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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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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.

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SERUM IL-6, IL-12, AND IL-10 LEVELS IN EARLY-STAGE, UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: INSIGHTS FROM GEORGIA

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Abstract.

Introduction: Chronic Lymphocytic Leukemia (CLL) is the most common adult leukemia in Western countries and is characterized by the clonal expansion of mature $CD19^+/CD5^+/CD23^+$ B lymphocytes. Immune dysregulation is a hallmark of CLL, predisposing patients to infections and significantly altering circulating cytokine profiles. While such changes have been extensively investigated in advanced stages, little is known about cytokine alterations in early-stage, untreated CLL patients in Georgia.

Methods: Serum levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-12 (IL-12), as well as the anti-inflammatory cytokine interleukin-10 (IL-10), were measured in 25 newly diagnosed, untreated CLL patients (Rai stage 0/I, Binet A) and 15 age- and sex-matched healthy volunteers. Cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Thermo Fisher Scientific). Statistical comparisons were performed between groups, and correlations with clinical parameters were analyzed.

Results: IL-6 levels were significantly higher in CLL patients compared to healthy volunteers (median 15 pg/mL vs. 4 pg/mL; $P < 0.0001$), with considerable inter-individual variability. IL-12 concentrations were also elevated in CLL patients ($P = 0.0019$) and showed a strong negative correlation with absolute lymphocyte counts ($r = –0.7$). In contrast, IL-10 levels did not differ significantly between groups ($P = 0.3432$).

Conclusions: Even at the earliest clinical stages, untreated CLL patients demonstrate a cytokine profile indicative of a pro-inflammatory and partially immunostimulatory microenvironment, which may influence disease pathogenesis and progression. The observed elevations in IL-6 and IL-12 suggest their potential utility as early biomarkers and possible therapeutic targets.

Key words. Chronic lymphocytic leukemia, IL-6, IL-10, IL-12, cytokines, immune dysregulation.

Introduction.

Chronic Lymphocytic Leukemia (CLL) is the most common adult leukemia in Western countries. This hematologic malignancy is defined by the clonal expansion of $CD19^+CD5^+CD23^+$ mature B lymphocytes, which accumulate abnormally in peripheral blood, bone marrow, and lymphoid tissues [1]. The disease progression relies on proliferative niches within lymph nodes and bone marrow, where leukemic cells receive survival signals through microenvironmental interactions, cytokine networks, and dysregulated signaling pathways [2]. It's well established that immune dysregulation is a trait of CLL, contributing to an increased susceptibility to infections [3,4]. These infections, along with suppressed immunity significantly alter circulating cytokine profiles. Conversely, profiling cytokine levels provides valuable insights into the status of immune dysregulation, the degree of microenvironmental support, and disease progression [5].

In this study we assessed the levels of pro-inflammatory cytokines Interleukin-6 (IL-6) and Interleukin-12 (IL-12), along with the anti-inflammatory cytokine Interleukin-10 (IL-10), in untreated CLL patients in Georgia (Rai stage 0/I, Binet A).

Interleukin-6 (IL-6), a pleiotropic cytokine, is synthesized by numerous cell types, including fibroblasts, endothelial cells, monocytes, normal hematopoietic cells, and lymphocytes, in response to infection or tissue injury [6]. It plays a crucial role in the activation of STAT3 and NF-κB signaling pathways, both of which regulate genes associated with tumor cell growth and survival [7]. In CLL, higher IL-6 levels were associated with a shorter lymphocyte doubling rate, earlier initiation of treatment, the presence of 17p or 11q chromosomal deletions, and shorter progression-free survival (PFS). By activating STAT3 and NF-κB, IL-6 may promote the survival of B-CLL cells, and blocking IL-6 signaling has been proposed as a potential therapeutic strategy [8].

Interleukin-12 (IL-12) is an immunomodulatory cytokine that has emerged as a potent inducer of antitumor immunity. It was first identified in 1989 as a natural killer (NK) cell stimulatory factor [9]. This interleukin is primarily produced by dendritic cells (DCs) and activated phagocytes, exhibiting pleiotropic effects that include the activation of both T cells and NK cells to produce interferon-gamma (IFN-γ). It also enhances their proliferation and cytotoxic functions [10]. It is well known that IL-12 promotes Th1-type immune responses and has demonstrated antitumor efficacy in preclinical cancer models [11]. Only a limited number of studies have investigated IL-12 in CLL, suggesting that its role is complex, with evidence indicating both pro-tumorigenic and anti-tumorigenic effects [12,13].

Interleukin-10 (IL-10) is a key immunosuppressive cytokine produced by several immune cell types, including T helper 2 (Th2) cells, monocytes, macrophages, B1 cells, and CLL cells themselves [14]. In CLL, IL-10 plays a significant role in shaping an immunosuppressive tumor microenvironment, which

facilitates leukemic cell survival and contributes to immune evasion. CLL cells can adopt regulatory B cell-like (Breg-like) features and actively secrete IL-10 [15]. This secretion has been shown to inhibit effector T-cell activation, impair antigen presentation by dendritic cells, and reduce pro-inflammatory cytokine production, thereby contributing to T-cell exhaustion and immune dysregulation observed in CLL patients [16].

To our knowledge, levels of above-mentioned cytokines in untreated CLL patients have not been previously investigated in Georgia.

Materials and Methods.

Patients and Sample Collection:

Peripheral blood samples were collected from 25 chronic lymphocytic leukemia (CLL) patients (age 50-75y, 14 males and 11 females) at the M. Zodelava Centre of Hematology in Tbilisi, Georgia (Table1), based on informed written consent and ethical approval (The ethics committee of National Center for Disease Control and Public Health of Georgia (NCDC) (approval number #2024-071)). All patients were newly diagnosed and remained untreated at the time of sample collection. Fiveteen healthy volunteers of matching age and sex were also enrolled in the study after providing informed consent. Blood samples were collected into serum separator tubes and allowed to clot at room temperature for 30–60 minutes. Samples were centrifuged at 1,500 × g for 10 minutes at 4 °C, and the serum was carefully aliquoted into polypropylene cryovials. Aliquots were stored at −80 °C until analysis. Thawed serum was kept on ice and used immediately, with all samples undergoing a single freeze–thaw cycle to preserve analyte integrity. All sample processing was performed at the Laboratory of Immunology and Microbiology, Faculty of Biology, Ivane Javakhishvili Tbilisi State University.

Table 1. *Clinical and hematological characteristics of patients with Chronic Lymphocytic Leukemia (CLL). Data are presented as mean ± standard deviation or number of patients (n). Patients were early-stage (Rai 0–I). WBC, white blood cells; NLR, neutrophil-to-lymphocyte ratio.*

Parameter	CLL Patients
Number of participants (n)	25
Mean Age (years)	70,9 ± 6.5
Gender (M/F)	14/11
CLL Stage (Rai)	Rai 0–I (early-stage)
Leukocytes (WBC)	41,3(± 9.8) 10^9/L
Lymphocytes	77,3(± 3.3) %
Monocytes	3,7(± 0.6) %
NLR	0,2(± 0.04)

Determination of serum levels of cytokines - IL-6, IL-10 and IL-12:

Serum concentrations of interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-12 (IL-12) were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits provided by Thermo Fisher Scientific, following the manufacturer's protocols.

Sera samples were added to individual wells of 96-well microplates that were pre-coated with monoclonal antibodies specific to IL-6, IL-10, or IL-12. The plates were incubated for 2 hours to allow cytokines in the samples to bind to the capture antibodies According to the manufacturer's instructions. After incubation, the wells were washed multiple times with commercial washing buffer (Thermo Fisher Scientific) to remove unbound components. Subsequently, a detection solution comprising polyclonal antibodies conjugated to horseradish peroxidase (HRP) was introduced into each well, followed by the addition of a chromogenic substrate solution, tetramethylbenzidine (TMB), the enzymatic reaction was stopped by the addition of a stop solution - 1N sulfuric acid.

The optical density (OD) was measured at 450 nm using a microplate reader (Tristar 5, Berthold, Germany). The concentration of each cytokine was determined by comparing sample absorbance values to a standard curve generated from known concentrations of recombinant cytokines. Final cytokine concentrations are expressed as picograms per milliliter (pg/mL).

Statistical Analysis:

Results are expressed as the mean ± SEM. As the assumption of equal variances between the two groups was violated, Welch's t-test was employed to evaluate the difference in mean values between CLL patients and healthy controls, as it provides a robust comparison under conditions of unequal variances. p values < 0.05 were considered statistically significant. For IL-10 (pg/ml), no significant difference was detected between groups, and the null hypothesis of homogeneity was accepted.

The correlation between IL-12 levels and total lymphocyte count was determined using Pearson's correlation coefficient.

Results.

Levels of IL-6, IL-12, and IL-10 were evaluated in the sera of 25 untreated CLL patients and 15 age- and sex-matched healthy volunteers. All patients were classified as Rai stage 0 to I/Binet A, corresponding to early-stage disease, and included 14 males (56%) and 11 females (44%), aged between 55 and 80 years.

Study results indicate that serum IL-6 levels were significantly higher in untreated CLL patients (Rai stage 0/I, Binet A) compared with healthy controls, with median concentrations of 15 pg/mL versus 4 pg/mL ($P < 0.0001$) (Figure 1). Notably, the range of IL-6 levels among CLL patients was broad, with several individuals exceeding 40 pg/mL, indicating substantial inter-patient heterogeneity.

Along with IL-6, IL-12 levels were also significantly elevated in untreated CLL patients13.3±2.5 compared to healthy controls 4.0±0.7 ($p = 0.0019$) (Figure 2), although the increase was less pronounced than that of IL-6. ($p = 0.0019$) (Figure 2), although the increase was less pronounced than that of IL-6.

In addition, we also evaluated correlations between interleukin concentrations and various leukocyte populations (data not shown). Notably, we observed a strong negative correlation between IL-12 levels and total lymphocyte counts in peripheral blood ($r = –0.7$).

In contrast to IL-6 and IL-12, no significant difference in IL-10 concentrations was observed between CLL patients 5.3±1.9 and healthy controls 3.4±0.3 ($p = 0.3432$, Figure 3).

Discussion.

Cytokines play a crucial role in the pathogenesis, progression, and immune evasion of chronic lymphocytic leukemia (CLL).

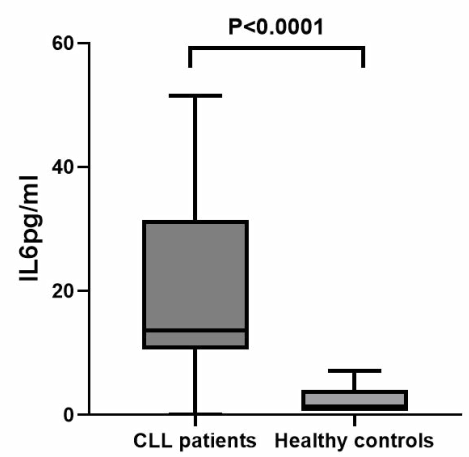

Figure 1. *Serum IL-6 levels in untreated CLL patients (Rai stage 0/I, Binet A, n = 25) and age- and sex-matched healthy controls (n = 15). Cytokine concentrations were measured using ELISA (Thermo Fisher Scientific) according to the manufacturer's instructions.*

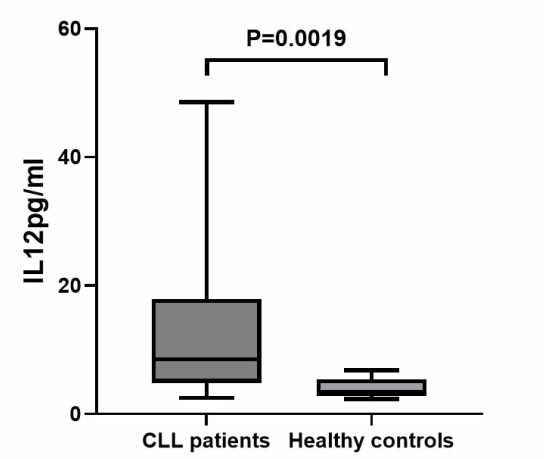

Figure 2. *Serum IL-12 levels in untreated CLL patients (Rai stage 0/I, Binet A, n = 25) and age- and sex-matched healthy controls (n = 15). Cytokine concentrations were measured using ELISA kits (Thermo Fisher Scientific) according to the manufacturer's instructions.*

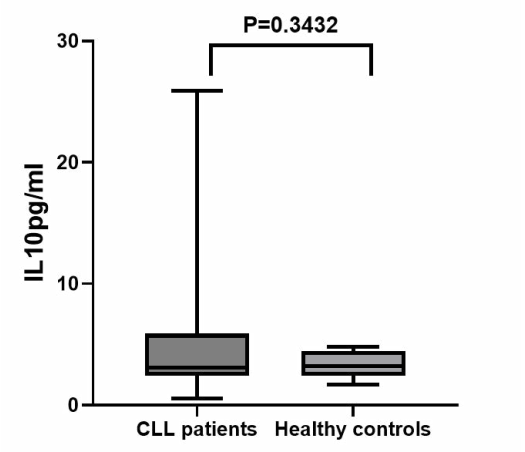

Figure 3. *Serum IL-10 levels in untreated CLL patients (Rai stage 0/I, Binet A, n = 25) and age- and sex-matched healthy controls (n = 15). Cytokine concentrations were measured using ELISA kits (Thermo Fisher Scientific) according to the manufacturer's instructions.*

They are not only involved in the creation of a pro-survival, anti-apoptotic microenvironment that supports leukemic cells but can also act directly on CLL cells, thereby promoting disease progression and contributing to the immune deficiencies characteristic of CLL [2,5]. A better and deeper understanding of this complex cytokine network will provide important insights into disease biology, offer novel prognostic markers, and help identify new avenues for therapeutic intervention.

In this study, we evaluated circulating levels of key inflammatory mediators—the pro-inflammatory cytokines IL-6 and IL-12 and the anti-inflammatory cytokine IL-10—in a cohort of untreated Georgian patients with early-stage CLL (Rai stage 0/I, Binet A).

According to our findings, the sera of the studied patients exhibited significantly elevated levels of IL-6 and IL-12 compared to those from healthy controls (Figures 1 and 2). This cytokine [IL-6] is known for its role in promoting inflammation, cell survival, and tumor proliferation, thereby contributing to the creation of a pro-tumorigenic microenvironment for CLL cells [8]. Previous studies have shown that elevated IL-6 levels are associated with enhanced B-cell survival and resistance to apoptosis, underscoring its importance as both a biomarker and a potential therapeutic target in CLL [17-18]. Patients enrolled in those studies had stage II or more advanced disease. Our findings are notable since we observed elevated IL-6 levels during the early stages of the disease. The underlying cause of elevated IL-6 levels in chronic lymphocytic leukemia (CLL) remains unclear, as it is challenging to discern whether these increases are primarily due to bacterial infections commonly associated with CLL [4] or a consequence of tumor-induced immune dysregulation. Nevertheless, it is well documented that elevated IL-6 concentrations facilitate CLL progression [19]. Moreover, early intervention with anti-IL-6 therapies has demonstrated potential in halting or decelerating disease advancement [18].

Generally, the elevation of IL-12 suggests activation of cellular immunity, potentially reflecting an anti-tumor response. IL-12 plays a pivotal role in stimulating Th1-type responses and natural killer (NK) cell activity, both of which are involved in tumor surveillance. However, the variability observed in IL-12 expression among CLL patients suggests that its upregulation may be influenced by individual immune status and other factors.

Although, IL-12 were discovered over 35 years ago, its precise role in the pathophysiology of various diseases, including CLL, remains incompletely understood. Several studies have highlighted the dual and context-dependent functions of IL-12 in CLL [12,13]. For instance, Schuhknecht et al. reported that IL-12 inhibits apoptosis in tumor-derived B lymphocytes ex vivo, suggesting a potential pro-tumorigenic effect under certain conditions [12]. It is important to emphasize, however, that in this study lymphocytes were isolated from their native tumor microenvironment, which may significantly influence their behavior. In contrast, IL-12 is widely recognized as a key antitumor cytokine that enhances cellular immunity by promoting Th1 differentiation and inducing interferon-gamma (IFN-γ) production. These mechanisms are critical for effective tumor surveillance and immune-mediated tumor clearance [20].

The conflicting data regarding the role of IL-12 in CLL underscore the complex and context-dependent nature of its function. The effects of IL-12 as well as those of other cytokines are modulated by a range of factors, including the tumor microenvironment, disease stage, cytokine concentration, and interactions within the broader cytokine network [13]. Whether the elevation of IL-12 serves a protective function or reflects an ineffective compensatory mechanism remains to be fully elucidated. Notably, we observed a strong negative correlation between IL-12 levels and total lymphocyte counts in peripheral blood ($r = –0.7$). Although this single finding is insufficient to draw definitive conclusions regarding the role of IL-12 in CLL pathogenesis, it nonetheless highlights the need for further investigation in this direction. Several and complex potential biological mechanisms may underlie this relationship. Several complex potential biological mechanisms may underlie this relationship. While dendritic cells (DCs) and macrophages are the major producers of IL-12, they are functionally suppressed by malignant cells in advanced stages of CLL [20]. However, in patients with early-stage disease, innate immune cells may remain less impaired, allowing them to produce more IL-12. This finding is consistent with IL-12's known role in enhancing Th1 immunity, activating NK and CD8+ T cells, and potentially limiting leukemic expansion. Alternatively, IL-12 itself may influence CLL cell apoptosis or tissue homing, thereby indirectly modulating lymphocyte counts in peripheral blood.

Unlike IL-6 and IL-12, there was no significant difference in IL-10 concentrations between CLL patients and healthy individuals (Figure 3). This finding is not unexpected, considering the elevated IL-12 levels detected. A well-established reciprocal regulatory relationship exists between IL-12 and IL-10. By promoting a strong Th1 response and IFN-γ production, IL-12 actively suppresses the development of Th2 cells. Since Th2 cells are a major source of IL-10 (among other cytokines like IL-4, IL-5, IL-13), this indirectly limits IL-10 production [9]. In CLL, the situation is more complex - CLL cells and their microenvironment often produce high IL-10, which promotes immune suppression and disease progression. However, our finding that IL-10 is not elevated in early-stage CLL, suggests that the elevated IL-12 might be suppressing IL-10 production in this phase. Over time, CLL cells and Tregs may override this balance by secreting IL-10, leading to immunosuppression in later stages.

Several studies have reported that serum IL-10 levels are increased in CLL patients with advanced disease and are associated with unfavorable prognoses [13,20-22]. Notably, the patients enrolled in our study were at an early stage of the disease at the time of sampling, therefore, our findings are not inconsistent with previous reports suggesting that as the disease progresses, CLL cells and Tregs might upregulate IL-10, which suppresses IL-12 and shifts the balance toward immune tolerance, allowing leukemic cells to evade immune control.

In addition, we do not exclude the possibility that IL-10's immunosuppressive effects in CLL may be localized within the tumor microenvironment.

Conclusion.

Our study showed that in untreated CLL patients in Georgia, the cytokine profile was characterized by elevated IL-6 and IL-12 levels, indicating the presence of a pro-inflammatory and partially immunostimulatory microenvironment in the early disease stage (Rai stage 0/I, Binet stage A). These findings underscore the critical need for comprehensive and systematic investigations to elucidate the mechanisms underlying cytokine activity and their role in CLL pathogenesis, with a view toward the potential development of cytokine-targeted therapeutic strategies.

Ethics statement.

The research project was approved by the ethics committee of National Center for Disease Control and Public Health of Georgia (NCDC) (approval number #2024-071). All participants were previously informed about the study, written informed consent was obtained and the confidentiality of the information provided was ensured.

Conflict of interests.

The authors have no conflicts of interest to disclose.

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