GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 11 (332) ноябрь 2022

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
No 11 (332) 2022

Содержание:

GEORGIAN MEDICAL NEWS
No 11 (332) 2022

NEUTROPHIL TRAPS AS AN IMMUNE RESPONSE MECANISM IN PETIENTS WITH EROSIVE DISEASES OF THE UPPER GASTROINTESTINAL TRACT

Kryshen V.[1], Garkava K.[2], Trofimov N.[1], Tatarchuk O.[3], Korpusenko I.[1], Nor N.[1], Kudryavtseva V.[4], Guzenko B.[1], Garkavy S.[5], Makarenko A.[5].

[1]Dneprovsk State Medical University, Dnipro, Ukraine.

[2]National Aviation Unìversity, Kyiv, Ukraine.

[3]SE "Institute of Gastroenterology" NAMS, Dnipro, Ukraine.

[4]Prydniprovskuy State Academy of Physical Culture and Sport, Dnipro, Ukraine.

[5]National Medical University. A.A. Bogomolets, Kiev, Ukraine.

Abstract.

To optimize the methods for determining neutrophil extracellular traps in peripheral blood and studying their level in the norm and in patients with candidiasis with inflammatory and erosive diseases of the upper gastrointestinal tract on the basis of the Institute of Gastroenterology of the National Academy of Medical Sciences and Clinical Association of Emergency Medicine, Dnipro, Ukraine, in 2019-2021. 42 patients with candidiasis with inflammatory and erosive diseases of the upper gastrointestinal tract were examined, including 10 women and 32 men aged 35-56 years, who made up the main group (MG). These patients were divided into two groups according to the results of microbiological examination of the mucous membranes of the gastrointestinal tract: group 1 consisted of 20 patients with oropharyngeal candidiasis of the oral cavity; Group 2 consisted of 22 patients with candidiasis in the esophagus and stomach with or without oropharyngeal candidiasis. The comparison group (ComG) included 9 patients without detection of yeast-like fungi. The control group (ConG) consisted of 25 practically healthy people (donors). Neutrophils were isolated from the leukocyte mixture on a double ficoll-verografin gradient. The resulting cells were incubated with E. coli, S. aureus, C. albicans. Control - neutrophils without activators. Cells were stained according to Romanovsky-Giemsa or acridine orange. A decrease in the level of NET was found in patients of groups 1 and 2 of the main group in relation to the control group and the comparison group. The results obtained showed that microorganisms induce the formation of neutrophil extracellular traps. The low level of neutrophil extracellular traps in patients indicates the inability of their neutrophils to form NET, which are important for antifungal protection, and the method of their determination can be used to analyze the functional state of neutrophils in various diseases.

Key words. Neutrophil extracellular traps, systemic fungal infections, erosive diseases of the gastrointestinal tract.

Introduction.

Neutrophil granulocytes, being cells of the innate immune system, use various strategies for the body's antimicrobial defense during the development of infectious inflammation. Depending on the nature of the activation signal and the efferent tasks they face, neutrophils use phagocytosis, degranulation, or the formation of extracellular trap networks, being active participants in the modulation of immune responses - the innate immune system and acquired adaptive immunity [1]. The formation of extracellular traps by neutrophils is an important mechanism of the innate immune response. In 2004, scientists working under the guidance of Arturo Zychlinsky of the Max Planck Institute for the Study of Infection Biology discovered a new function of neutrophilic granulocytes - extracellular capture of pathogens, by forming extracellular traps, which are DNA strands with antimicrobial factors of neutrophil granules adsorbed on them. The process of formation of extracellular traps by neutrophils is an alternative function to phagocytosis [2]. The mechanism of antimicrobial protection carried out by neutrophils through the formation of extracellular networks has been termed "NETosis". Microorganisms that fall into these traps die [2,3]. Extracellular traps can only form active neutrophils. The formation of neutrophil extracellular traps (NET) can occur in two ways: lytic and vesicular. In the lytic variant, the neutrophil undergoes morphological changes. Numerous vacuoles are formed in it, the characteristic lobed shape of the nuclei is lost, then the nuclear membrane is destroyed, and chromatin occupies the entire cell. The granules dissolve and the components of the future trap are distributed throughout the volume, the cell contracts until its membrane bursts, the neutrophil dies, and the highly active mixture is thrown out. The lytic pathway of NET formation requires reactive oxygen species. The vesicular mechanism of NET formation is oxygen-independent, while neutrophils retain their viability. In this variant, the formation of NET begins with the formation of vesicles. Vesicles surrounded by a nuclear membrane move into the intercellular space without destroying the plasma membrane. Outside the cell, the membranes of the vesicles rupture, releasing chromatin. This scenario unfolds within minutes of neutrophil stimulation. If the action of the activator continues, the mechanisms of lytic formation of traps are triggered [4,5]. Structurally, neutrophil extracellular traps are DNA strands capable of non-specifically trapping all possible extracellular particles potentially to be destroyed. The biochemical features of the processes occurring during the release of DNA by cells are under study. Ideas about the important role of bactericidal products secreted by these cells outside in antimicrobial protection were substantiated. However, there are still no clear ideas about the formation of NET by neutrophils. There are no standardized methods for detecting NET. For visualization of NET, V. Brinkmann et al. scanning electron microscopy was used [3,6-8]. However, this method is expensive and time-consuming; special equipment and reagents are required.

Purpose of research. To optimize methods for detecting NET in peripheral blood and determine their level in the norm and in patients with candidiasis in inflammatory and erosive diseases of the upper gastrointestinal tract (GIT).

Materials and methods.

To achieve this goal on the basis of the Institute of Gastroenterology of the National Academy of Medical Sciences and Clinical Association of Emergency Medicine, Dnipro, Ukraine, in 2019-2021. 42 patients with candidiasis with inflammatory and erosive diseases of the upper gastrointestinal tract were examined, including 10 women and 32 men aged 35-56 years, who made up the main group (MG). These patients were divided into two groups according to the results of microbiological examination of the mucous membranes of the gastrointestinal tract: group 1 consisted of 20 patients with oropharyngeal candidiasis of the oral cavity; Group 2 consisted of 22 patients with candidiasis in the esophagus and stomach with or without oropharyngeal candidiasis. The comparison group (ComG) included 9 patients without detection of yeast-like fungi. The control group (ConG) consisted of 25 practically healthy people (donors).

A suspension of neutrophils was obtained using 15.0 ml of heparinized (10-15 U/ml of heparin) peripheral venous blood, which was settled in a sterile test tube at a temperature of +37°C for 30 minutes for the purpose of erythrocyte sedimentation, using the Dolgushin I. I. method [5]. Neutrophils were isolated from leukocyte suspension on a double density gradient of sterile ficoll-verografin solutions. The density of the upper layer of the gradient was 1.075-1.077, and the lower layer was 1.093-1.095. The volume of each gradient was 1.5 ml. After 40 minutes of centrifugation at 1500 rpm, a ring of granulocytes with a purity of 98-100% appeared at the boundary between the gradients. The neutrophil ring was carefully collected, transferred to sterile centrifuge tubes, washed from the gradient three times with sterile saline sodium chloride solution by centrifugation at 1500 rpm for 10 minutes, and adjusted to a concentration of 5 106 cells/mL [6,7,9].

The resulting cell suspension was incubated at a temperature of +37°C for 30 minutes in the presence of 0.1 ml of a suspension of a daily culture of control strains (E. coli (strain O55K59), S. aureus, C. albicans), brought to a concentration of 106 E. coli , 106S. aureus, 106 C. albicans in 1 ml per 1 ml of neutrophil suspension. To determine the optimal activation time for neutrophils, neutrophils with culture suspension were incubated for 30 min, 40 min, 3 hours, 24 and 48 hours. A suspension of neutrophils incubated under the same conditions, but without activators, was used as a control. After incubation, the cell suspension was placed on glass and dried. For fixation, 96% ethyl alcohol was used, which was applied to the smear in an amount of 100 µl; after evaporation of the alcohol, the smear was considered fixed.

The smears were stained according to Romanovsky-Giemsa and acridine orange using the method developed by I. I. Dolgushin and A. V. Zurochka [5]. 200 µl of acridine orange working solution was applied to the fixed preparation and kept at room temperature for 2 minutes in the dark. A solution of acridine orange was preliminarily prepared according to the following scheme: 5 mg of dry dye was dissolved in 5 ml of saline, the mother solution thus obtained (1 mg/ml) was stored at T=4°C. Before use, 0.2 ml of the solution was mixed with 4.8 ml of saline to obtain a working solution. Accounting was performed using a light (immersion) and luminescent microscope (using filters that provide exciting light with a wavelength of not more than 490 nm and emission with a wavelength of 520 nm).

Statistical data processing was carried out using a personal computer using software products STATISTICA 6.1 (StatSoftInc., serial no. AGAR909E415822FA) and Microsoft Excel (Microsoft Office 2016 Professional Plus, Open License 67528927) using methods of descriptive and analytical biostatistics and multivariate methods of statistical analysis [10].

Results and Discussion.

In the course of the work, we determined the optimal conditions for the incubation of neutrophils for their activation and the formation of NET in vitro. It was found that after 30 minutes of incubation, the number of NET was 18.2 ± 1.2%, after 40 minutes - 25.3 ± 1.8%, after 3 hours - 12 ± 0.9%, after 24 hours - 8, 4±1.0% and 48 hours - 2.1±0.5%. The optimal incubation time for determining neutrophil activity is 40 minutes and the temperature is 37°C (Figure 1). At .the same time, this phenomenon is observed only in neutrophils isolated from peripheral blood on a double ficoll-verografin gradient.

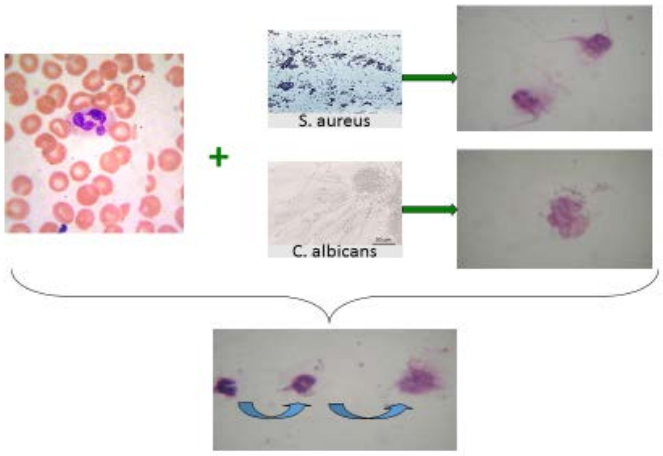

Figure 1. *Determination of the optimal conditions for the incubation of neutrophils for their activation and the formation of NET in vitro.*

Next, we determined the effect of microorganisms (E. coli, S. aureus, and C. albicans) on the formation of NET in normal healthy donors. The formation of NET in vitro is induced by the microorganisms E. coli, S. aureus and C. albicans (Figure 2).

The analysis of the obtained results showed that the highest level of NET is formed during the activation of S. aureus. Thus, S. aureus is the strongest inducer of NET.

In order to determine the optimal method for detecting NET, in the course of our work, we compared two smear staining methods: Romanovsky-Giemsa and acridine orange (Figure 3).

When stained with acridine orange and fluorescent microscopy, neutrophil nuclei are stained bright green, granulocyte cytoplasm is not stained, neutrophil traps are represented by thin bright green filaments, and activator bacteria are bright orange.

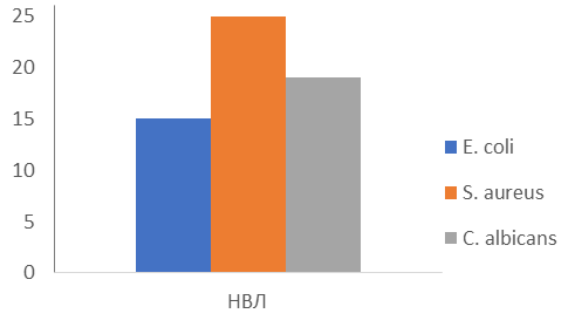

Figure 2. *The level of NET in donors with various neutrophil inducers, in vitro.*

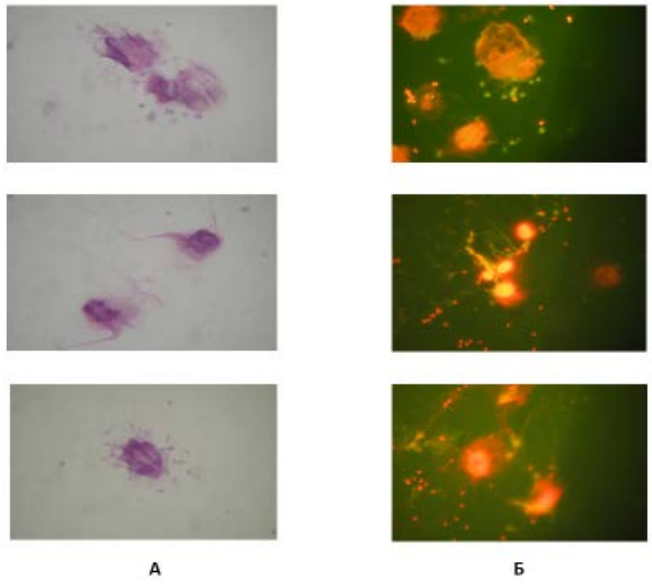

Figure 3. *Methods for staining NETosis formed by neutrophils isolated from the peripheral blood of donors. (A - Light microscopy, Romanovsky-Giemsa stain; B - Luminescent microscopy, acridine orange stain).*

To determine the role of NET in antifungal immunity, we determined their level in patients with varying degrees of C. albicans damage (Table 1).

Table 1. *The level of NET in the examined groups of patients, (M±m).*

Indicators	1 gr (MG) (n=20)	2 gr (MG) (n=22)	Com G (n=9)	ConG (n=25)
NET, %	10,1±1,2* **	5,2±0,4* **#	17,2±1,5	19,2±0,85

Notes:
1. - p <0.05 - differences between the parameters of MG and ConG.*
*2.** - p <0.05 - differences between the parameters of MG and ComG.*
3.# - p <0.05 - differences between the indicators of groups 1 and 2 of the MG.

A decrease in the level of NVL in patients of group 1 (MG) was found to be 1.9 times (p <0.05) and 1.7 times (p <0.05) compared with ConG and ComG, respectively. In patients of group 2 (MG), the level of NET was reduced by 3.7 times (p <0.05) and 3.3 times (p <0.05) compared with ConG and ComG, respectively. In addition, in group 2 (MG), their level was significantly lower by 1.9 times (p <0.05) compared to group 1 (MG), which may be due to the inability of their neutrophils to form NET. Because comparison and control patients can eradicate yeast-like fungi by forming NET, they can prevent the spread of C. albicans and are at lower risk of systemic fungal infection.

In the course of our work, we determined the effect of E. coli, S. aureus, and C. albicans on the formation of extracellular traps by neutrophils. It was found that S. aureus is the strongest inducer of neutrophil extracellular traps. Normally, the level of NET upon activation of S. aureus is (25.1±1.04)%, C. albicans (19.2±0.85)% and E. coli (15.4±0.68)%. The optimal incubation time was 40 minutes. The data obtained indicate that the formation of neutrophil extracellular traps is induced by microorganisms, which coincides with the data of other studies [11]. At the same time, this phenomenon is observed only in neutrophils isolated from peripheral blood on a double ficoll-verografin gradient.

Neutrophils fulfill their host defense role by phagocytosing pathogens, secreting their granules full of cytotoxic enzymes, or removing NETosis during the process of their formation. To effectively manipulate this process to combat a particular disease, researchers must work to understand the mechanisms that govern the formation of NET. Such an understanding would allow the creation of means to stimulate or prevent it as needed. While the (patho)physiological significance of NET formation has been demonstrated, the molecular, cellular, and biophysical mechanisms driving this process have only begun to be unraveled. Much of the current literature is devoted to the study of which stimuli can induce NETosis and which proteins can inhibit their release without a clear understanding of how these factors affect cellular genesis events. For decondensed DNA stained with nuclear, granular, and cytosolic contents to be released extracellularly, the process must be initiated either extracellularly or intracellularly; chromatin must be decondensed and released from the nucleus; and the cytoskeleton, organelles, intracellular and nuclear, as well as plasma membranes must be reconstructed [4,11]. The data obtained indicate that the formation of neutrophil extracellular traps is induced by microorganisms, which coincides with the data of other studies [2,4,11,12]. At the same time this phenomenon is observed only in neutrophils isolated from peripheral blood on a double ficoll-verografin gradient. The developed methods for detecting NET open up possibilities for studying the significance of NET for diagnosing various diseases, assessing the severity of the course of diseases and the effectiveness of treatment, and can also be used in clinical practice to assess the functional status of neutrophils.

Conclusions.

1. The formation of neutrophil extracellular traps is induced by microorganisms, of which Staphylococcus aureus was the strongest inducer.
2. The optimal incubation time for neutrophils isolated from peripheral blood on a double density gradient of ficoll-verografin with various microorganisms reached to 40 minutes.

3. Normally, the level of neutrophil extracellular traps during the activation of St. aureus was (25.1±1.04)%, C. albicans (19.2±0.85)% and E. coli (15.4±0.68)%.

4. The low level of neutrophil extracellular traps in patients of the main group 1 was associated with neutrophils inability to form themselves which was a sign of neutrophil extracellular traps importance for antifungal protection.

REFERENCES

1. Smirnova TG, Savochkina AY, Dolgushin II, et al. Changes in Functional Activity of Neutrophils and Monocytes Isolated from the Peripheral Blood of Women at Different Phases of the Menstrual Cycle. Bull Exp Biol Med. 2018;166:222-224.
2. Thiam HR, Wong SL, Wagner DD, et al. Cellular Mechanisms of NETosis. Annu Rev Cell Dev Biol. 2020;36:191-218.
3. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018;18:134-147.
4. Castanheira FVS, Kubes P. Neutrophils and NETs in modulating acute and chronic inflammation. Blood. 2019;133:2178-2185.
5. Dolgushin II, Andreeva Yu. S, Savochkina A Yu. Neutrophil traps and methods for assessing the functional status of neutrophils. M. 2009;126.
6. Brinkmann V. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532-1535.
7. Brinkmann V. Beneficial suicide: why neutrophils die to make NETs. Nat Rev Microbiol. 2007;5:577-582.
8. de Buhr N, von Köckritz-Blickwede M. Detection, Visualization, and Quantification of Neutrophil Extracellular Traps (NETs) and NET Markers. Methods Mol Biol. 2020;2087:425-442.
9. Korotina OL Generals II. Neutrophil extracellular traps: mechanisms of formation, function. Immunology, allergology, infectology. 2012;4:23-32.
10. Lang TA, Sesik M. How to describe statistics in medicine. A guide for authors, editors, and reviewers. 2nd ed. per. from English V.P. Leonova, editor. Moscow: Practical Medicine. 2016:480.
11. Honda M, Kubes P. Neutrophils, and neutrophil extracellular traps in the liver and gastrointestinal system. Nat Rev Gastroenterol Hepatol. 2018;15:206-221.
12. Ravindran M, Khan MA, Palaniyar N. Neutrophil Extracellular Trap Formation: Physiology, Pathology, and Pharmacology. Biomolecules. 2019;9:365.

РЕФЕРАТ

НЕЙТРОФИЛЬНЫЕ ЛОВУШКИ КАК МЕХАНИЗМ ИМУННОГО ОТВЕТА У БОЛЬНЫХ С ЭРОЗИВНЫМИ ЗАБОЛЕВАНИЯМИ ВЕРХНИХ ОТДЕЛОВ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА

Крышень В.П.[1], Гаркава К.Г.[2], Трофимов Н. В.[1], Татарчук О. М.[3], Корпусенко И.В.[1], Нор Н.Н.[1], Кудрявцева В.Е.[4], Гузенко Б.В.[1], Гаркавый С.С.[5], Макаренко А.Н.[5].

[1]Днепровский государственный медицинский университет. Днепр. Украина.

[2]Национальный авиационный университет. Киев. Украина.

[3]ДУ «Институт гастроэнтерологии» НАМН. Днепр. Украина.

[4]Приднепровская государственая академия физической культуры и спорта. Днепр. Украина.

[5]Национальный медицинский университет им. А.А. Богомольца. Киев. Украина.

Для оптимизировации методов определения нейтрофильных внеклеточных ловушек в периферической крови и исследования их уровеня в норме и у больных с кандидозом при воспалительных и эрозивных заболеваниях верхнего отдела желудочно - кишечного тракта на базе ДУ «Институт гастроэнтерологии» НАМН и Клинического объединения скорой медицинской помощи Днепр, Украина, в 2019-2021г.г. было обследовано 42 больных с кандидозом при воспалительных и эрозивных заболеваниях верхнего отдела ЖКТ, из них 10 женщин и 32 мужчин в возрасте 35-56 лет, которые составили основную группу (ОГ). Эти больные были разделены на две группы по результатам микробиологического исследования слизистых оболочек ЖКТ: 1 группа составили 20 больных с орофаренгиальным кандидозом ротовой полости; 2 группу составили 22 больных с определением кандидоза в пищеводе и желудке с наличием или отсутствием орофаренгиального кандидоза. В группу сравнения (ГС) вошли 9 больных без выявления дрожжеподобрых грибов. Контрольную группу (КГ) составили 25 практически здоровых людей (доноров). Нейтрофилы выдиляли из лейкоцитарной смеси на двойном градиенте фиколла-верографина. Полученные клетки инкубировали с E. Coli , S. aureus, C. albicans. Контроль - нейтрофилы без активаторов. Клетки красили по Романовскому – Гимзе или - акридиновым оранжевым. Было выявлено снижение уровня НВЛ у больных 1 и 2 группы основной группы в отношении контрольной группы и группы сравнения. Полученные результаты показали, что микроорганизмы индуцируют образование нейтрофильных внеклеточных ловушек. Низкий уровень нейтрофильных внеклеточных ловушек у больных указывает на неспособность их нейтрофилов образовывать НВЛ , которые имеют важное значение для противогрибковой защиты, а метод их определения может быть использован для анализа функционального состояния нейтрофилов при разнличных заболеваниях.

Ключевые слова: нейтрофильные внеклеточные ловушки, системные грибковые инфекции, эрозивные заболевания, желудочно-кишечного тракта.