ацетилирование, токсическое воздействие, поллютант, хронические заболевания, секвенирование, редактирование генома.

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A pollutant is a natural or synthetic chemical that causes environmental pollution when present in the environment at levels exceeding background values. The organs and systems that have direct contact with the pollutant sustain the most damage. Gases and suspended particulate matter affect the respiratory tract. Pollutants ingested with food or drinks are harmful to the gastrointestinal tract. Blood cells are affected as the main transport system of the body. The liver and kidneys can be damaged because of their leading role in the metabolism and excretion of toxic substances from the body.

Systemic effects of pollutants on the human body include irritation; disrupted mucociliary clearance, which results in the increased permeability of the bronchial epithelium to allergens and infection and promotes the risk of asthma; neurogenic inflammation; lipid peroxidation activation and depression of the ROS metabolism system; hyperactivity of neutrophil elastase, which causes lung tissue damage; increased production of inflammatory mediators, like metabolites of arachidonic acid, cytokines and adhesion molecules.

Basic concepts of epigenetics

Epigenetic studies the rules and patterns of epigenetic inheritance, i.e. changes in gene expression and cell phenotypes caused by mechanisms other than changes in DNA sequences. When exploring environmental effects on the epigenome, the primary focus is placed on the regulatory mechanisms of gene expression. The most common mechanisms are listed in Table 1.

DNA methylation at cytosine residues is the most prevalent epigenetic mark. The most abundant form of methylated cytosine is 5-methylcytosine (5-mC) found in GC-rich sequences, which are known as CpG islands. These regions

Table 1. Types of epigenetic markers regulating DNA transcription

Molecular signal	Example	Reference
Histone post-translational modifications	Repressive histone H3 lysine 9 trimethylation (H3K9me3)	[1]
Histone variants	Histone variant macroH2A.1	[2]
Nucleosome positioning	Nucleosome-free regions of gene promoters	[3]
Chromatin loops	Modulation of gene expression at the *Kit* locus by Gata1/Gata2	[4]
DNA modifications	DNA methylation at cytosine position 5	[5]
Structural DNA variants	R-Loop Formation	[6]
RNA-mediated pathways	Antisense RNA transcription	[7]

are typically located in the regulatory areas of the genome. In the absence of external influences, the pattern of DNA methylation is inherited by offspring from their parent. The inability to maintain this pattern leads to the death of the organism. Methylation of cytosine residues is carried out by a family of DNA-(cytosine-C5)-methyltransferases (DNMT) [8], the enzymes that transfer methyl groups from a donor S-adenosyl methionine to cytosine. DNMT1 maintains the level of methylation inherited from a parent. When complexed to UHRF1 (a chromatin protein), it can recognize methylated sites in a parental chromosome and reproduce a "methylation mark" at the equivalent locus on the new DNA. DNMT3a and DNMT3b establish methylation patterns *de novo*. DNMT3b is responsible for the hypermethylation of genes encoding DNA repair enzymes, which is believed to play the key role in malignant transformation in some cancer types [9]. Mutations in the DMNT3a gene are associated with acute myeloid leukemia in one-fifth of leukemia patients. Demethylation of cytosine bases occurs through iterative oxidation reactions of 5-mC to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5caC), followed by the excision and substitution of these modified residues with unmodified cytosine; this process is mediated by thymine DNA glycosylase (TDG) and enzymes participating in the base excision repair (BER) mechanism [10].

Histone modifications constitute the second most common type of epigenetic marks. Histones are highly conservative proteins responsible for packaging and ordering DNA into nucleosomes. Histone modifications that modulate gene expression include lysine acetylation, which induces transcriptional activation, and lysine methylation, which, depending on the methylation site, can either act as an activating or repressing mechanism [11]. Lysine acetylation is regulated by 2 families of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are categorized into 4 classes. Class I comprises HDAC 1, 2, 3 and 8 expressed in the nucleus; class IIA includes HDAC 4, 5, 7 and 9, which shuttle between the cytoplasm and the nucleus; class IIB encompasses HDAC 6 and 10, which remain in the cytoplasm; class IV is constituted by HDAC 11.

Both DNA methylation and histone modifications (methylation and acetylation) can be affected by exogenous factors. For example, the activity of NAD+-dependent HDAC (sirtuin 1) can be modulated by a number of bioactive compounds, including resveratrol. HDAC inhibitors cancel transcriptional repression and gene silencing; this may result in untimely gene activation and trigger pathology. By contrast, HAT inhibitors restore epigenetic control, preventing unwanted gene transcription.

Summing up, epigenetic mechanisms of gene regulation per se constitute a complex multi-tiered system that remains understudied to this day. Environmental factors only add to its complexity, creating extra challenges for the analysis.

Methods for epigenetic landscape analysis

At present, two major types of epigenetic inheritance are known. With direct inheritance, epigenetic modifications are acquired at the germinal or embryonic stages [12]. They are manifested in phenotypes as early as the first generation and persist into the second or third generation of offspring. With indirect inheritance, phenotypic changes reveal themselves in the second or third generation of offspring, long after the causative epimutagen has been removed from the organism. If an epimutation is severe and affects critical genes, its consequences can manifest themselves during the lifetime of the organism.

Currently, there are a few methods for rapid methylation measurement in individual genes. Peripheral blood DNA methylation profiles hold promise as biomarkers of multiple small metastases [13]. Abnormal cellular content of the certain protein may be associated with cancer: levels of glycolytic and mitochondrial proteins (alpha-enolase, glyceraldehyde-3-phosphate dehydrogenase, ATP synthase) are substantially elevated in human breast cancer induced by exposure to benzo[a]pyrene [14]. However, information about the proteome has value only when it is analyzed together with transcriptome data. Besides, cells can change their proteome to compensate for the effects elicited by the pollutant. For example, MCF-7 cells exposed to benzo[a]pyrene, dibenzo[a,i]pyrene or coal tar extract were shown to hyperexpress heat shock proteins HSP-70 and HSP-27 [14]. Also, antibodies specific for the native protein may fail to recognize its mutant variant. An experimental study tested the reactivity of p53 with conformation-specific monoclonal antibodies PAb1620 and PAb240 in MCF-7 cells treated with cadmium salts. Exposure to cadmium resulted in the incorrect folding of the protein, disrupted its conformational structure and affected its recognition by antibodies [15]. Such analysis can be carried out using two-dimensional polyacrylamide gel electrophoresis.

In the simplest model, the gene would have only 3 distinct levels of methylation: 0 — no methylation, 50% — methylation of 1 allele, 100% — methylation of both alleles. In practice, this is not the case due to the heterogeneity of samples collected from real populations; most studies estimate DNA methylation at only 10–30%. Only quantitative methods are suitable for this type of analysis.

At present, there are two very alike groups of methods suitable for the analysis of genomes and transcriptomes (Table 2). The first is DNA-RNA hybridization in which short DNA molecules are immobilized on a microarray, the studied DNA/RNA is hybridized to the immobilized DNA and then used as a template for DNA synthesis with fluorescent tagged nucleotides. Fluorescence intensity measured during DNA synthesis correlates with the amount of the analyzed DNA/RNA. This rapid analytical method for measuring gene transcription is, however, not free of errors associated with faulty hybridization.

Table 2. Currently known types of epigenetic regulation of DNA transcription and methods of detecting epigenetic marks

Transcriptional regulator	Detection method	Advantages (+) and downsides (–)	
Recommended			
DNA methylation	Bisulfite sequencing	MethylC-seq [17]	(+) Single-base resolution; encompasses a majority of cytosine bases in the genome (–) Expensive
		RRBS [18]	(+) Single-base resolution, relatively cheap (–) Encompasses a limited pool of cytosine bases mainly occurring in CpG islands
	Analysis with methylation-sensitive restriction enzymes	HELP-tagging [19] MSCC [20]	(+) Relatively cheap, does not depend on CpG density (–) Encompasses a limited pool of cytosine bases
DNA methylation	Affinity analysis	meDIP-seq [21]	(+) Can be genome-wide (–) Quantitative analysis in CpG-impoverished regions
	Microarrays	Infinium Methylation BeadChip [22]	(+) Cheap; targets the region responsible for the studied function (–) Targets a limited pool of cytosines; reliability depends on the nucleotide sequence
siRNA	Sequencing	[23]	(+) Quantitative analysis; can be used to scan for previously unknown siRNA (–) Complex library preparation
mRNA	Sequencing	[23]	(+) Quantitative analysis; can be used to analyze co-transcriptional events, e. g. alternative splicing (–) Approaches to data analysis are still unoptimized
Alternative			
Posttranslational chromatin modifications	Sequencing on microarrays	[24]	(+) Whole-genome analysis (–) Low resolution
Chromatin structure	Sequencing with DNases	[25]	(+) Identifies regulatory DNA regions outside of annotated promoters (–) Non-quantitative analysis

Chromatin immunoprecipitation is another common analytical method. It consists of a few stages: formation of DNA-protein complexes, DNA purification, elution and sequencing. It is used to determine the proportion of DNA fragments with the target sequences in the mixture. The main constraint of massively parallel sequencing (MPS) is associated with the length of DNA fragments subject to sequencing: during immunoprecipitation, DNA is normally cut into short 100–500 bp fragments because longer fragments can give rise to sequencing errors. If the level of gene expression and the level of modification differ between the epimutated and the intact sites by only 10–20%, they will not be detected by chromatin immunoprecipitation. Interestingly, benign tumors are usually characterized by 10–20% difference in the levels of methylation at a studied locus [16]. At the same time, MPS can be employed to sequence both individual genes and whole genomes; the procedure can be sped up by using automated MPS. Unlike data from microarrays, MPS can be used to identify allelic variants, detect alternative splicing events, study DNA methylation at single-base resolution, and obtain information about previously unsequenced genomic regions, which makes MPS data only more valuable over time. The advantage of this method stems from its potential for further development: MPS is becoming faster and cheaper, whereas microarray-based sequencing has almost exhausted its potential. Besides, sequencing ensures higher accuracy of methylation measurements than microarrays.

Methylome sequencing is performed using the same approaches. However, in order to be applied to methylomes, sequencing techniques have been modified. Classically, unmodified cytosine is converted to uracil through sodium bisulfite-mediated covalent modification; in contrast, the methylated form of cytosine (5-mC) doesn't react with sodium bisulfite. Differences in the obtained sequences allow identifying cytosine methylation sites. Novel luminometric methylation assays are based on DNA cleavage by methylation-sensitive restriction enzymes and subsequent DNA pyrosequencing accompanied by fluorescence detection. One of the platforms exploiting this technique is Pyrosequencer by Qiagen [26]. Pyrosequencing is a quantitative, reproducible and scalable method that doesn't require any genomic DNA modification and is, therefore, time-saving. Besides, it works with as little as 200–500 ng of genomic DNA and includes internal controls to trace errors associated with differences in the amounts of initial DNA. Pyrosequencing has a few downsides: only relatively short DNA sequences can be sequenced without errors, and the probability of error increases for sequences with repeated bases.

The search for possible associations between the effects exerted by pollutants and genetic/epigenetic marks relies on the analysis of genome-wide, epigenomic and transcriptomic data. For the purpose of systematization, epigenomic data are arranged into databases (Table 3), like ENCODE and Roadmap in Epigenomics. Challenges facing epigenomic data analysis pertain to the choice of the reference epigenome: even within one organism, the epigenome varies across tissues [27], changing over time and at different phases of the cell cycle [28]. Epigenomic databases will continue to expand as new data are accumulated. In the future, epigenomic databases will become an effective tool for uncovering the pathogenesis of human diseases associated with pollutants.

Challenges facing epigenomic data analysis

The diversity of epigenetic alterations caused by a pollutant is a serious obstacle in the development of models simulating the effects of the pollutant on the organism. It is reported that exposure to dioxin derivatives leads to the hypermethylation of CpG islands located in the imprinting control region of the murine *Igf2* gene, whereas differential histone retention sites located upstream of the adjacent noncoding regions of the H19 gene are hypomethylated in comparison with the control group [29].

The second challenge pertains to the way epigenetic modifications are interpreted by the organism depending on tissue type, age, and the context in which the modification occurs. For example, histone 3 lysine 9 trimethylation (H3K9me3)

Table 3. Databases of epigenetic marks and related browsers

Project	Websites
ENCyclopedia Of DNA Elements (ENCODE, modENCODE)	http://www.genome.gov/10005107 http://genome.ucsc.edu/ENCODE/ http://www.modencode.org/ http://www.genome.gov/modencode/
Roadmap in Epigenomics	http://www.roadmapepigenomics.org/ http://www.epigenomebrowser.org/ http://www.ncbi.nlm.nih.gov/epigenomics
BPA The Cancer Genome Atlas (TCGA)	http://www.genome.gov/17516564 http://cancergenome.nih.gov/ http://tcga-data.nci.nih.gov/tcga
International Human Epigenome Consortium (IHEC)	http://www.ihec-epigenomes.org/
NGSmethDB	http://bioinfo2.ugr.es/NGSmethDB
The Smith Lab MethBase	http://smithlabresearch.org/software/methbase

is recognized by the transcription system as repressive in cases when H3 is not only bound to heterochromatin at individual sites but affects chromatin packaging globally within a cell [30] or is located in a gene promotor. However, H3K9me3 is also found in the bodies of actively transcribed genes [31]. DNA methylation inhibits transcription when it occurs in a gene promoter and has the opposite effect when it occurs in the gene body, which is characteristic of actively transcribed genes [20]. Besides, patterns of nucleosome positioning [32] and DNA methylation detected at intron-exon boundaries are different [33]. So, epigenetic modifications can affect the choice of splicing pathways and modulate the functions of the synthesized protein. Thus, transcriptome analysis is essential in developing a model of epigenetic modifications.

The dynamic nature of the epigenetic landscape, which transforms throughout the cell cycle, makes the analysis more complicated. At the same time, epigenomic signatures can be retained long after the causative factor has been removed [34]. This property of epigenomic signatures has given rise to an intrauterine growth restriction (IUGR) paradigm: a past event induces epigenetic changes that transform cellular memory into phenotypic consequences. The increased risk of morbidity and type 2 diabetes at older age long after the exposure to a toxic agent speaks in favor of this hypothesis [16, 35]. A caloric deficit in the uterus is presumed to evoke an adaptive response, causing the embryo to reorganize its metabolism in order to accumulate more calories; this adaptation becomes harmful once the baby is born and has access to a balanced diet [36].

Another problem that complicates the analysis arises from the existence of a non-linear interplay between several metabolic pathways, which get affected by a pollutant. For instance, bisphenol A directly interacts with S-adenosyl-methionine and at the same time modulates miRNA-29 expression via estrogen receptors [37]. This results in the decreased expression of DNA methyltransferases and the elevated expression of histone methyltransferase EZH2 implicated in repressive histone modification [38]. This means that the cumulative effect of all changes happening to the methylome is hard to predict.

Outside the laboratory, organisms are exposed to a medley of pollutants, which produce an unpredictable interplay of effects, complicating the analysis of real populations vs. model objects. This problem can be solved by using data on the epigenetic modifications that are caused by known pollutants and produce known effects [39].

Biological models for genomic and epigenomic analysis

A high-quality study of the epigenome must adhere to the fundamental principles of toxicologic research, including proper dosing, injection routes and the duration of toxic exposure [40, 41].

Epigenetic deregulation events are traditionally considered to be somatic; therefore, epigenome studies should be carried out on cells in which genetic, epigenetic and phenotypic changes can be detectable and distinct. This poses a serious difficulty for human studies because they can only rely on small biopsy specimens. Besides, even within one tissue specimen collected from a living organism cells may be in different states and affect each other.

The available biological models can be classified into three major groups. The first group is represented by cell cultures. Primary cell cultures collected during animal/human biopsies are very close to living organism cells in terms of their epigenome and transcriptome; however, primary cell cultures are fastidious and can undergo a limited number of passages. Besides, the stability of their methylome cannot be maintained without synthetic organoids that require a lot of time and resources to grow. Cancer cells are less capricious and can survive over 200 passages [18]. However, their epigenome, transcriptome and sometimes genome (unstable number of chromosomes) significantly differ from those of *in vivo* healthy tissue; so, the possibility of extrapolating the characteristics of healthy cell methylomes from the methylome of cancer cells is unlikely to be reliable. Primary cultures immortalized by viruses [42] are a tradeoff: they do not differ drastically from conventional primary cultures in their metabolism, can undergo an infinite number of passages and are easy to maintain. Another solution lies in the use of primary cultures obtained from embryonic or inducible stem cells.

The second group includes animal models. Epigenetic modifications are known to bring about the same effects in model mammals and humans [43], i. e. the results of a murine study can be extrapolated to humans. Advantageously, animal models allow exploring the inherited effects of pollutants [44–47]. Yellow agouti mice Avy are a great example of animal lines whose phenotype correlates directly with DNA methylation levels. However, sometimes these animals do not respond to a known epimutagen used as positive control [48, 49]. The zebrafish (*Danio rerio*) is another popular model object: it breeds rapidly, allowing researchers to study the inheritance of epigenetic marks within a short time [50]. The genome of *Danio rerio* has been fully sequenced, so its changes are easy to track. Mechanisms underlying epigenetic regulation in these fish only slightly differ from those in mammals [51]. Zebrafish embryos are a successful model for studying the toxic effects of pollutants at early developmental stages.

The third group comprises cell cultures that are generated by animals throughout their lives and can be obtained without

killing the animal. For example, the methylome of parental reproductive cells can be used to assess susceptibility to disease in offspring [52].

Candidate drugs against diseases caused by pollutants

The main therapeutic strategy against epigenetic disorders includes the following steps: removing the detrimental factor and neutralizing its residual effects. Often, prescribing a therapeutic diet is enough. For instance, the demethylating effect of BPA can be compensated for by ingesting foods rich in methyl donors (folic acid and vitamin B_{12}). Natural and synthetic chemotherapeutics are being increasingly used to reverse epigenetic modifications associated with cancer [53]. They usually act as inhibitors of DNA methyltransferases and histone deacetylases. For example, green tea polyphenols (GTP) and epigallocatechin gallate (EGCG) were shown to inhibit DNMT activity and expression; thus, GSTP1 [54] and the onco-suppressor gene RARβ2 were reactivated, which led to the inhibition of proliferation of esophageal cancer cells [55], breast cancer cells [56] and lung cancer cells [57] in model cell cultures and mice. On the one hand, the anti-cancer effects of the listed compounds have been proved; on the other hand, genome-wide demethylation may reactivate genes whose activity per se may have serious side effects.

Improved selectivity of synthetic drugs targeting the enzymes implicated in epigenomic regulation is an important research goal. N-hydroxy-N'-feniloctandiamide, which has been approved in the USA for treating cutaneous T-cell lymphoma [58] and thyroid cancer [59] and is available on the Russian market as Vorinostat or Zolinza, inhibits class I and II HDAC but ignores class III HDAC. Romidepsin, also known as Istodax, has a similar effect. Another promising chemical is DIM (3,3'-diindolylmethane), which selectively inhibits class I HDAC and thereby leads to the increased transcription of *p21* and *p27* (genes coding for cyclin-dependent kinases) [60], the termination of the cell cycle at the G2/M phase, inhibition of papillomavirus-associated neoplastic growth [61], induction of apoptosis in breast cancer cells [62], and inhibition of prostate cancer growth [63]. DIM has the potential to prevent acute radiation syndrome caused by technogenic disasters and radiation therapy and alleviate its symptoms [64]. DIM precursors have therapeutic potential, too. For example, indole-3-carbinol (I3C) can regulate methylation levels in the promoter region of the *p16* INK4a gene in a dose-dependent manner [65] and terminate cell division. I3C suppresses production of estrogen mediators, and therefore can be used to mitigate the course of some autoimmune diseases [66]. On the other hand, the overuse of I3C poses a risk for endocrine disorders. Another group of drugs that are currently undergoing clinical trials is represented by histone methyltransferase (HMT) inhibitors. One of them, tazemetostat, blocks EZH2-methyl transferase [67]. Pinometostat is another member of this group. Pinometostat inhibits (DOT1L) HMT [68] and GSK3326595, which, in turn, inhibits arginine methyltransferase 5 (PRMT5) [69].

Summing up, drugs that target the methylome and have been already approved for use in a clinical setting modulate the level of DNA methylation across the entire genome [70, 71] or by inhibiting one particular enzyme [72] involved in methylation. They are intended for symptomatic treatment of progressing diseases but cannot correct epimutations.

Prospects of genetic and epigenetic therapy

Development of *de novo* drugs that can penetrate into the cell nucleus, selectively bind to a specific DNA locus and recruit or carry enzymes regulating DNA methylation is the most promising area of drug research. Systems for targeted genome editing are thought to have the greatest potential. Initially, hopes were laid on endonucleases with zinc-containing DNA recognition domains (ZFN or TAL) [73]. Later it became clear that each target site requires a unique protein to be synthesized, resulting in increased costs. A more versatile CRISPR/Cas9 system is based on the immune system of bacteria that specifically recognizes nucleotide sequences typical of viruses. This protein complex can be modified to disable its endonuclease activity, incorporate an RNA molecule responsible for the recognition of the target site and thus obtain an RNA-guided DNA-binding protein. Using genetic engineering techniques, the modified complex can be equipped with an enzyme exerting an intended effect on the epigenetic mark. With short Cas9 molecules it becomes possible to package the enzymatic complex into adeno-associated viral particles and thus integrate it into a recipient's genome. Cpf1 is another promising endonuclease: it is smaller than a CRISPR/Cas complex but exerts similar activity. However, its potential is yet to be investigated.

Conclusion

Genetic and epigenetic changes are interrelated. Under certain conditions, replication/transcription enzymes recognize an epigenetic mark as a different nucleotide, which poses a risk of mutations. Epigenetic modifications can interfere with DNA repair by suppressing the expression of proteins involved in this process. In turn, genetic aberrations can disrupt the normal functioning of epigenome editing systems.

The analysis of epigenetic effects of pollutants poses a more serious challenge than genetic analysis due to the varied nature of epigenetic tags, their plasticity, the context in which they occur and the complex interplay of transcriptional regulatory pathways. Applied epigenetics requires a systemic approach. Bioinformatic projects may be very useful in systematizing epigenomic data.

Most methods of studying epigenetic marks rely on the analysis of the most common covalent modifications of DNA (methylation) and histones (methylation and acetylation); DNA isolation and epigenome analysis are the modifications of similar methods used in genomic studies and involve detection of modified sites.

There are a lot of limitations impeding the study of pollutant-associated effects on human genomes and epigenomes. The list of model objects exploited to investigate and predict the detrimental effects of pollutants includes cell cultures from organs and tissues, embryonic stem cells, embryonic tissue analysis and model animals, like mice, rats and *Danio rerio* fish.

Most of the currently available epigenetic drugs only alleviate the symptoms of epigenetic disorders. Research focus is placed on the targeted editing of pathogenic epigenetic sites.

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CHRONIC URTICARIA ASSOCIATED WITH HIGH-RISK OCCUPATIONS

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In their line of duty, firefighters and rescuers are exposed to a combination of adverse factors, which necessitates monitoring their health. This review covers the most common variants of urticaria associated with high-risk occupations, such as cholinergic urticaria, food-induced anaphylaxis and exercise-induced urticaria, cold and stress-induced urticaria. The analysis includes the relevant research results published up to 2020; the discussion outlines the possible pathogenesis mechanisms of chronic urticaria.

Keywords: urticaria, stress, substance P, high-risk occupations, rescuers

Author contribution: Mikryukova NV — information collection, manuscript authoring; Kalinina NM — editing and reviewing

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ХРОНИЧЕСКАЯ КРАПИВНИЦА У ПРЕДСТАВИТЕЛЕЙ ПРОФЕССИЙ ВЫСОКОГО РИСКА

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Совокупность неблагоприятных факторов условий труда пожарных и спасателей требует контроля за состоянием их здоровья. В обзоре представлены варианты крапивниц, наиболее часто встречающиеся у представителей профессий высокого риска, такие как холинергическая крапивница, пищевая анафилаксия и крапивница, вызванная физической нагрузкой, холодовая, стрессиндуцированная крапивница. Сделан анализ опубликованных результатов исследований до 2020 г. и обсуждены возможные механизмы патогенеза хронической крапивницы.

Ключевые слова: крапивница, стресс, субстанция P, профессии высокого риска, спасатели

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The growing number of emergency situations and disasters necessitates increasing the headcount of first responders while making the health requirements for them more stringent. The measures taken to preserve health of the rescuers in the context of responding to emergency situations (ES) are especially important, since such measures ensure their maximum effectiveness in the line of duty. The combination of adverse factors rescuers are exposed to professionally substantiates the need for monitoring of their health [1].

Human factor (individual characteristics and capabilities, including those related to personality) and the level of physical fitness (the state of the cardiovascular and pulmonary systems) cause many problems in the work of the rescuers. There are also situations involving contact with highly toxic substances, e.g., in the context of disinfection in the pandemic. In addition to emergency response, firefighting, piloting and military professions are considered to be extreme. High-risk occupations are such that have the worker exposed to harmful production factors (chemical, excessive physical, biological), life-threatening and increasing the risk of development of somatic pathologies. Contributing to the psychological stress are the long periods of relative inactivity accompanied by anxiety, and the stress load associated with rescue operations, all of which find reflection in the rescuers' clinical and laboratory examination results. For example, firefighters have been shown to be at risk of depression and post-traumatic stress disorder [2].

Russian researchers have not paid due attention to the state of immune system of rescuers, which is why we analyzed the available data describing individuals from other countries whose working conditions are similar to those of the employees of the Russian Ministry of Emergency Situations (EMERCOM): police officers responding to emergencies and fires, as well as world-class athletes. The data on the state of their health are fragmentary; it is shaped by chronic stress, the influence of chemically active substances, increased or excessive physical exertion, lack of sleep, 24-hour shifts. In this connection, it was decided to use the data from these studies for comparison purposes, since understanding of the immunopathological mechanisms enables prevention, timely identification and elimination of the cause of pathology.

Respiratory pathology and cardiovascular diseases are the most common subjects of research targeting high-risk occupations. Only a small number of papers cover dermatological pathologies in rescuers. For example, in 2016 it was shown that young people performing high-risk work often have skin disease symptoms [3]. Various types of urticaria have been described along with dermatitis.

"Chronic urticaria" is a term describing a group of disorders characterized by itching blisters and/or angioedema persisting for more than 6 weeks. Worldwide, 1–3% of the population suffers from this disease, with women developing it twice as often as men [4]. Chronic urticaria can be spontaneous, without obvious triggers, and induced, with triggers being physical and chemical. The physical triggers are pressure (urticaria with delayed pressure), radiation (solar urticaria), friction (symptomatic dermographism), temperature (cold and warm urticaria) and

vibration (vibratory angioedema). The chemical triggers are water (aquagenic urticaria), sweat (cholinergic urticaria) and other chemical compounds (contact urticaria) [5]. The disorders that call for differential diagnosis are those that were historically considered urticaria and syndromes that include urticaria/angioedema, such as urticaria pigmentosa (mastocytosis), urticaria vasculitis, bradykinin-mediated angioedema, exercise-induced anaphylaxis and some autoinflammatory syndromes [4]. The symptoms of some of these disorders manifest in childhood, so it is unlikely that patients with such pathologies will choose the considered occupations in their adulthood. The disorders may also be divided by the causative factor, e.g., into pseudo-allergic and stress-induced urticaria.

There are no reliable data on the prevalence of most of chronic urticaria. Chronic induced urticaria affects 0.5% of the population, but up to 70% of patients experience systemic reactions, including severe anaphylaxis [5]. The literature has 4–11.2% of the population suffering from cholinergic urticaria, while a third of all induced urticaria cases are cold urticaria [6].

Physical exercise-induced urticaria

Cholinergic urticaria (CU) occurs when the body's temperature increases following physical exercise, a stressful situation, being in a stuffy room, taking a hot bath. It has characteristic clinical manifestations, but the exact pathogenetic mechanism is still not fully clear. There are four subtypes of CU distinguished: CU with occlusion of pores; CU with acquired generalized hypohidrosis; CU with sweat allergy; idiopathic CU [7].

To understand the mechanisms behind occurrence of CU, some authors studied the role of hypersensitivity to the autologous sweat antigens in the chronic CU's pathogenesis [8, 9]. In 2010, researchers conducted an experiment that allowed discovering that acetylcholine induces degranulation in a dose-dependent manner, which conditions disturbance of expression of cholinergic receptor muscarinic 3 (CHRM3). CHRM3 is not expressed in the area of anhidrosis, but its expression persists to a small extent in the hypohydrotic area. Histological analysis revealed an infiltrate of $CD4^+$- and $CD8^+$-T-cells around endocrine glands in the anhydrotic area. The authors suggested that in the hypohydrotic area of the skin, exercise induces release of acetylcholine, which is not completely captured by the receptors of sweat glands (as in normal sweating) and affects the neighboring mast cells (MC), which can produce histamine in response to acetylcholine, since MC in the hypohydrotic area express CHRM3 [10].

The subtypes of urticaria most commonly seen in athletes are acute forms caused by physical stimuli such as exercise, temperature, sunlight, water, or certain levels of external pressure. CU is the most common type of physical urticaria registered in athletes under 30 [11].

Unfortunately, in addition to skin rashes, a number of patients developed anaphylaxis and bronchial obstruction. In the first place, the aggravation was seen in young people who were actively practicing heavy physical exercise, e.g. military personnel [12].

Exercise-induced anaphylaxis is a specific life-threatening reaction that occurs very unpredictably in susceptible individuals with CU [13]. People whose jobs are considered extreme do not perform optimally against the background of severe hypotension, fainting, or laryngeal edema. Typically, such symptoms are effectively controlled with appropriate medications, which, however, often have side effects that are unacceptable in high-risk situations. The literature describes four cases of CU in US Air Force pilots [13].

Other authors resorted to differential diagnosing while examining individuals presenting dermatological and systemic symptoms post-exercise. In particular, they aimed to differentiate between CU and exercise-induced anaphylaxis. In both cases, the symptoms were triggered by MC degranulation with the release of vasoactive substances. The exercise-induced anaphylaxis and CU were differentiated between relying on the urticaria morphology, anaphylaxis reproducibility, progression and response to passive warming. The diagnosis was made after a thorough history study and examination of the morphology of the lesions. Treatment for acute episodes of exercise-induced anaphylaxis included cessation of exercise, adrenaline and antihistamines. Further therapy required changes to or abstinence from exercise, prevention of co-factors and prophylactic use of drugs (antihistamines, MC stabilizers etc) [14].

There are recorded cases of food anaphylaxis and exercise-induced urticaria. This is a rare condition, which has postprandial exercise causing anaphylaxis. One of the reviews presents the definition, etiology and pathogenetic mechanisms underlying this disease [15]. The review reports a number of foods, including wheat, eggs, chicken, shrimp, shellfish, nuts, fruits and vegetables, that can trigger this pathology; it also declares that exercising after meals can stimulate the release of mediators (mast cell mediators) from IgE-dependent MC, which leads to urticaria and anaphylaxis once a certain exercise level threshold has been exceeded. Also, it is reported that high-intensity physical loads are more likely to provoke an attack than low-intensity and low-frequency exercise. Several other factors, such as physical and mental stress, fatigue, dry air, inadequate sleep, runny nose, wet weather and low temperatures, aggravate anaphylaxis. Some researchers have stated that intense and prolonged exercise promotes the conversion of Th1 lymphocytes to Th2 lymphocytes, with an increase in the production of Th2 cytokines. However, the exact pathogenesis underlying exercise-induced anaphylaxis is unknown. It has been suggested that exercise lowers the MC degranulation threshold. Another study showed that exercise disrupts digestion, and eating food abundant in allergens leads to an increase in the concentration of allergenic proteins in blood and to IgE-mediated sensitization of MC. Continued consumption of allergenic food led to MC degranulation, release of histamine, development of urticaria, angioedema, decreased blood pressure and fainting [15].

There are some conditions that modulate the onset of anaphylaxis as concomitant or potentiating factors that trigger it even when the allergen is consumed in small doses. The most frequently described factors of this kind are physical exercise, alcohol, certain foods, nonsteroidal anti-inflammatory drugs (NSAIDs) and concomitant infectious diseases [16].

One study describes skin tests with food allergens, which suggests the assumption the disease has an IgE-mediated mechanism underlying it. However, regardless of the food taken, some patients were recorded to experience anaphylaxis intensified with additional exercise [17].

Another study reports registering various clinical symptoms in the course of examination of patients with chronic exercise-induced urticaria [18]. Some patients developed only a periorbital angioedema; others had giant urticaria, wheezing and hypotension; yet another group of patients exhibited clear signs of CU. Those with cutaneous or subcutaneous manifestations only had normal plasma histamine levels. The complement component levels (C3, C4) remained normal, regardless of the form of urticaria considered. The elevated plasma histamine levels were detected only against the background of systemic symptoms (hypotension etc) manifesting simultaneously [18].

In another study, some CU and anaphylaxis patients showed signs of activation of the alternative complement pathway, while other patients had CU at the outset and then saw it developing into angioedema and vascular collapse. Plasma histamine levels were elevated during anaphylaxis, but there was no evidence of complement activation [19].

Authors of study [20] have described the physical manifestations of two states closely resembling each other. The first is CU, that is, chronic urticaria caused by increased body temperature. The second is exercise-induced anaphylaxis. Anaphylaxis can be idiopathic, following a specific trigger (food, medication, or insect bite), or exercise-induced. Cholinergic urticaria is caused by exercise, increased body temperature, strong emotions, hot or spicy food, hot water shower. The disease is characterized by generalized erythema, urticaria (a blister of 2–4 mm surrounded by erythema) and pruritus. Many patients report tingling, pruritus, or burning of the skin before blistering. As the reaction progresses, the macula may coalesce to form large areas of erythema that become increasingly difficult to recognize as urticaria. The lesions can appear anywhere on the body, but usually they first manifest on the torso and the neck and then spread distally to the face and the limbs. In rare cases, the progression of urticaria includes systemic symptoms, such as hypotension, angioedema, and bronchospasm. Urticaria appears 6 minutes into a session of physical activity. The symptoms increase within 12–25 minutes. The pathogenesis was associated with elevated serum histamine levels during the attack. There was described a group of patients with type I allergy to their own sweat. Twenty patients underwent autologous sweat testing and showed an immediate cutaneous reaction. A subset of patients with CU symptoms had allergic urticaria, which manifested only with perspiration. Exercise-induced urticaria and anaphylaxis began 45 minutes into an exercise session. The major symptoms other than urticaria included bronchospasm, laryngospasm, and/or vascular collapse. Some other symptoms were sudden fatigue, feeling of fever, hot flushes, sudden pruritus, gastrointestinal upset, squeezed throat, voice changes, troubled breathing. In contrast to a CU situation, the size of the blister reached 10–15 mm. In the absence of control, urticaria, bronchospasm, and airway edema progressed to vascular collapse. The pathogenesis was conditioned by the sudden release of basophil and MC mediators, which was confirmed by the increased serum tryptase level. With time, patients developed exercise tolerance, i.e. the frequency of manifestations has decreased. It is explained by the fact that, over time, physical exercising lightens the leukocyte inflammatory response, slows the release of pro-inflammatory cytokines and dampens the regulation of expression of toll-like receptors 4 on the surface of immune cells. These are the mechanisms that reduced the systemic immune response to exercise [20].

Another work reports exercise-induced anaphylaxis accompanied by anaphylactic symptoms (cutaneous, respiratory, gastrointestinal and cardiovascular) after physical activity. Cofactors were identified in about a third of all such cases: food, temperature (warm or cold), drugs (especially NSAIDs). The researchers postulated some pathophysiological mechanisms, such as changes in gastrointestinal mucosa permeability (including growth thereof), changes in the level of tissue transglutaminase that enables IgE cross-linking, increased production of cytokines, blood redistribution during exercise that leads to alteration of the MC degranulation process, changes in acid-base balance and sensitization to wheat omega-5-gliadin (O5G) [21]. In 2020, the studies investigating O5G allergy were published. Patients with idiopathic urticaria and anaphylaxis were diagnosed to be sensitive to O5G. In both groups, the most common cofactor were physical exercises, followed by alcohol and NSAIDs [22].

Cold urticaria

Cold urticaria is of no less interest. In the overall population, the prevalence of cold urticaria is 0.05% [23]. The key pathophysiological mechanism behind the onset of chronic induced urticaria is the activation of skin MC. It is assumed that the factor triggering the said activation in chronic induced urticaria cases is the formation of autoantigens under the influence of physical factors [24]. Early studies of cold urticaria showed local release of histamine following cold stimulation [25].

Cold urticaria is characterized by blisters and angioedema developing after exposure to cold. According to a retrospective analysis, the mean temperature threshold of cold urticaria patients was 13.7 ± 6.0 °C (4–26 °C) [26]. Anaphylaxis may develop when swimming in water. The list of atypical cold urticarias includes atypical acquired cold urticaria, delayed cold urticaria, cold dermographism, cold cholinergic urticaria, systemic atypical cold urticaria. The distinction is made between primary and secondary cold urticaria. The possible causes of secondary cold urticaria are systemic diseases, monoclonal (IgG) or mixed (IgG/IgM, IgG/IgA) cryoglobulinemia, viral and/or bacterial infections, parasitic invasions, vasculitis [27].

Pseudoallergen-induced urticaria

There is a relationship between chronic urticaria and pseudoallergens. Pseudoallergens are low molecular weight compounds that can bind to the X2 receptor bound to G-protein on the MC membrane and lower the threshold for other factors to fully activate the MC mediator release capacity. The small size of these molecules renders direct IgE binding impossible, and there is no evidence that they act as haptens. However, it was established that the level of intestinal mucosa permeability increases under the influence of pseudoallergens and, consequently, diets limiting the intake thereof [28].

Stress-induced urticaria

Nociceptor neurons use many of the same molecular threat recognition pathways as immune cells. Responding to danger, peripheral nervous system cooperates directly with immune system to form an integrated defense mechanism. In combination with the high rate of neuronal transduction, the dense network of nerves in sensory and autonomic fibers of peripheral tissues enables rapid local and systemic neurogenic modulation of the immunity. Peripheral neurons also play an important role in immune dysfunction in cases of autoimmune and allergic diseases [29].

Human skin MC are closely associated with sensory nerve endings that release neuropeptides following antidromic stimulation by physical or chemical factors and stress. Recently, it has been again proposed to focus on the role of substance P (SP) in the development of chronic urticaria [30]. SP is involved in the activation and degranulation of MC. In turn, MC mediators, histamine and tryptase, can activate sensory nerves, supporting the interaction between MC and sensory fibers in MC-induced skin inflammation. Current data has the biological activity of SP manifesting not only through the Neurokinin-1 receptor (NK-1), but also through the Mass-related G-protein coupled receptor member X2, or MRGPRX2, with the subsequent activation of MC. MRGPRX2 was found

to be activated in the skin of severe chronic urticaria patients [31]. It has been noted that persisting stress and infectious processes in chronic urticaria patients can activate MC through activation of several neuropeptides and antimicrobial host defense proteins acting through MRGPRX2 [28].

Many authors confirm the involvement of SP in the pathogenesis of urticaria, since SP can cause pruritus and angioedema, degranulation of MC and basophils, and act as a MC sensitizer, i.e. increasing their sensitivity to various triggers [32]. Recent studies of urticaria patients have shown them to have significantly higher SP circulation levels, clearly dependent on the disease severity [33]. They also had a higher number of circulating SP-positive basophils [34]. SP has been shown to induce degranulation in basophils obtained from the chronic urticaria patients. Besides, SP may be involved in pseudo-allergic reactions and act as a histamine-releasing factor in patients with urticaria.

As early as in 2004, it was shown that cytosolic Ca^{2+} concentration triggers release of neuropeptides from the sensory nerve [35]. Cutaneous sensory nerves express MRGPR in addition to tension regulated Ca channels, the activation of which increases the concentration of cytosolic Ca^{2+}. MRGPR are involved in histamine-independent pruritus pathways. Their activation on MC causes severe itching, which subsequently leads to the destruction of skin cells and progression of the inflammatory process therein [36].

In addition, cationic channels expressed on sensory nerve endings include some transient receptor potential (TRP) channels that are involved in the release of neuropeptides. Triggering them results in the Ca^{2+} influx and the release of neuropeptides, such as SP and calcitonin gene-related peptide (CGRP), followed by neurogenic inflammation. TRP-mediated Ca^{2+} influx in the skin can regulate the proinflammatory cytokine gene expression by influencing the immune cells in addition to the neuropeptide release. TRPV1 is also found in skin cells that function as pain sensors for chemical stimuli, including keratinocytes, MC, dendritic cells, sebocytes, dermal blood vessels, hair follicles, and sweat glands [27]. In endothelial and smooth muscle cells, TRPV1-mediated Ca^{2+} influx induces vasodilation by releasing nitric oxide (NO). At the same time, TRPA1 is a non-selective Ca^{2+} channel that responds to cold sensations (< 17 °C), unlike TRPV1. TRPA1 is localized in about 60–75% of sensory C-fibers, which are also TRPV1-positive. Topical application of cinnamaldehyde (TRPA1 agonist) in human skin aggravates itching significantly, suggesting that TRPA1 plays a central part in the mechanism of pruritus [37]. The studies have investigated the role of TRPA1 in chronic skin inflammation. It is believed that TRP channels, especially TRPA1, act as a "gatekeeper" that mediates the transition of cytokine skin inflammation into the sensory nerve activation [38].

Much attention has been paid to the connection between urticaria and a high prevalence of depression, anxiety, and poor sleep quality. A TatTS study confirmed higher levels of depression and anxiety in individuals with chronic urticaria [19]. It was shown that urticaria has a negative effect on the quality of life and working efficiency [36].

The expression of the serotonin transporter protein (SERT) in the skin of chronic spontaneous urticaria patients was studied for association with depression and anxiety. The research uncovered the role played by SERT in the pathophysiological processes of inflammatory skin diseases. Chronic urticaria patients had higher SERT expression levels than patients from the control group [39]. There is mounting evidence that ongoing stress prolongs and worsens the course of chronic urticaria.

ACTH and its releasing factor were shown to activate basophils in chronic urticaria patients [40]. It was established that NLRP-3 inflammation grows against the background of depression and stress, which proves a link between psychological factors and exacerbation of urticaria as a result of emotional stress [41].

Another study [42] demonstrated that stress and severity of the disease condition the growth of level of C-reactive protein, interleukin 18 and the significant drop of the level of basal cortisol. Thus, chronic stress can contribute to the formation of a vicious circle in the urticaria pathogenesis [42].

In this connection, it should be noted that stress, leading to the release of sensory nerve neuropeptides, can alter the behavior of Langerhans cells in the dermis and the immune system of the skin, directing the immune response towards certain T-helper cells. In particular, CGRP stimulates Th17 cells, promoting inflammation by recruiting T cells and neutrophils [43].

Neuropeptides such as CGRP and VIP can activate dendritic cells to direct Th2-type immune response and suppress the Th1-type response by stimulating the production of certain cytokines and decreasing or increasing the migration of dendritic cells to the local lymph nodes [44, 45].

Considering that signaling molecules released from the peripheral sensory nerve fibers regulate not only the lumen of small blood vessels but also chemotaxis of the immune cells, their return to original state, maturation and activation, it becomes clear that neuroimmune interactions are much more complex [29].

Features of immune inflammation in rescuers and firefighters

The intensity of work of professional rescuers and the duration of their employment contribute to the development of dysfunctions of organs and systems. According to the somatic pathology laboratory diagnosing guidelines for rescuers and firefighters, common for immunological indicators are the increase in the relative and absolute number of cells with CD25, HLAII, CD95 markers, the IL1β spontaneous production level, an increase of the absolute number of lymphocytes, T-cells and T-helpers, a shift of the immune response towards Th2. Spontaneous production of TNF, IL1β is higher than the reference values, which contributes to the formation of chronic inflammation in the absence of an infectious agent. Progressive increase in the ultimate level of immunoglobulin E, which depends on the duration of active service, shows that rescuers grow sensitive to the inhaled and contact allergens [1].

Studying systemic inflammation in firefighters, researchers revealed that blood serum concentrations of IL8, VEGF and TNFα are significantly higher after participating in fire extinguishing than during rest periods [46]. After exposure to smoke, the levels of circulating cytokines were higher than usual, which stimulated bone marrow [47] and initiated a systemic inflammatory response to smoke inhalation. IL8, being a potent bone marrow stimulant, promoted the migration of neutrophils into the lung tissue [48], which intensified and prolonged neutrophilic inflammation in the bronchi and maintained systemic inflammation related to smoke inhalation.

Conclusion

This literature review presents the available data on the mechanisms of occurrence of chronic urticaria, which are most common in high-risk occupations. The formation of chronic inflammation in the absence of an infectious agent is beyond doubt, and the search for informative biomarkers continues. There is no doubt about the role of SP in the development of

inflammatory process. However, further research is required to clarify its significance. Understanding the pathogenetic mechanisms of immune inflammation will improve the diagnosing of these conditions and optimize the related prevention measures.

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RESULTS OF THE 67-TH SESSION OF THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF THE ATOMIC RADIATION (UNSCEAR)

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The 67-th Session of the United Nations Scientific Committee on the Effects of the Atomic Radiation (UNSCEAR) took place in the form of videoconferences during 2-6 November 2020. Within the framework of the meetings of the Working group and subgroups the documents of the following projects were discussed: R.741 «Evaluation of medical exposure to ionizing radiation»; R.742 «Levels and effects of radiation exposure due to the accident at the Fukushima Daiichi nuclear power station: implications of information published since the UNSCEAR 2013 report»; R.743 «Biological mechanisms relevant for the inference of cancer risks from low-dose and low dose rate radiation»; R.744 «Evaluation of occupational exposure to ionizing radiation»; R.745 «Second primary cancer after radiotherapy»; R.746 «Epidemiological studies of radiation and cancer»; R.747 «Evaluation of public exposures to ionizing radiation from natural and man-made sources»; Project 67/7 «Implementation of the Committee's strategy to improve collection, analysis and dissemination of data on radiation exposure». The Committee also discussed the future research program (2020–2024); report to the UN General Assembly; public outreach activity including the strategy for the period 2020–2024.

Keywords: 67th UNSCEAR Session, low doses, biological effects, epidemiology, medical exposure, occupational exposure

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ИТОГИ 67-Й СЕССИИ НАУЧНОГО КОМИТЕТА ПО ДЕЙСТВИЮ АТОМНОЙ РАДИАЦИИ ООН

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В период со 2 по 6 ноября 2020 г. в формате видеоконференции прошла 67-я сессии Научного комитета по действию атомной радиации ООН (НКДАР ООН). В рамках совещаний Рабочей группы и подгрупп состоялось обсуждение документов по ряду проектов: R.741 «Оценка облучения пациентов от медицинских источников ионизирующего излучения»; R.742 «Уровни и эффекты радиационного облучения, обусловленного аварией на атомной станции «Фукусима-1»: последствия информации, опубликованной после выхода отчета НКДАР ООН 2013 г.»; R.743 «Биологические механизмы, влияющие на прогнозируемые риски рака при воздействии радиации в малых дозах и с низкой мощностью дозы»; R.744 «Оценка облучения персонала от источников ионизирующего излучения»; R.745 «Вторичные раки после радиотерапии»; R.746 «Эпидемиологические исследования радиации и рака»; R.747 «Облучение населения от естественных и искусственных источников»; проект 67/7 «Реализация стратегии Комитета по совершенствованию сбора, анализа и распространения данных по радиационному облучению». Комитет также обсудил будущую программу исследований (2020–2024); отчет Генеральной Ассамблее ООН; информационную и просветительскую деятельность НКДАР ООН, включая стратегию на период 2020–2024 гг.

Ключевые слова: 67-я сессия НКДАР ООН, малые дозы, биологические эффекты, эпидемиология, медицинское облучение, профессиональное облучение

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The 67th session of UNSCEAR took place from 2 to 6 November 2020, attended by more than 150 experts from 25 UNSCEAR Member-States (the Russian Federation, Argentine, Australia, Belarus, Belgium, Brazil, the UK, Germany, Egypt, India, Indonesia, Spain, Canada, China, the Republic of Korea, Pakistan, Peru, Poland, Slovakia, the USA, Ukraine, Finland, France, Sweden, and Japan), 4 observer-countries (Algeria, the Islamic Republic of Iran, Norway, and the United Arab Emirates) and also representatives of 8 international organizations:

– United Nations Environmental Programme (UNEP);
– International Atomic Energy Agency (IAEA);
– International Labour Organization (ILO);
– International Agency for Research on Cancer (IARC);
– World Health Organization (WHO);
– European Commission (EC);
– Food and Agriculture Organization (FAO);
– International Commission on Radiation Units and Measurements (ICRU).

The Session took place as an online meeting. The Russian delegation included 11 experts: A. V. Akleyev (the RF representative in the UNSCEAR, URCRM of the FMBA of Russia), T. V. Azizova (deputy RF representative in the UNSCEAR, SUBI of the FMBA of Russia) and S. A. Romanov (SUBI of the FMBA of Russia), V. K. Ivanov (A.Tsyb MRRC – NMRRC branch of the FMBA of Russia), S. M. Kiselev and S. M. Shinkarev (SRC-FMBC of the FMBA of Russia), E. M. Melikhova (the Nuclear Safety Institute of the Russian Academy of Sciences), S. G. Mikheenko and V. Yu. Usoltsev (Rosatom), R. M. Takhauov (SBRC of the FMBA of Russia) and S. V. Fesenko (RIRA of the Ministry of Science and Higher Education of the Russian Federation).

Gillian Hirth (Australia) was Chair, Anna Friedl (Germany), Jing Chen (Canada) and Jin Kyung Lee (Republic of Korea) were Vice-Chairs, and Anssi Auvinen (Finland) was Rapporteur for the 67th UNSCEAR Session. Borislava Batandjieva-Metcalf was UNSCEAR Secretary.

Within the framework of the 67th UNSCEAR Session final scientific documents, progress reports, future programme of work of the Committee for 2020–2024, and Report to the UN General Assembly have been reviewed and discussed.

The Committee deliberated the following scientific documents which are to be finalized in 2020:

– R.741 «Evaluation of medical exposure to ionizing radiation»;
– R.742 «Levels and effects of radiation exposure due to the accident at the Fukushima Daiichi nuclear power station: implications of information published since the UNSCEAR 2013 report»;
– R.743 «Biological mechanisms relevant for the inference of cancer risks from low-dose and low dose rate radiation»;
– R.744 «Evaluation of occupational exposure to ionizing radiation»;

The Committee also reviewed the current status of the following reports:

– R.745 «Second primary cancer after radiotherapy»;
– R.746 «Epidemiological studies of radiation and cancer»;
– R747 «Evaluation of public exposure to ionizing radiation from natural and man-made sources»;
– Project 67/7 «Implementation of the Committee's strategy to improve collection, analysis and dissemination of data on radiation exposure».

Besides, public outreach activity of the Committee including the strategy for the period 2020–2024, and new projects which are planned to begin in 2021–2024 were also discussed.

Preparation for the session was carried out in two stages. At the first stage (13, 14 and 16 July, 2020), delegates of the UNSCEAR State members deliberated the following documents online: R.741 «Evaluation of medical exposure to ionizing radiation»; R.742 «Levels and effects of radiation exposure due to the accident at the Fukushima Daiichi nuclear power station: implications of information published since the UNSCEAR 2013 report»; R.743 «Biological mechanisms relevant for the inference of cancer risks from low-dose and low dose rate radiation»; and R.744 «Evaluation of occupational exposure to ionizing radiation». After discussion, the Working Groups finalized the documents and introduced their updated versions during the 67th Session.

The RF delegation took an active part in the preparation of the scientific documents, progress reports and future programme of work sending their comments and suggestions on the topics discussed before the 67th Session. The members of the Russian delegation also participated in the discussion of the session materials during the 67th UNSCEAR Session. In general, consideration and discussion of the scientific documents, progress reports and future programme of work went productively with an active engagement of all UNSCEAR Member-states.

Results of the discussion of the scientific documents
Document R.741 «Evaluation of medical exposure to ionizing radiation»

The document «Evaluation of medical exposure to ionizing radiation» was approved for publication at the 67th UNSCEAR Session. Compared with the previous publication, the current report tested a new approach to data stratification of global assessment of medical exposure of population. Previously, all countries were stratified into four categories according to the number of physicians per 1,000 people and for each, average frequency and mean dose were calculated for each type of procedure according to the available data. Then the average values in this category were extrapolated to the whole of the country. This approach is applicable provided that the world's population is more or less evenly distributed across all categories. However, over the past 10 years, there has been a major demographic shift in the world and this condition is no longer met. Therefore, an alternative approach has been used. It is based on the World Bank (WB) classification. Countries are divided according to the level of gross national income per caput into four groups: low, lower middle, upper middle and high. Over the past decade, these groups included 9%, 39%, 36% and 16% of the world's population, respectively. Since WHO also uses the WB classification, it becomes possible to compare data on medical exposure with other indicators of public health services collected by WHO. An important feature of the methodological part of the prepared document is the development of a methodology for assessing the uncertainty of the data presented. The document presents errors (standard deviations) for global indicators of frequencies of different examinations and collective effective dose.

The Committee reviewed the results of the assessment of medical exposure in the light of its previous UNSCEAR 2008 report and made the following conclusions. Medical exposure of patients in quantitative terms remains the most significant source of radiation exposure of the population. The annual collective effective dose to the world population is 4.2 million man Sv, the annual per caput effective dose is 0.58 mSv. In general, the population exposure data are comparable to the results from the UNSCEAR 2008 document (0.65 mSv) taking into account the uncertainty which is about 30%. When analyzing UNSCEAR documents since 1988, there is

a tendency to an increase of annual collective effective doses due to sources of medical exposure (0.37 mSv in 1988). As expected, the greatest contribution to a collective dose is made by computed tomography (CT) (62%) with a 10%-contribution to the structure of medical diagnostic studies associated with patient exposure. The second place, taking into account the dominant number (63%) in the structure of medical diagnostics, is taken by X-ray and radiological procedures; their contribution to the collective dose is 23%. Interventional radiology accounts for only 0.6% of all procedures, but it accounts for 8% of the total collective dose. Diagnostic nuclear medicine takes 1% of all procedures and 7% of the total collective dose. The contribution of CT to the total collective effective dose increased from 37 to 62%, the share of interventional radiological procedures in the structure of the collective dose increased 8 times if compared to the estimates of 2008. At the same time, the contribution of research related to the application of nuclear medicine increased by only 1.4 times, remaining a minor component in the structure of medical diagnostics and treatment (1%).

Despite the fact that the Committee did not take into account the contribution to the collective dose from therapeutic procedures, the intensity of their use in medical practice has sharply increased. The number of radionuclide therapy procedures increased by 60%, radiation therapy — by 22% compared to the data of the previous UNSCEAR report.

It should be emphasized that medical exposure of the population is clearly correlated with the level of well-being of countries. Thus, in countries with high per capita income, the number of diagnostic procedures is 18 times higher than in low-income countries: the former account for about 70% of all medical radiological studies and 75% of the collective dose. This is reflected in the indicators of the annual dose and the collective dose to the population of the country as a whole (13 and 22 times higher, respectively).

To obtain assessments, the Committee for the first time used a system for collecting information in the form of questionnaires on medical exposure, which were sent to the participating countries for filling in and submitting national data to the UNSCEAR. This system allowed a significant increase in the amount of data for making estimates, but the uncertainty of the results also increased significantly. This is due to the inability of countries to provide the full amount of data requested, for example, on gender and age distributions of patients and on measured dose characteristics due to the lack of centralized data collection systems in most countries. Therefore, the key data sources in the preparation of the report were the results of the analysis of scientific literature, as well as the WHO resource base. Representatives of different countries, including Russia, proposed to optimize the questionnaire, focusing on the structure of radiation diagnostics and dosimetric parameters for calculating effective doses, and using dose coefficients for basic X-ray and radionuclide studies. An important result of the activities carried out by the Committee on data collection in the field of medical exposure was the understanding of the need to improve the existing data collection system in Russia, which is currently based on No.3-DOZ statistical reporting form (a form of Federal State Statistical Observation used by Rospotrebnadzor to collect information on exposure doses of patients during medical X-ray and radiological studies in order to protect the well-being of citizens of the Russian Federation) and form No. 30 of the Ministry of Health of Russia (Federal Statistical Observation form № 30 "Information about a medical organization"). The existing domestic statistical forms can be updated taking into account the presented methodology of data collection within the UNSCEAR project, which will significantly increase the reliability and volume of information provided, including for internal use.

As for the scientific aspect of the issue of discussing the exposure doses of the population from medical radiation sources, it is necessary to emphasize the importance of the discussion about the correctness of using the concepts of “effective dose” and “collective effective dose” in the analysis of medical exposure. Taking into account the irregularity of the exposure of patients during diagnostic procedures, it is advisable to use the absorbed dose in the organ. This issue is especially acute in the field of nuclear medicine. It is pointed out that when considering the issues of medical exposure, special attention should be paid to the assessment of individual rather than collective exposure doses. The position of the Committee on this issue is that the report does not aim at assessing the risks from medical exposure, but solves the problem of identifying trends in medical exposure of the population and comparing different types of radiation exposure procedures. It is recommended to emphasize in the text of the document that the estimate of the collective effective dose should not be used to assess the risk of medical exposure in epidemiological studies.

In conclusion, a few words should be said about the new methodology for assessing uncertainties. It is clear that this innovation is of fundamental importance, since we are talking about the correct accounting of statistical errors and the statistical significance of the identified global trends. The methodology presented in the document is the first step on this path. Therefore, it is not surprising that the final estimates of the uncertainties of global indicators in some cases look overly optimistic, if we take into account the quantity and quality of the initial data, the accuracy of modelling and some other points. The new methodology needs to be improved, and it should be done in parallel with the improvement of the initial data collection system. However, the discussion showed that experts working on the topic of medical exposure still consider the estimates of uncertainties as irrelevant information, which is clearly discordant with the Committee's approach to the use of statistical errors in medical records, for example, in radiation epidemiological studies. This situation will require correction in the very near future.

Document R.742 «Levels and effects of radiation exposure due to the accident at the Fukushima Daiichi nuclear power station: implications of information published since the UNSCEAR 2013 report»

The structure of the document follows the structure of the UNCEAR 2013 report. The new document contains seven thematic sections in addition to the introduction and conclusion:

– Releases of radionuclides to the atmosphere, their dispersion and deposition.

– Releases to the marine environment, their dispersion and deposition.

– Transfer in terrestrial and freshwater environments.

– Exposure of members of the public.

– Exposure of workers.

– Health implications for the public and workers.

– Exposures and effects for non-human biota.

The document confirms the main conclusion made in the UNSCEAR 2013 report that the radiation doses to the public and workers and, accordingly, the radiation risks were very low. It is expected that the health effects of radiation exposure will not be discernible against the background of spontaneous diseases in the population. At the same time, a number of changes were made to the text of the document R.742 in

comparison with the UNSCEAR 2013 report, including clarifications on the assessment of exposure doses to the population.

The latest estimates of the total release of radioactive substances into the environment as a result of the accident, taking into account the analysis of all currently available data, have not fundamentally changed and do not contradict the previously published estimates. According to recent studies, ^{131}I and ^{137}Cs releases (two of the most significant radionuclides from the perspectives of population and biota exposure) are estimated to be 120 PBq and 10 PBq, respectively. And it is considered that about 80% of the total release was dispersed over, and deposited on to, the Pacific Ocean.

It should be noted that during the preparation of the UNSCEAR 2013 report, the group of specialists preparing the report was tasked to make realistic assessments of the levels of exposure of workers, public and biota. However, very limited information on the results of measurements of the radionuclide content in environmental media, food, human body after the accident did not enable in the previous report to fully rely on objective measurement data when calculating radiation doses. The assumptions used turned out to be conservative and led to overestimation of doses. In this document, the most significant changes in models, methods and data that influenced the calculation results were related to the following aspects.

– A more realistic description of the dynamics of the release of radioactive material, their dispersion and surface deposition. At the same time, a large number of measurement results of the radionuclide content in environmental objects accumulated over the past years were used.

– An improved empirical model to estimate doses of external exposure of the population from radionuclide deposits was developed and validated by actual data. The model is based on long-term measurements of dose rate on the ground with different landscape and soil types typical of Japan.

– A special biokinetic model was developed specific to the Japanese population, taking into account the high content of stable iodine in the traditional daily diet of the Japanese. This model made it possible to more realistically estimate the exposure doses to the thyroid in the population from inhaled and ingested radioiodine. Within the framework of this model, for the same intake of radioiodine, the thyroid exposure doses are reduced by about two times

– When assessing the exposure doses to the population the numerical values of the parameters were corrected in the calculation models, taking into account the real picture of both the conditions of external exposure and the pathways of radionuclide intakes into the body when assessing internal exposure doses. For example, it was taken into account that the concentration of radionuclides inside dwellings was reduced by about two times compared to their concentration outside buildings, which led to a twofold decrease in internal exposure doses through the inhalation pathways.

– Instead of the previously used conservative parameters of the population's dietary intake of radionuclides, the new document adopted a more realistic approach based on the actual composition of the foodstuffs of the residents of the contaminated area in the months immediately following the accident. According to refined calculations, internal exposure doses of the population from ingestion declined by about 10 times.

The refinements introduced into the computational models led to a decrease in the average effective doses of the population for municipalities and prefectures by several tens of percent, and in terms of thyroid doses — by about two times. It is important to emphasize that the structure of revised thyroid doses in the population has changed. The contribution from the intake by radioiodine inhalation turned out to be more significant compared to the dietary intake after the Fukushima accident, which differs significantly from the picture of the formation of thyroid doses in the population after the Chernobyl accident, where the dominant pathway of radioiodine intake was the consumption of contaminated fresh cow milk by the residents from pasture grazed cows.

For the population of municipalities that was evacuated in the first days after the accident, the mean effective doses to 1-year-old infants in the first year after the accident were in the range of 0.2–8.0 mSv, and the average absorbed thyroid doses were in the range of 2.0–30.0 mGy. Mean exposure doses to adults were 70% lower than to 1-year-old infants in relation to the effective dose and 50% in relation to the absorbed thyroid dose. For municipalities in Fukushima Prefecture that were not evacuated in the first days after the accident, the estimated mean effective doses to 1-year-old infants in the first year were in the range of about 0.1 mSv–5 mSv, and the mean absorbed thyroid doses were in the range of 1.0–20.0 mGy. The dose ratios between adults and 1-year-old infants were similar to those obtained for evacuated residents. According to the calculations, in general, in the whole of Fukushima Prefecture (population about 2 million people) a few hundred infants have been estimated to have received absorbed doses to the thyroid of more than 100 mGy.

The document notes that the UNSCEAR 2013 reported dose estimates of workers involved in mitigation at the Fukushima Daiichi Nuclear Power Station site remain generally valid. The average effective dose of the 21,135 workers from March 2011 to the end of March 2012 was about 13 mSv. About 36% of the workforce received total effective doses of more than 10 mSv over that period, while 0.8% received doses of more than 100 mSv. The new document revised the individual dose estimates for six workers with effective doses over 250 mSv. As a result of individual measurements of thyroid size for the six workers and taking these data into account in the dose estimates, the calculated values of the absorbed thyroid dose increased for five of them, and declined for one person. For one of the workers, the thyroid exposure dose increased by almost a factor of three. The maximum value of the thyroid dose of workers due to the inhaled radioiodine is estimated as 32 Gy.

The UNSCEAR 2013 report documented no adverse health effects among Fukushima residents that could be directly attributed to radiation exposure from the Fukushima Daiichi Nuclear Power Station accident. The updated estimates of doses to members of the public have either decreased or are comparable with the Committee's previous estimates. The Committee therefore continues to consider that future health effects directly related to radiation exposure are unlikely to be discernible from pre-existing diseases. The document stresses that although a large number of thyroid cancers have been detected among the Fukushima Prefecture residents exposed in childhood, increase in the number of thyroid cancers results not from radiation exposure, but rather from ultrasensitive thyroid screening procedures.

Despite the fact that red bone marrow dose estimates in the population did not increase, estimates of leukemia risk per mGy increased somewhat compared to the UNSCEAR 2013 estimates. However, the application of risk models based on the experience of analyzing the consequences of the atomic bombing of Hiroshima and Nagasaki, any cases of an increase in the number of cases of leukemia with the obtained estimates of exposure doses to red bone marrow after the Fukushima

accident are unlikely to be reliably identified in any age group of the population. However, at such low doses of red bone marrow exposure statistically significant increase of the leukemia risk in population is hardly to be observed in the future either. Likewise, the levels of public exposure have been too low to expect discernible increases in the incidence of breast cancer or other solid cancers.

With regard to the exposure of clean-up workers, it was also noted that since most workers received low exposure doses (effective doses for the first year are less than 10 mSv), it is unlikely that an increase in the incidence of leukemia, solid cancers, including thyroid cancer, will be reliably detected in clean-up workers. The report points out that the information is rather limited to make a reliable judgment about the risk of cataract.

In the section "Radiation exposures and effects on non-human biota" it is stated that regional impacts on wildlife populations would have been unlikely although detrimental effects on individual organisms might have been possible. A few scientific publications have indicated various cytogenetic, physiological and morphological (sublethal) effects in some plants and animals that have been observed in areas of increased radiation levels following the Fukushima accident in the absence of any reported wide-scale group impacts. In contrast, substantial population- and ecosystem-level impacts on selected wildlife groups were observed in areas of increased radiation level due to fallout following the Chernobyl accident.

An obvious drawback of this section is the use of criteria that exclude the effects of chronic low-dose exposure of biota. This is primarily due to gaps in the recommendations of the International Commission on Radiological Protection (ICRP) regarding biota protection. It was noted that many of the effects in plants and animals described in publications were incorrectly associated with exposure after the Fukushima Daiichi Nuclear Power Station accident, and the obtained data need to be reassessed. It should also be noted that the follow up period after the Fukushima Daiichi Nuclear Power Station accident is insufficient to detect many population effects in biota, such as radioadaptation or effects associated with radiation-induced genome instability. It explains the necessity to conduct further studies in the near zone of the Fukushima Daiichi Nuclear Power Station, taking into account the experience gained in similar Chernobyl studies.

Document R.743 «Biological mechanisms relevant for the inference of cancer risks from low-dose and low dose rate radiation»

The expert group chaired by Simon Bouffler have done great job on preparing the final document. The experts were able to compile in a document sufficient data on the low-dose and low dose rate effects that could be involved in carcinogenesis. The document comprises mainly the new data obtained over the last 10 years. The presented results of the studies are well arranged in accordance with the levels of organization of living systems. The focus in the document is made on the response of the cells, including the stem cells, to low doses: DNA damage, damage of signaling pathways, epigenetic changes, chromatin remodeling, changes in the gene and protein expression. DNA reparation, adaptive response and non-targeted effects have been considered. Some attention has been paid to the low-dose effect on non-nucleated cellular components.

Despite great job done it is clear that nowadays it is still not possible to form a systemic view of cancer mechanisms following exposure at low doses. The authors centered on the cellular response to low doses. However, the reactions of tissues, organs and body as a whole practically were not considered. No data are presented on the status of the local anti-tumor immunity which has a profound impact on the tumor progression. The low-dose effect on the endocrine system which contributes to the induction of such hormone-dependent cancers as breast cancer has not been given any consideration.

Another issue which has not been covered by the report concerns the specificity of carcinogenic mechanisms of the low doses. This issue is rather complicated and requires thorough analysis. The authors declared in the document that they examined only the role of dose-dependent effects in carcinogenesis, i.e. of the effects that from their point of view are radiation-induced. Such approach seems dubious for a number of reasons. First of all, dose dependence is most often registered for the effects of high doses. Moreover, low-dose effect and especially of functional responses of the cells (for example, changes in the gene expression and apoptosis) is characterized by triggering mechanism. That is why the analysis of the carcinogenesis mechanism at low doses based only on the dose dependent effects does not seem justified.

The weakest part of the document is the analysis. It is obvious that with the accumulation of the new facts on the low-dose effects it is necessary to analyze them. In this regard, it would be important to analyze whether the newly revealed effects of low doses that from the point of view of the authors are relevant to the carcinogenic mechanisms conform to the present day radiobiological and oncological concepts and theories of the carcinogenesis.

Unfortunately, such issue as radioadaptation (in published scientific papers it is also known as radiation adaptive protection) which is crucial for the understanding of the mechanism of low doses was not touched upon in the document. Today it is evident that without understanding of this phenomenon it is basically impossible to understand the mechanisms of biological effects of low doses. The document discusses the radioadaptive response of the cells. However the mechanisms of the adaptation of tissues, organs, and body as a whole were not considered. Even though the document has one section devoted to the mechanistic models of cancer, which addresses initial and very limited processes of malignant transformation of a cell following the exposure, but apparently it is not enough.

Nevertheless, despite the noted weak points of the Report which are objective and are connected to the shortage of such data, the document could be viewed as the basis for future studies. It should be stated that today it is impossible to develop a comprehensive view of the cancer mechanisms following the low-dose and low dose rate exposure. Taking into account the fact that the report uses a lot of special terminology and abbreviations that are accepted in immunology, molecular genetics and other fields of studies it is important to recommend to make a list of abbreviations and terms. In general the document should be regarded as very important for the future work of the Committee.

Document R.744. «Evaluation of occupational exposure to ionizing radiation»

Since 1977, UNSCEAR has been publishing reports on the assessment of occupational exposure levels. Estimates presented in the given document are based on data obtained from UN Member States over the period from 2010 through 2014, supplemented by information published in national reports and open sources. The principal objective of the report

is to assess the exposure of workers of various professional groups from sources of ionizing radiation based on the criterion of the average annual effective exposure dose.

To achieve the set goal, it was intended:

– to estimate the worldwide level of occupational exposure within various professional sectors;

– to identify new groups of workers receiving high doses of radiation in connection to the introduction of new technologies using radiation sources;

– to assess the impact of changes in regulatory standards or requirements on the tendency of dose formation.

Unlike previous reports, much attention is paid to the effects of natural sources of ionizing radiation. The document presents the analysis of the information on four sectors related to the impact of natural radiation sources on:

– aircrew and space crew;

– workers in mining and processing industry;

– workers engaged in gas and oil extraction industries;

– radon exposure in workplaces other than mines.

Data on occupational exposure to man-made sources of radiation include:

– nuclear fuel cycle;

– medical use of radiation (including veterinary medicine);

– industrial use of radiation;

– various groups of workers not included in the sectors described previously, including educational establishments; management of radiation sources used in industry, science and medicine; transport of radiation sources and radioactive materials. It also includes an assessment of the impact of man-made radiation sources when used for military purposes.

The approach used by the expert group was to provide a basic rationale for the methodology for estimating doses of cosmic ray exposure, external and internal exposure doses, radon exposure doses and doses to the lens of the eyes, based on the recommendations of the International Commission on Radiation Units and Measurements (ICRU) and ICRP.

Criteria for selecting the workers to be monitored and exposure to be recorded differ considerably between countries. Some countries monitor only exposed workers, while others include non-exposed workers in their individual monitoring programs for various reasons. Moreover, exposure due to radon is often underestimated, as in many countries the exposure dose is registered only if the concentration of radon in the air exceeds 1,000 Bq/m3 in the workplace.

As it has been mentioned, four groups of countries are distinguished based on the income level: low income, lower-middle, upper-middle and high income. At the present stage of data collection, it seems possible to assess trends in personnel exposure only for countries with a high level of economic development. Attempts by the working group to extrapolate the resulting methodology to low- and middle-income countries were unsuccessful. First of all, this can be explained by the low efficiency of collecting data on occupational exposure in these countries.

By the end of 2020, it is planned to complete work on the assessment of exposure levels during the reprocessing of spent nuclear fuel, oil and gas extraction, and in veterinary medicine. The report is scheduled to be published in 2021. The UNSCEAR Secretariat expressed gratitude to international organizations for their contribution to the preparation of the report.

Document R.745 «Second primary cancer after radiotherapy»

The project was approved by the Committee for inclusion in the program of work at the 65th session of UNSCEAR. At the 66th session, the document was designated a “high priority” document and a group of experts was established to prepare it. At the 67th session of UNSCEAR, a group of experts presented to the Committee a progress report and a clear work plan with specific timelines.

The presented report includes a detailed description of the principles of literature search by agreed and approved keywords; selection criteria for literary sources; structure of tables to describe the results of studies of individual organ-specific cancers and meta-analyses performed to refine risk estimates; as well as the full content of the report. From the point of view of the experts, the document should contain the following main sections:

– dosimetry, including an overview of dosimetry quantities, calculation of exposure doses in various treatment protocols; dose reconstruction using physical and computational phantoms, detectors and dosimeters; methods for measuring and calculating the uncertainty of exposure doses;

– radiobiology, including the description of the molecular mechanisms of radiation-induced cancer development, assessment of the contribution of other factors (sex, age, lifestyle, environmental factors, etc.); the role of the microenvironment in the development of second cancer; mathematical models allowing to predict radiotherapy induced cancer risk; biological dosimetry;

– oncology, including definitions and diagnostic criteria for second primary cancers after radiotherapy; description of genetic tests; type and incidence of certain second cancers (breast cancer, lung cancer, malignant neoplasms of lymphoid and hematopoietic tissue, sarcoma, thyroid cancer, brain cancer, etc.); prognosis and prevention of second cancers after radiotherapy;

– epidemiology, including the literature review of data on the incidence and lifetime risk of second primary cancers following radiotherapy, including for the selected cancer sites listed above; risk prediction models; comparison of second primary cancer risk from radiotherapy to cancer risk from other radiation exposures; limitations of existing evidence of risk, major uncertainties and gaps in knowledge;

– conclusion and recommendations.

The Committee unanimously endorsed the work done, emphasizing the urgency and importance of this problem for society in connection with the increase in the number of diagnostic methods and therapy using sources of ionizing radiation and the increase in life expectancy after treatment of the first cancer.

When discussing further work plans, the Committee recommended that the experts in a future report: a) present clear criteria for the differential diagnosis of second cancers following radiotherapy and metastatic neoplasms; b) consider issues related to individual radiosensitivity; c) clarify the effect of additional types of treatment (chemotherapy, hormone therapy, etc.) on the risk of second cancer development; d) evaluate the impact of diagnostic methods (CT, nuclear magnetic resonance imaging, etc.) on the risk of second cancer development following radiotherapy.

Document R.746 «Epidemiological studies of radiation and cancer»

Currently, the Committee is carrying out another project devoted to the study of the relationship between radiation and cancer, which testifies to the urgency of this problem. Over the 75 years after the atomic bombing of Hiroshima and Nagasaki, as a result of the rapid development of atomic power engineering and extensive use of radiation sources, considerable amount of scientific data has been accumulated

on the consequences of radiation exposure to human health as a result of radiological emergencies, occupational and medical exposure, as well as exposure as a result of radioactive contamination of the environment. The aim of the project is to publish a comprehensive scientific review prepared using the evidence obtained after the release of the UNSCEAR 2006 report (Annex A. Epidemiological studies of radiation and cancer). Risk estimates from the study of the Japanese cohort have not changed appreciably over the last several decades, and there has been minimal change in estimates of radiation detriment. However, updated data are important and necessary. During the 66th session in 2019, a decision has been made to analyze epidemiological studies conducted in various research centers. Out of several thousand scientific papers 561 relevant articles have been selected to be used in preparation of the evidence-based scientific review in accordance with the quality criteria of scientific epidemiological studies provided in Annex A to the UNSCEAR Report 2017. The screening criterion was the research question, the answers to which could be found in the articles. This research question was formulated as PECO statement (Populations, Exposures, Comparators, and Outcomes) used in the evidence based medicine. Results of the studies should fit this statement. Research questions related to the effect of low doses or low-LET radiation on cancer development, excess risk dependence on the cancer site; the effect of exposure dose rate, sex, age at exposure and time elapsed after the exposure, on excess risk value. It is also necessary to assess the risks of developing cancer of various localizations when exposed to low doses or low dose rates, and to carry out the uncertainty analysis. Moreover, it is essential to evaluate quality, reliability of information sources, the feasibility and ways of updating the 2006 report by combining it with new data.

New information obtained as a result of the study is important for the preparation of recommendations on radiation protection, the forecast of radiation risks caused by the use of radiation in various fields of human activity.

Document R.747. «Evaluation of public exposure to ionizing radiation from natural and man-made sources»

The study of the effects of natural and man-made sources of ionizing radiation (IRS) on the population has been the subject of constant attention of UNSCEAR since 1955, which resulted in a number of publications of the Committee. The latest document on this issue was published in 2008, and there is a need to update it. In 2019, at the 66th session of UNSCEAR, the decision has been made to prepare an updated report. For this purpose, an expert group consisting of experts from 17 member states (including Russia) and observers from four international organizations (European Commission, IAEA, NEA/OECD and WHO) was established. The updated report is scheduled for publication in 2024.

The aim of the project is to provide a comprehensive and independent assessment of the effect of all major sources of public exposure. The main objectives of the project are to analyze and update, if necessary, the methodology of radiation dose assessment, assess the variability and uncertainty of public exposure, identify temporal trends in exposure, geographic patterns and environmental features in public exposure worldwide. There will be established working groups in the following areas of public exposure: 1) natural radiation sources; 2) radon exposure; 3) nuclear fuel cycle (nuclear power production); 4) nuclear fuel cycle (spent fuel and radioactive waste management); 5) other applications of radioactive materials; 6) past military use of IRS and nuclear legacy sites; 7) residential areas contaminated as a result of past nuclear and radiological accidents and other incidents. A separate group of experts has also been established to assess the quality of incoming information, including the assessment of data uncertainties and exposure doses to the population. Information for analysis will be collected from two main sources: review of literature data for the period from 2007 to 2022 and data obtained from UN member states in the form of completed questionnaires in all areas of the expert group's work. Questionnaires have been prepared to collect information in this format. It is planned that completed survey forms will be submitted via the National Contact Persons nominated by the UN Member States to carry out the work on this project.

Document UNSCEAR/67/7 «Implementation of the Committee's strategy to improve collection, analysis and dissemination of data on radiation exposure»

The project to collect data on radiation exposure of personnel and the public is a long-term strategic task that the Committee implements in three areas: analysis of data provided in peer-reviewed scientific literature, interaction with international organizations (WHO, ILO, IAEA, etc.), collection of data from UN member-states in the form of national questionnaires. The information collected by the Committee serve as the scientific underpinning of UNSCEAR documents on occupational and public exposure from sources of ionizing radiation. In 2019, an ad-hoc working group was established to optimize the collection and analysis of data from UN member states and stratify global estimates of radiation doses for the world's population. As part of the group's activities, interactions with national contact persons were arranged in a form of a survey to understand the difficulties or challenges in data collection and submission. The results of the conducted study showed that the main problem is related to the fact that the systems for collecting data on public exposure existing in the countries do not allow providing the information requested by UNSCEAR in full. Therefore, the main sources of data for the preparation of UNSCEAR reports at the moment are still scientific publications and databases of international organizations. And here there is a number of problems. In some cases, the data collected from the scientific publications are not representative. For example, when assessing environmental contamination levels, there are significantly more data provided by areas with high background levels of exposure. This imbalance in data needs to be thoroughly investigated, especially in case of public exposure assessment. Similarly, whereas there are sufficient data on external exposure, there is notably less information on internal exposure of the population. One of the important questions is how to ensure consistency in the estimates of population exposure, for example, in terms of the effective dose. Experience has shown that countries often use different values of tissue coefficients when assessing population exposure doses, which could be difficult to determine when analyzing exposure doses. In the opinion of the working group, this issue should be addressed in a survey manual or glossary explaining the methodology used to measure, calculate or report effective dose. In 2020, work on collection of data on natural and man-made exposure of the population was started. For this project, the Committee recommended the appointment of National Contact Points (NCPs) from UN Member States. To effectively carry out this activity, the working group recommends improving the interactive format of the UNSCEAR online platform as a tool for submitting information to the NCL, optimizing cooperation

with international organizations and other institutions for the completeness of data collection in this area. It should be emphasized that in view of the fast technical development in the medical field, the working group recommends starting the collection of new data on medical exposure of the population already in 2024.

Scientific communication and public relations — a new area of UNSCEAR activities

In 2021, UNSCEAR will celebrate the 65th anniversary from the date of its foundation. The tasks assigned to the Committee in the late 1950s included a comprehensive assessment of the levels and effects of atomic radiation and the preparation of reports so that governments and international organizations could rely on them when making decisions in the field of radiation protection, regulation and other matters related to the ionizing radiation effect. For 60 years, UNSCEAR has regularly issued scientific reports and reviews, on the basis of which the ICRP and the IAEA prepared their publications and recommendations. In the professional environment, the authority of UNSCEAR is extremely high, the UN General Assembly invariably appreciates its work and emphasizes the importance of the tasks it solves, including the assessment of the consequences of severe radiation accidents [1]. However, over the past decade, UNSCEAR has been experiencing serious funding problems and a lack of staff.

Recognizing that in present-day conditions the solution of these problems hinges on the success of scientific communication and public relations, in 2014 the UNSCEAR Secretariat developed an outreach strategy for the next decade (document UNSCEAR/67/8). The goal was defined as follows: to deepen the understanding of the levels and effects of radiation among all stakeholders, not only researchers and scientists, but also decision-makers, their advisers, the scientific community, students and journalists. New target audiences include decision makers and their advisers, academia, students and journalists. In the context of a limited budget and top priority of scientific work, traditional tools of scientific communication were chosen — a public website, topic-specific information materials and news releases for the media, contacts with governments and international organizations, etc. In addition, the Secretariat planned to prepare for the 10th anniversary Fukushima accident an updated version of Appendix A to the 2013 UNSCEAR report

The results of the work performed in 2014–2019 and plans for the future were presented for discussion at the 67th session. They did not cause much discussion. The suggestion of Abel Gonzalez, who is wise from experience in scientific communication, to focus on preparing a popular brochure on Fukushima, which should become a hit for the general scientific community like the well-known 1985 brochure “Radiation. Doses and Effects”[2] for the 10th anniversary, did not receive support due to the forced postponement of the preparation of the updated Annex A to the UNSCEAR 2013 report. The Secretariat's report was approved, the work plan and the request for additional funding for communication activities in 2020–2024 were endorsed.

Nevertheless, not everything is as good as it may seem. For most of the participants in the session, as well as for the members of the Secretariat, the very notion of scientific communication is far from their purely scientific interests. If we analyze UNSCEAR's attempts to move in a new direction from the standpoint of the experience accumulated by the IAEA, ICRP, WHO and other international and national expert organizations in the field of communication of scientific knowledge on health risks, the conclusion is not comforting.

To begin with, the UNSCEAR communication strategy aims to deepen the understanding of scientific information outside the professional community. In fact, there is nothing to deepen in this area — public perceptions are very far from scientific knowledge. All target groups with whom UNSCEAR is going to work, as well as society as a whole, are convinced that serious damage to health from radiation is inevitable, regardless of the dose, and that medical consequences of Chernobyl and Fukushima accidents were disastrous. For example, in Russia, the gap between respondents' assessments and scientific data on the number of deaths from radiation exposure as a result of these accidents reaches 3–4 orders of magnitude. Moreover, the distribution of respondents' answers basically does not depend on age, education, social status and place of residence of the respondents [3]. There is reason to believe that the situation is similar in other countries. We see that the gap between public opinion and scientific knowledge does not decrease over time, traditional approaches to scientific communication are not effective. One of the main barriers is the disagreement in the professional environment regarding the scientific validity and expediency of using the linear non-threshold hypothesis in the range of fundamental scientific uncertainty, but the professionals themselves still clearly underestimate the importance and influence of their consolidated opinion on the public perception of risk

The preservation of the "status quo", as we can see from the example of the UNSCEAR, is already beginning to threaten with a decrease in the financial stability of scientific activity. The reasoning is simple: if over 65 years scientists have not been able to answer the question how serious the risk for mankind from additional man-made exposure is relative to the natural background, then is it worth continuing to divert financial resources to continue this work when more pressing problems are on the agenda, the solution of which is required here and now. Not surprisingly, the Governing Council of the United Nations Environmental Program (UNEP), consisting of representatives from 58 countries, is no longer reallocating resources in favor of UNSCEAR. In this situation, apparently, the main thing for the expert community is not to stand still, to break the internal inertia, to recognize new challenges, since a well-formulated problem is a half-solved problem.

Conclusion

The 67th session of UNSCEAR took place as an online meeting from 2 to 6 November 2020. During the Session the Scientific Committee discussed 7 Scientific Reports, Future Programme of Work of the Committee (2020–2024), Implementation of public information and outreach strategy, Report to the General Assembly, and organizational aspects of the Committee’s activities. Based on the results of the discussion decision has been made to finalize and publish the following documents in 2020: R.741 «Evaluation of medical exposure to ionizing radiation»; R.742 «Levels and effects of radiation exposure due to the accident at the Fukushima Daiichi nuclear power station: implications of information published since the 2013 UNSCEAR report»; R.743 «Biological mechanisms relevant for the inference of cancer risks from low-dose and low dose rate radiation»; The Comittee planned R.744 «Evaluation of occupational exposure to ionizing radiation for publication in 2021».

The Committee decided to extend the mandate of the ad-hoc working group on the effects of radiation exposure and the

biological mechanisms by which they occur for one year, and established the second ad-hoc working group on sources and exposure of the population. The next session of UNSCEAR is planned to take place from 21 to 25 June 2021.

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PAGET-SCHROETTER SYNDROME IN FEMALE WATER POLO PLAYER

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This article describes a case of Paget-Schroetter syndrome in a female water polo player. The condition was associated with strenuous exercise. The initial treatment strategy was limited to a 14-day heparin regimen followed by a course of diosmin and sulodexide. The article discusses the high risk of post-thrombotic syndrome in this cohort of patients and the rationale for a surgical intervention.

Keywords: thrombosis, thrombolysis, Paget–Schroetter syndrome, sports

Author contribution: Rodionovskaya SR supervised the study, wrote and edited the manuscript; Torosian GG collected data for the study and compiled the reference list; Aksenova NV collected data for the study.

Compliance with ethical standards: the patient gave voluntary informed consent to participate in the study.

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СИНДРОМ ПЕДЖЕТА–ШРЕТТЕРА У ПАЦИЕНТКИ, ЗАНИМАЮЩЕЙСЯ ВОДНЫМИ ВИДАМИ СПОРТА

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Представлен случай синдрома Педжета–Шреттера у пациентки, занимающейся водным поло. Развитие заболевания отмечено на фоне интенсивных физических нагрузок. Тактика ведения пациентки была ограничена консервативным методом лечения с применением двухнедельного курса гепарина и последующими курсами диосмина и солудексида. Рассмотрены вопросы высокого риска посттромбофлебитического синдрома у данной категории пациентов, целесообразности применения хирургических методов лечения.

Ключевые слова: тромбоз, тромболизис, синдром Педжета–Шреттера, спорт

Вклад авторов: С. Р. Родионовская — общее руководство, написание и редактирование рукописи; Г. Г. Торосян — сбор информации, оформление списка литературы; Н. В. Аксенова — сбор информации.

Соблюдение этических стандартов: пациентка подписала добровольное информированное согласие на участие в исследовании.

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Vascular injuries of the upper extremity are rarely seen in athletes. However, sports involving extreme physical exertion, like baseball, water polo, hockey, and swimming, increase the risk of upper extremity deep vein thrombosis, also known as effort thrombosis, or Paget–Schroetter syndrome (PSS) [1].

PSS is axillosubclavian vein thrombosis provoked by repetitive strenuous exercise of the upper extremity. The condition was first described by the French anatomist Jean Cruveilhier in 1816. In 1875, Sir James Paget provided a detailed account of its clinical presentations. In 1894, Leopold von Schroetter discovered that vascular injury due to physical strain was a potential factor implicated in the etiology of the disease. In the Russian literature, PSS has been known as effort thrombosis since 1934 when it was described by Anton Pytel.

The pathogenesis of the disease is linked to excess strain on the subclavian vein associated with shoulder hyperadduction, abduction or external rotation that result in endothelial microtrauma with subsequent coagulation cascade activation. Because of muscle hypotrophy common for athletes, the space between the clavicle and the first rib is narrowed, exposing the vein to compression. In turn, chronic compression of the vein or its branches provokes aseptic phlebitis (obliterative or mural) commonly accompanied by mural thrombus formation [1, 2].

Clinical case

On July 3, 2019, patient L., a 17-year-old female athlete (10 years in professional water polo; training load of up to 6 h a day) was admitted to the Federal Research and Clinical Center for Children and Adolescents, FMBA, for edema in the left shoulder region, which reportedly increased during physical exercise.

The patient had a sudden onset on August 30, 2018 during a water polo competition, when she developed a swelling in the left shoulder. The swelling extended to the chest and was accompanied by acute pain in the arm, limiting the range of motion in the upper extremity. A day before the competition, the patient had noticed a tingling sensation in her shoulder. During the next 5 days, the patient continued playing for the team. She tried using NSAIDs to relieve pain but the medications did not help much. An ultrasound examination of soft tissues was suggestive of axillary vein thrombosis. The patient was hospitalized for upper left extremity deep vein thrombosis to the Vascular Surgery Unit in her local clinic on September 04, 2018 and discharged home on September 14, 2018. A venous duplex scan performed on admission revealed signs of thrombosis in the axillary, subclavian, brachial and forearm veins, showing no signs of recanalization. During the hospital stay, the patient was receiving anticoagulants (the regimen was not specified in the discharge record). The patient responded to treatment and her edema began to resolve. A venous duplex scan conducted on September 10 revealed signs of early thrombosis recanalization. The patient was screened for hereditary thrombophilia. Polymorphisms were detected in the fibrinogen gene (C10034NT) and the plasminogen activator inhibitor gene (PAI-1). Because the girl was improving, she was

discharged home. She was also recommended to take diosmin + hesperidin (1000 mg/day), wear compression garments, and make an appointment with a vascular surgeon. In September 2018, the vascular surgeon issued the following prescription: 10 IM injections of sulodexide 600 LSU (1 ampoule a day), followed by a course of oral sulodexide 500 LSU a day. The patient agreed with the recommendations, but went on and off her medications without consulting the doctor, and continued exercising for 2 h a day.

In June 2019, the patient's condition deteriorated following her stay at a water polo training camp. She noticed that her edema had returned, was growing bigger, and was accompanied by pain. By that time, the patient had discontinued her medications. She was referred to the Federal Research and Clinical Center for Children and Adolescents to rule out recurrent thrombosis and make adjustments to her anticoagulation therapy.

The patient's medical history was unremarkable, with no family history of thrombosis. On examination, the left upper extremity showed no signs of discoloration. There was moderate edema in the shoulder region (the diameter of the affected shoulder differed by 1–15.cm from the diameter of the contralateral extremity). The infraclavicular fossa appeared full. Peripheral pulses were palpable and symmetrical. Secondary thrombosis associated with rheumatoid diseases (systemic lupus erythematosus, antiphospholipid syndrome), hereditary thrombophilia or connective tissue dysplasia was ruled out.

A triplex ultrasound examination of upper extremity veins conducted on July 7, 2019 revealed no evidence of superficial or deep vein thrombosis or significant venous insufficiency. Coagulation tests were normal: D-dimer 451.0 ng/ml (reference values < 500 ng/ml); aPTT 29.2 s (24.6–31.2 s); fibrinogen 3.1 g/L (1.70–4.20 g/L), antithrombin III 126% (75–125%); Quick 100.6% (70–130%); thrombin time 20.3 s (15.8–24.9 s); INR 0.99; lupus anticoagulant 41.9 s (30.4–45.3 s). Natural body anticoagulants: protein S 59.1% (> 56.10%) and protein C 130.0% (70.0–140.0%); homocysteine 7.5 μmol/L (5.0–12.0 μmol/L). Autoimmunity tests were negative: antinuclear antibodies (ANA-HEp-2) 1 : 80 (reference values < 1 : 160); anti-double stranded DNA antibodies 1.9 IU/ml (0–20 IU/ml); IgG cardiolipin antibodies 1.90 IU/ml (reference values < 20 IU/ml); IgM cardiolipin antibodies were not detected; β_2-glycoprotein antibodies (IgG) 2.30 un/ml (reference values < 5.0 un/ml); IgM β_2-glycoprotein antibodies were not detected. In addition, the patient underwent a cervical MRI scan to rule out anatomic abnormalities predisposing to subclavian vein damage.

Having analyzed the patient's medical history and clinical tests (symptoms of acute-onset deep vein thrombosis of the left upper extremity in a professional female athlete due to high training load and an increase in muscle bulk in the shoulder girdle) and ruled out secondary (autoimmunity-associated) thrombosis, we arrived at the diagnosis of Paget–Schroetter syndrome. Angioprotective antithrombotic therapy with sulodexide 500 LSU/day was resumed, and the patient was instructed to follow up with a vascular surgeon. Sports were allowed.

Clinical case discussion

Secondary deep vein thrombosis (DVT) of the upper extremity is a well-known clinical syndrome. It is common for patients with implanted pacemakers, central venous catheters and cancer. The group of primary DVT disorders is constituted by PSS and thoracic outlet syndrome. The annual incidence of DVT is 1 case per 1,000 population. Upper extremity thrombosis accounts for 4–10% of DVT cases, of which PSS makes up 20% [2, 3]. Early diagnosis and adequate therapy are crucial to avoiding the life-threatening complication of PSS (pulmonary thromboembolism) and expediting recovery. A metanalysis demonstrated that upper extremity DVT was most commonly observed in baseball players and weightlifters (26.8% and 19% of the total 123 DVT cases, respectively); 26.7% of patients developed pulmonary thromboembolism [4].

At present, DVT is diagnosed based on clinical and instrumental tests. In our case, acute-onset edema and pain in the upper extremity irradiating to the axillary fossa and accompanied by redness were suggestive of acute DVT of the left upper extremity. This provisional diagnosis was later confirmed by an ultrasound scan. Ten months after onset, the teenager was hospitalized to the Federal Research and Clinical Center for Children and Adolescents where the following criteria and risk factors for thrombosis were identified: being a professional athlete (a water polo player), frequent air travel (because of competitions), recurrent injuries during training or competitions, muscle hypertrophy in the upper girdle. Further tests identified risk factors for PSS, including congenital anomalies (a cervical rib), autoimmune diseases increasing the risk of thrombosis, and hereditary thrombophilia.

According to the Russian clinical guidelines on the diagnosis, treatment and prevention of venous thromboembolic complications, patients under the age of 50 should be screened for congenital thrombophilias when thrombosis-inciting events have not been identified or the patient presents with recurrent thromboembolic complications [5]. Polymorphisms detected in the fibrinogen gene (C10034NT) and the plasminogen activator inhibitor gene (PAI-1) are associated with increased risk for thromboembolic complications and indicate that the patient's condition should be closely monitored.

Instrumental tests play the key role in the diagnosis of DVT [2]. Venous Doppler is an accessible, portable and cheap diagnostic test and should be preferred when establishing a preliminary diagnosis. Contrast venography allows a sonographer to visualize all venous lumens in the upper extremity, identify the sites of vein compression by osseous structures, and detect stenosis and fibrotic changes to the subclavian and axillary veins [6, 7]. Of all non-invasive diagnostic modalities, MRI has the highest sensitivity (100%) and specificity (97%). Although venography is not essential for diagnosing DVT, it is almost always performed as part of the multimodal treatment strategy, which includes catheter-directed thrombolysis and surgical decompression [6].

Currently, there is no unified treatment for PSS due to its rarity, the lack of awareness and the absence of large-scale randomized trials [8]. Recommendations given to the patient at her local clinic (14 days of heparin followed by a course of detralex) differed from standard conservative treatment regimens that normally include at least 3 months of anticoagulants [5]. Perhaps, the absence of adequate anticoagulation therapy was the risk factor for the post-thrombotic syndrome developed by our patient.

Among PSS complications are pulmonary embolism, recurrent thrombosis and post-thrombotic syndrome, which occurs in up to 45% of patients [9]; this urged the development of active treatment strategies with thrombolysis, thrombectomy, percutaneous and surgical venoplasty, venous bypass grafting and stenting. A more aggressive approach (thrombolysis or catheter-directed thrombolysis) surpasses conservative treatment in effectiveness if performed within 2 weeks after the onset of acute thrombosis. According to some authors, early catheter-directed thrombolysis is effective in 75–84% cases and significantly reduces the risks of post-thrombotic disease and disabilities [10, 11].

Our opinion differs from that of the vascular surgeons who decided to put the patient on short-term anticoagulation monotherapy. A study [1] analyzed the outcomes of 41 athletes with upper extremity DVT (44% of them were female; the mean age was 19 years), including 5 water polo players. PSS was diagnosed in 14 patients; all of them underwent thrombolysis/anticoagulation therapy followed by a first rib resection. This strategy was successful: 93% of patients were able to return to professional sports within an average of 4.6 months after surgery. Only 2 patients (14%) relapsed.

CONCLUSION

Raising awareness about the risk of thrombosis in the described cohort of patients among primary and emergency health care providers will facilitate early diagnosis and timely treatment; such patients should be referred to a vascular or thoracic surgeon for thrombolysis or surgery. Further research should explore the advantages of thrombolytic therapy in cases of diagnostic delay, identify factors that render thrombolysis ineffective and raise the need for surgery.

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