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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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T LYMPHOCYTE LEVELS PRE AND POST VITAMIN C INFUSION IN PEOPLE NOT INFECTED WITH SARS-COV-2

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Abstract.

Introduction: Vitamin C is a micronutrient with immunomodulatory potential, involved in lymphocyte differentiation and activation, as well as in the regulation of oxidative stress and inflammation. During the COVID-19 pandemic, interest in its use as a therapeutic adjuvant has emerged. However, there is little evidence regarding its effects on healthy individuals not infected with SARS-CoV-2.

Methods: A quasi-experimental study was conducted in healthy adults without respiratory symptoms who received intravenous vitamin C at a dose of 7.5 g for three consecutive days. Leukocyte population levels, including CD4+ and CD8+ T lymphocytes, were assessed before and after the intervention using flow cytometry. Statistical analysis included significance tests for pre- and post-intervention differences.

Results: Statistically significant increases in CD4+, CD8+ T lymphocyte, and eosinophil levels were observed after vitamin C administration. No significant differences were found based on sociodemographic variables. The results remained within accepted biological variability ranges, supporting their consistency.

Conclusion: High-dose intravenous vitamin C may have an immunostimulatory effect in healthy individuals, particularly on CD4+ and CD8+ T lymphocytes. Controlled clinical trials with greater methodological rigor are needed to confirm these findings and explore their applicability in immunocompromised populations.

Key words. Vitamin C, T lymphocytes, antioxidants, ascorbic acid.

Introduction.

Ascorbic acid, commonly known as vitamin C, is a water-soluble molecule with multiple physiological functions, whose intake has been associated with various health benefits [1]. In the oncological context, it has been suggested that, administered in high doses intravenously, vitamin C could act as a selective pro-oxidant agent on neoplastic cells [2]. However, its main biochemical characteristic is its action as a reducing agent, which makes it a potent antioxidant [3].

From an immunological perspective, vitamin C plays a fundamental role in modulating the immune system. It has been described as stimulating the production, differentiation, and function of neutrophils, lymphocytes, and phagocytes, cells that store high concentrations of this vitamin [4]. Hernández et al. reported that vitamin C promotes the proliferation of T lymphocytes and natural killer (NK) cells, in addition to regulating their effector functions. It is also attributed anti-inflammatory, antimicrobial, antiviral, and immunostimulatory properties, as well as a possible antimutagenic effect [5].

Van Gorkom et al. [6] observed that vitamin C influences the function and development of lymphocytes; however, they found that leukocyte restoration is slower in deficiency states, which could predispose to infections. In turn, Carr et al. [7] detailed that this vitamin supports both the innate and adaptive immune systems, in addition to acting as an antioxidant and cofactor for key biosynthetic enzymes. During states of stress or infections, plasma concentrations of ascorbic acid decrease significantly.

Preclinical studies and clinical trials evaluated by Nabzdyk et al. [8] highlight the therapeutic potential of vitamin C as a modulator of immune cell biology. However, a conclusive effect on B and T cell proliferation and differentiation has not been demonstrated.

Clinically, intravenous administration of vitamin C at doses up to 1.5 g/kg of body weight has shown a favorable safety profile. In viral infections such as COVID-19, a significant reduction in CD4+ and CD8+ T cells has been documented, along with exacerbated immune activation [9-11]. T lymphocytes are divided into three main subpopulations: helper (CD4+, which activate Th1 and Th2 cells), cytotoxic (CD8+), and regulatory. These cells mediate immune responses through cytokines with both pro-inflammatory (Th1) and anti-inflammatory (Th2) functions.

Since vitamin C may contribute to preventing or modulating respiratory and systemic infections, it is important to explore its immunological effects. Therefore, the objective of this study was to evaluate intravenous vitamin C infusion on T lymphocyte stimulation (CD4+ and CD8+) in healthy individuals, as a prophylactic strategy potentially applicable to patients with respiratory diseases.

Materials and Methods.

Study design:

A two-arm quasi-experimental study was conducted with assessment at three points: baseline, first follow-up (day 8), and second follow-up (day 15) post-intervention. The objective was to evaluate changes in T lymphocyte counts (CD4/CD8) after intravenous administration of vitamin C. The duration of the study was 30 days in total.

Population and sample:

The study population consisted of 40 people (professors from the Escuela de Tecnología Médica and administrative staff of the Facultad de Medicina de la Universidad Nacional Mayor de San Marcos – UNMSM), between the ages of 19 and 69. Participant selection was conducted during the COVID-19

pandemic, using virtual invitations via mobile applications, phone calls, and institutional email. Sampling was non-probabilistic by convenience, and 20 participants were assigned to the intervention group and 20 to the control group, in order of arrival and compliance with inclusion and exclusion criteria.

Inclusion criteria: Faculty and UNMSM personnel of both sexes, without symptoms of acute or chronic respiratory illness, and without chronic or metabolic disease, who agreed to participate in the study and signed the informed consent form.

Exclusion criteria: Teachers with symptoms of acute or chronic respiratory illness and/or chronic or metabolic disease. Teachers with a previous positive COVID-19 test.

Data collection instruments:

Data collection was conducted using a structured form created using Google Forms. The database was built in Microsoft Excel, with a unique code assigned to each participant.

Immunological analysis was performed by flow cytometry. A cocktail of anti-CD45-Percp, CD3-FITC, CD4-APC, and CD8-PE antibodies was used, according to the protocol of the Kombitest reagent (Exbio, Czech Republic), processed in a flow cytometer Novocyte Advanteon (Agilent, EE.UU.). Cytometry data analysis was performed with Infinicyt software, using file merging and automatic population separation tools (APS).

Study variables:

Sociodemographic variables (age and sex), clinical variables (asthma, high blood pressure, diabetes, obesity, cataracts, hypothyroidism, and osteoarthritis), body mass index (classified as normal, overweight, or obese), COVID-19 vaccination history (number of doses), vitamin C-rich diet (frequency and duration of consumption) (Table 1) and immunological parameters (absolute CD4 and CD8 lymphocyte counts in peripheral blood) were collected. The primary endpoint was the percentage change in absolute CD4/CD8 T lymphocyte counts, assessed at three time points: baseline, day 8, and day 15 post-intervention, categorized according to biological variability scenarios (±10% and ±20%).

Procedure:

On day 0 of the study, a blood sample was drawn (baseline sample) for T lymphocyte counts (CD4 and CD8). Subsequently, the intervention group received a daily intravenous infusion of 7.5 g of vitamin C dissolved in 100 ml of 9% saline solution for three consecutive days. The control group received 9% saline solution under the same regimen.

Two additional venous blood draws were performed (days 8 and 15) to reassess the absolute CD4/CD8 lymphocyte count.

Table 1. *Characteristics of Vitamin C and control groups.*

Characteristics	**Control (n=20)**	**Vit. C (n=20)**	**P value**
Age (years)*	48.5 (24.5)	55 (22.0)	0.279
Women	12 (60.0)	14 (70.0)	0.741
Weight (Kg)*	68 (11.0)	69 (19.3)	0.892
BMI (Kg/m^2)*	24.8 (2.8)	25.2 (5.1)	0.735
Categorized BMI			0.483
Normal	10 (50.0)	9 (45.0)	
Overweight	6 (30.0)	9 (45.0)	
Obesity	4 (20.0)	2 (10.0)	
No comorbidity	11 (55.0)	14 (70.0)	0.327
Asthma	1 (5.0)	2 (10.0)	1.000
Diabetes	1 (5.0)	2 (10.0)	1.000
High blood pressure (HBP)	3 (15.0)	2 (10.0)	1.000
Metabolic syndrome	1 (5.0)	0 (0.0)	1.000
Cataract	1 (5.0)	0 (0.0)	1.000
Osteoarthritis	1 (5.0)	0 (0.0)	1.000
Hypothyroidism	2 (10.0)	0 (0.0)	0.487
COVID-19 vaccine doses			1.000
1 dose	1 (5.0)	0 (0.0)	
2 dose	7 (35.0)	7 (35.0)	
3 dose	12 (60.0)	12 (60.0)	
4 dose	0 (0.0)	1 (5.0)	
Diet rich in Vitamin C	14 (70.0)	16 (80.0)	0.716
Days a week with Vit C			0.173
1 day	5 (35.7)	1 (6.2)	
2 to 3 days	4 (28.6)	9 (56.3)	
3 to 4 days	2 (14.3)	4 (25.0)	
Every day	3 (21.4)	2 (12.5)	
* Median (Interquartile Range)			
BMI: Body Mass Index			
Values expressed in percentage (%)			

The samples were transported at room temperature to the reference laboratory within a maximum of two hours after collection and analyzed by flow cytometry according to the described protocol.

Cytometric analysis included cell labeling, sequential gating, file merging, population analysis using dot plots, and automatic separation of cell subpopulations (Figure 1). Infinicyt analysis software was used, and analysis was performed using file merging (Table 2). Monocytes (weak CD4+, medium SSC), neutrophils, and eosinophils (high SSC, autofluorescence in FITC, PE, and Percp) were also evaluated.

Table 2. *Immunophenotype parameters to define leukocyte populations.*

Leukocyte Population	Immunophenotypic Profile
T lymphocytes	FSC low/ SSC low / CD45+++/ CD3++
T lymphocytes CD4	FSC low / SSC low / CD45 +++/ CD3 ++/ CD4 ++/ CD8-
T lymphocytes CD8	FSC low / SSC low / CD45 ++/ CD3 ++/ CD8 +/++/ CD4-
T lymphocytes CD4+/ CD8+	FSC low / SSC low / CD45 +++/ CD3 ++/ CD4+/ CD8+
T lymphocytes CD4-/ CD8-	FSC low / SSC low / CD45 +++/ CD3 ++/ CD8-/ CD4-
Other lymphocytes	FSC low / SSC low / CD45 +++/ CD3-, CD4-, CD8-/+d.
Neutrophils	FSC medium-high/ SSC high/ CD45+d/ CD3-, CD4-, CD8-
Monocytes	FSC medium/ SSC medium/ CD45++/+++/ CD4+débil
Eosinophils	FSC medium/SSC high/ autofluorescence in Percp, FITC and PE

Analysis plan:

The percentage changes in absolute CD4 and CD8 lymphocyte counts were estimated at two follow-up times with respect to the baseline measurement: first follow-up (Δ1) and second follow-up (Δ2), using the following formula Δ 1 or 2 = (No. of absolute cells t 1 or 2 * 100) / No. of absolute cells tbasal.

Values were transformed using a base-10 logarithm to normalize their distribution. Based on the literature on biological variability of peripheral blood T lymphocytes, two post hoc scenarios were established: one with a threshold of ±10% and another with a threshold of ±20%. In both cases, changes were categorized as "no change" (≤10% or ≤20%), "increase" (>10% or >20%), or "decrease" (<-10% or <-20%).

Numerical variables were described as mean or median and standard deviation or interquartile range, depending on their distribution. Categorical variables were summarized as absolute and relative frequencies.

For bivariate analysis, Fisher's exact test was used for categorical variables. Intra-individual comparisons were performed using the Student t test for paired samples, and between-group comparisons were performed using the Student t test for independent samples. Sankey diagrams were constructed to visually represent categorical changes across the defined scenarios.

Statistical analysis was performed using Stata 17 software (StataCorp LLC, EE.UU.) and RStudio. A p value < 0.05 was considered significant.

Ethical considerations:

The study was approved by the Research Ethics Committee of the Facultad de Medicina de la Universidad Nacional Mayor de San Marcos with the approval **N. º 05753-R-21 UNMSM**. All participants were fully informed about the study's objectives, procedures, benefits, and risks, and signed informed consent prior to participation.

Results.

The baseline characteristics of the participants showed no significant differences between the intervention and control groups. Overall, 50% of the participants had a body mass index within the normal range. In the intervention group, 45% were overweight and 10% were obese. Comorbidities were rare, with asthma, diabetes mellitus, and high blood pressure reported in up to 30% of participants.

Both groups consisted of 20 people each, predominantly female (65%) and with a predominantly higher educational level (95%). All participants resided primarily in central Lima, and none reported smoking. Furthermore, all were vaccinated against COVID-19, with 60% of each group having received three doses. The majority had no comorbidities and reported maintaining a diet rich in vitamin C.

When comparing both groups in the first delta (Δ1), a significant difference was observed in the CD4+ T lymphocyte count. In the second delta (Δ2), the significant differences widened, also being found in total T lymphocytes, CD4+ lymphocytes, CD8+ lymphocytes, and eosinophils (Figure 2). Within the intervention group, a significant difference was identified in eosinophils between Δ1 and Δ2.

When the biological variability scenario of ±10% was applied, significant differences were found between the control and intervention groups in Δ2 for total, CD4+, and CD8+ T lymphocytes (Table 3). These findings remained consistent when the second scenario was applied, with a biological variability of ±20%. Furthermore, under this second scenario, significant differences were observed in CD4+CD8+ lymphocytes when comparing both groups in both Δ1 and Δ2.

Analysis of the longitudinal behavior of the categories (increases, decreases, no change) according to the variability scenarios showed that individuals in the intervention group maintained a consistent trend over time. In the ±10% scenario, participants classified as "increased" at Δ1 mostly remained in that same category at Δ2. This trend was even more consistent in the ±20% scenario, where greater stability was observed in the classification of the cell subpopulations evaluated (Figure 3).

Discussion.

This study evaluated the effect of intravenous vitamin C administration on certain leukocyte populations, especially CD4+ and CD8+ T lymphocytes, in healthy adults without respiratory symptoms. Unlike previous studies focused on hospitalized patients with acute respiratory illness, this study

Table 3. *Biological variability due to differences in times according to control and intervention groups.*

		Biological variability ± 10%							Biological variability ± 20%					
		Difference 1			Difference 2				Difference 1			Difference 2		
		Control	Vit. C		Control	Vit. C			Control	Vit. C		Control	Vit. C	
Lymphocytes				1.000			0.141				0.590			0.138
	Decreases	4 (20.0)	3 (15.0)		6 (30.0)	1 (5.0)			4 (20.0)	2 (10.0)		2 (10.0)	0 (0.0)	
	No change	11 (55.0)	11 (55.0)		7 (35.0)	10 (50.0)			13 (65.0)	13 (65.0)		16 (80.0)	14 (70.0)	
	Increases	5 (25.0)	6 (30.0)		7 (35.0)	9 (45.0)			3 (15.0)	5 (25.0)		2 (10.0)	6 (30.0)	
T lymphocytes				0.409			**0.016**				0.227			**0.029**
	Decreases	6 (30.0)	2 (10.0)		7 (35.0)	0 (0.0)			4 (20.0)	1 (5.0)		3 (15.0)	0 (0.0)	
	No change	8 (40.0)	11 (55.0)		8 (40.0)	12 (60.0)			14 (70.0)	14 (70.0)		16 (80.0)	14 (70.0)	
	Increases	6 (30.0)	7 (35.0)		5 (25.0)	8 (40.0)			2 (10.0)	5 (25.0)		1 (5.0)	6 (30.0)	
CD4 lymphocytes				0.219			**0.005**				0.068			**0.014**
	Decreases	7 (35.0)	2 (10.0)		9 (45.0)	1 (5.0)			5 (25.0)	1 (5.0)		6 (30.0)	0 (0.0)	
	No change	7 (35.0)	9 (45.0)		8 (40.0)	9 (45.0)			14 (70.0)	14 (70.0)		13 (65.0)	15 (75.0)	
	Increases	6 (30.0)	9 (45.0)		3 (15.0)	10 (50.0)			1 (5.0)	5 (25.0)		1 (5.0)	5 (25.0)	
CD8 lymphocytes				0.841			**0.033**				0.421			**0.010**
	Decreases	5 (25.0)	3 (15.0)		6 (30.0)	0 (0.0)			4 (20.0)	1 (5.0)		2 (10.0)	0 (0.0)	
	No change	8 (40.0)	9 (45.0)		9 (45.0)	12 (60.0)			12 (60.0)	14 (70.0)		18 (90.0)	14 (70.0)	
	Increases	7 (35.0)	8 (40.0)		5 (25.0)	8 (40.0)			4 (20.0)	5 (25.0)		0 (0.0)	6 (30.0)	
CD4+CD8+ lymphocytes				0.544			0.356				**0.036**			**0.040**
	Decreases	7 (35.0)	9 (45.0)		5 (25.0)	6 (30.0)			7 (35.0)	4 (20.0)		5 (25.0)	2 (10.0)	
	No change	3 (15.0)	5 (25.0)		3 (15.0)	6 (30.0)			3 (15.0)	11 (55.0)		6 (30.0)	14 (70.0)	
	Increases	10 (50.0)	6 (30.0)		12 (60.0)	8 (40.0)			10 (50.0)	5 (25.0)		9 (45.0)	4 (20.0)	
CD4-CD8- lymphocytes				0.841			0.904				0.819			0.251
	Decreases	4 (20.0)	3 (15.0)		5 (25.0)	3 (15.0)			3 (15.0)	2 (10.0)		3 (15.0)	0 (0.0)	
	No change	8 (40.0)	10 (50.0)		4 (20.0)	4 (20.0)			11 (55.0)	13 (65.0)		8 (40.0)	11 (55.0)	
	Increases	8 (40.0)	7 (35.0)		11 (55.0)	13 (65.0)			6 (30.0)	5 (25.0)		9 (45.0)	9 (45.0)	
CD4/CD8 ratio				0.558			0.331				0.605			1.000
	Decreases	4 (20.0)	2 (10.0)		7 (35.0)	3 (15.0)			3 (15.0)	1 (5.0)		1 (5.0)	0 (0.0)	
	No change	14 (70.0)	14 (70.0)		11 (55.0)	13 (65.0)			17 (85.0)	18 (90.0)		18 (90.0)	18 (90.0)	
	Increases	2 (10.0)	4 (20.0)		2 (10.0)	4 (20.0)			0 (0.0)	1 (5.0)		1 (5.0)	2 (10.0)	
DP/DN ratio				0.423			0.050				0.437			0.050
	Decreases	11 (55.0)	8 (40.0)		10 (50.0)	9 (45.0)			10 (50.0)	7 (35.0)		10 (50.0)	9 (45.0)	
	No change	3 (15.0)	7 (35.0)		3 (15.0)	9 (45.0)			5 (25.0)	9 (45.0)		3 (15.0)	9 (45.0)	
	Increases	6 (30.0)	5 (25.0)		7 (35.0)	2 (10.0)			5 (25.0)	4 (20.0)		7 (35.0)	2 (10.0)	
Other lymphocytes				1.000			0.366				1.000			0.366
	Decreases	6 (30.0)	6 (30.0)		6 (30.0)	3 (15.0)			6 (30.0)	6 (30.0)		6 (30.0)	3 (15.0)	
	No change	8 (40.0)	7 (35.0)		5 (25.0)	9 (45.0)			8 (40.0)	7 (35.0)		5 (25.0)	9 (45.0)	
	Increases	6 (30.0)	7 (35.0)		9 (45.0)	8 (40.0)			6 (30.0)	7 (35.0)		9 (45.0)	8 (40.0)	
Neutrophils				0.827			0.292				0.827			0.292
	Decreases	5 (25.0)	4 (20.0)		3 (15.0)	6 (30.0)			5 (25.0)	4 (20.0)		3 (15.0)	6 (30.0)	
	No change	11 (55.0)	13 (65.0)		10 (50.0)	11 (55.0)			11 (55.0)	13 (65.0)		10 (50.0)	11 (55.0)	
	Increases	4 (20.0)	3 (15.0)		7 (35.0)	3 (15.0)			4 (20.0)	3 (15.0)		7 (35.0)	3 (15.0)	
Basophils				0.671			0.633				0.671			0.633
	Decreases	6 (30.0)	8 (40.0)		5 (25.0)	3 (15.0)			6 (30.0)	8 (40.0)		5 (25.0)	3 (15.0)	
	No change	2 (10.0)	3 (15.0)		3 (15.0)	2 (10.0)			2 (10.0)	3 (15.0)		3 (15.0)	2 (10.0)	
	Increases	12 (60.0)	9 (45.0)		12 (60.0)	15 (75.0)			12 (60.0)	9 (45.0)		12 (60.0)	15 (75.0)	
Eosinophils				1.000			0.472				1.000			0.472
	Decreases	9 (45.0)	9 (45.0)		10 (50.0)	14 (70.0)			9 (45.0)	9 (45.0)		10 (50.0)	14 (70.0)	
	No change	6 (30.0)	6 (30.0)		2 (10.0)	1 (5.0)			6 (30.0)	6 (30.0)		2 (10.0)	1 (5.0)	
	Increases	5 (25.0)	5 (25.0)		8 (40.0)	5 (25.0)			5 (25.0)	5 (25.0)		8 (40.0)	5 (25.0)	
Monocytes				0.310			0.380				0.310			0.380
	Decreases	6 (30.0)	4 (20.0)		9 (45.0)	5 (25.0)			6 (30.0)	4 (20.0)		9 (45.0)	5 (25.0)	
	No change	7 (35.0)	12 (60.0)		4 (20.0)	7 (35.0)			7 (35.0)	12 (60.0)		4 (20.0)	7 (35.0)	
	Increases	7 (35.0)	4 (20.0)		7 (35.0)	8 (40.0)			7 (35.0)	4 (20.0)		7 (35.0)	8 (40.0)	
Values expressed in percentages														

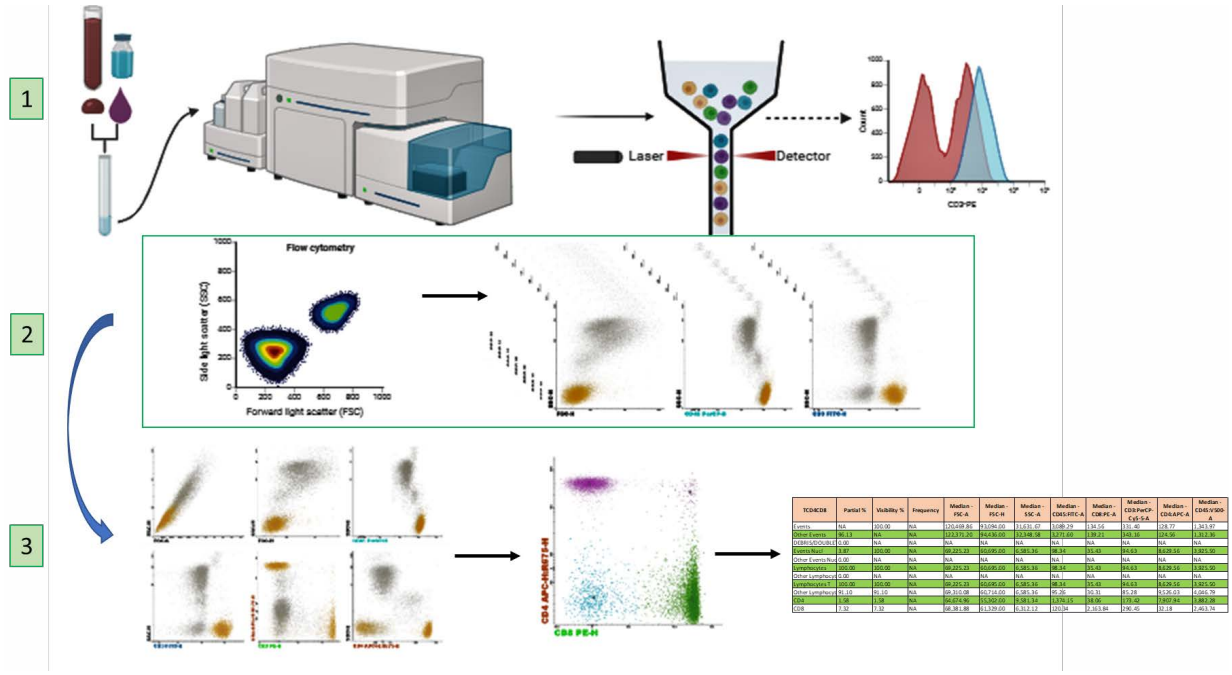

Figure 1. *Steps used for the flow cytometry process and subsequent analysis for the detection and characterization of T lymphocytes and their subpopulations. Step 1 shows the labeling of each sample for subsequent acquisition in the flow cytometer. Step 2 shows the merging of files for group file analysis, because they are files processed with the same sample adjustment and processing technique. Step 3 shows the separation and characterization of populations for export in statistical tables. This figure was created using Biorender.*

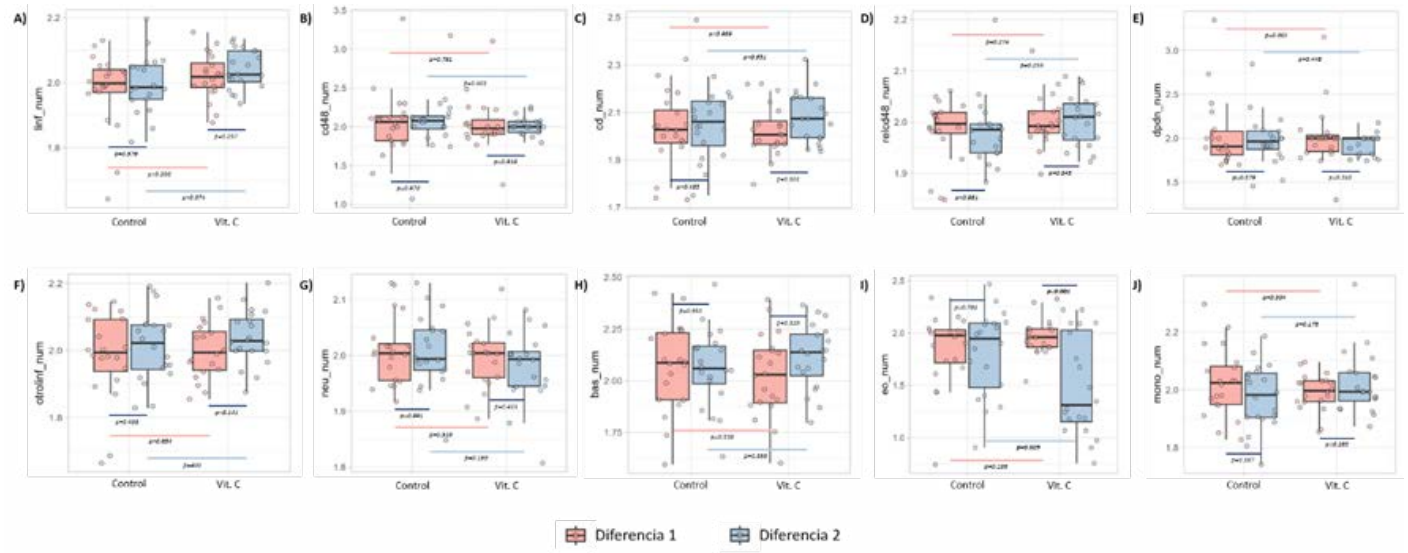

Figure 2 y Sup. 1. *Biological variability in total T lymphocytes, CD4+ lymphocytes, CD8+ lymphocytes, and eosinophils.*

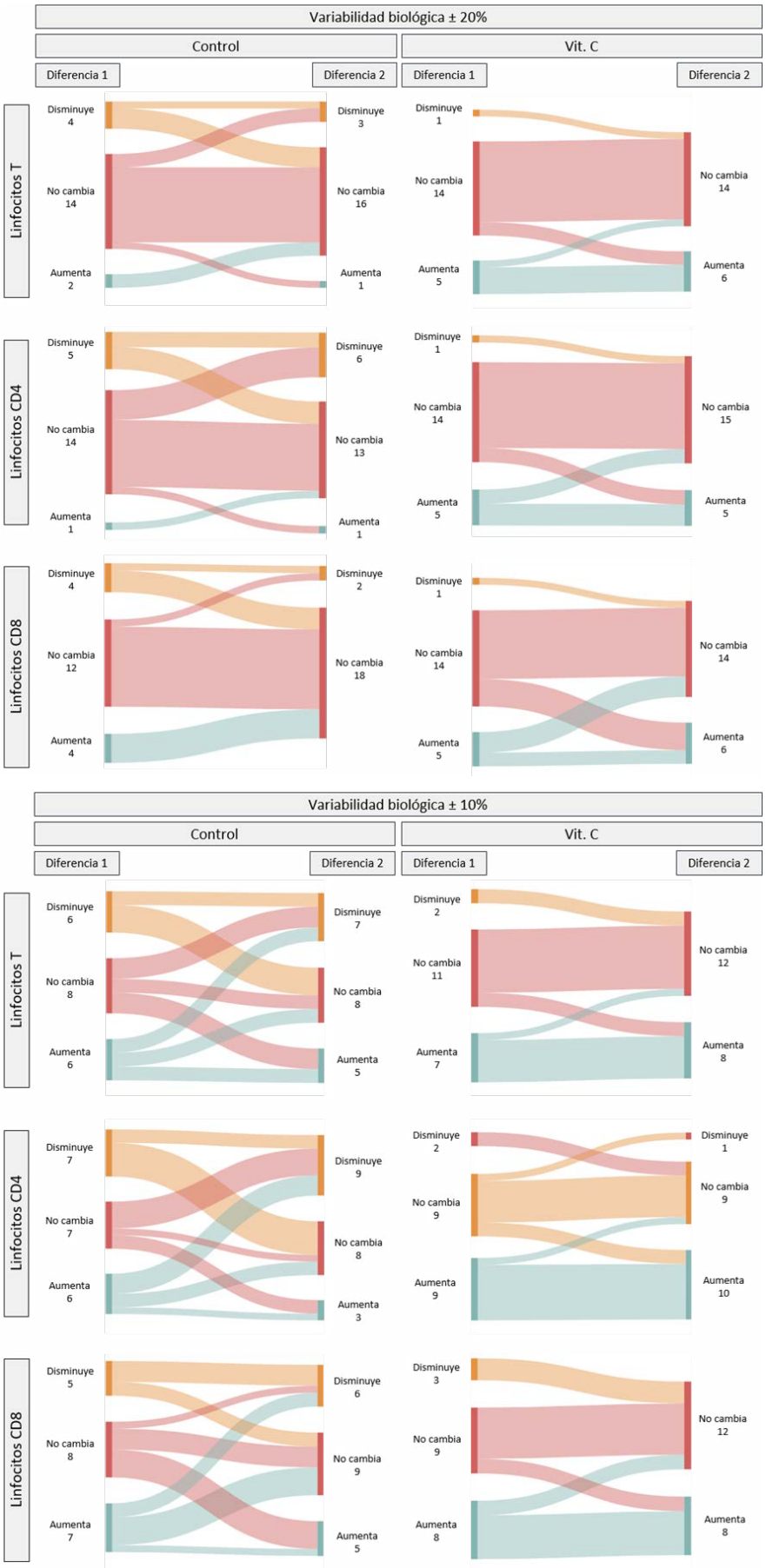

Figure 3. *Consistency of findings according to biological variability. Biological variability ± 20% and 10%.*

provides preliminary evidence of immune modulation in subjects without active infection.

Vitamin C has been widely studied for its immunomodulatory potential. It is recognized for its role in the differentiation and proliferation of T and B lymphocytes, the enhancement of phagocytosis in neutrophils and macrophages, and the regulation of inflammation and oxidative stress [4,12,13].

In this study, statistically significant differences were observed in the levels of CD4+, CD8+ T lymphocytes and eosinophils after the intervention, reinforcing the immunostimulatory role of vitamin C. While the elevation of T cells and eosinophils due to vitamin C intake can enhance overall immune function, it is important to note that this alone may not be sufficient to provide complete protection against viral disorders like COVID-19. The immune response to such infections is complex and involves multiple factors, including the presence of memory T cells and the overall health of the individual. Van Gorkom et al. confirmed that T and NK lymphocytes are sensitive especially to the effects of vitamin C, while the evidence in B lymphocytes is still contradictory [6,14]. Kouakanou et al. reported an increase in the expansion of T lymphocytes in the production of gamma interferon after supplementation with vitamin C [15].

These results were consistent even when applying margins of biological variability (±10% and ±20%), supporting the robustness of the findings. However, these effects could vary depending on the dose administered, the duration of treatment, and the individual characteristics of the participants. In in vitro studies, Cerullo et al. described that its action is dose-dependent and favors the maturation of precursor cells into functional lymphocytes, also modulating the Th2-type immune response to Th1 [14].

Longitudinal trend analysis showed remarkable stability in the immunological behavior of the intervention group, suggesting that the effect of vitamin C is not transient and could induce a sustained response at least until day 15. This finding is particularly relevant considering that, due to its water-soluble nature, vitamin C is usually rapidly excreted through the kidneys, which limits its time of action in the body.

However, intravenous administration allows higher plasma concentrations to be reached than those obtained orally, favoring its accumulation in leukocytes and other immunocompetent tissues, which could explain the prolongation of the observed effect [2]. Furthermore, it has been documented that vitamin C plays a fundamental antioxidant role in maintaining the intracellular redox state, thus modulating the function of various immune cells, including T lymphocytes, and promoting a more sustained immune response over time [11].

Although our participants were not acutely ill, the results raise the possibility that vitamin C could be considered an immunomodulatory tool in populations with immunosenescence, mild immunodeficiencies, or in the stages of post-infectious recovery. Vitamin C plays a fundamental role in the function of immune cells such as neutrophils, T and B lymphocytes, and in the integrity of epithelial barriers. It also exerts an antioxidant action that protects against damage induced by oxidative stress in infectious processes [16]. In older adults or health personnel exposed to a high infectious load, its supplementation could have immunoprotective benefits and be considered as a preventive strategy, given its immunomodulatory properties, low cost and minimal adverse effects [17]. Clinical trials and systematic reviews have documented benefits in specific populations, such as people with vitamin C deficiency or under intense physical stress, in whom a lower incidence of respiratory infections and a reduction in the duration and severity of symptoms were observed [13].

The study has several limitations. The main one is the small sample size, which limits the generalizability of the results and the statistical power. Furthermore, plasma vitamin C levels were not measured before and after the intervention, which would have allowed for correlation of immunological findings with the compound's actual bioavailability. Another limitation is the lack of a placebo group, which may introduce interpretation bias.

Furthermore, although data on diet, lifestyle, and comorbidities were collected, these factors, which could influence immune response, were not strictly controlled. Recruitment was difficult due to the pandemic context, and limited financial resources limited the number of tests processed.

Despite these limitations, the findings support the potential of vitamin C as a modulator of the adaptive immune response. Although its efficacy in the prevention or treatment of specific diseases has not been demonstrated to date, several authors suggest its use in the management of acute respiratory illnesses, sepsis, or septic shock, with high and safe doses exceeding 1 g/kg of body weight [18].

Elevated immune cell numbers correlate positively with enhanced infection defense. These cells play distinct yet complementary roles in identifying, attacking, and eliminating pathogens. A well-coordinated increase in immune cell activity can significantly improve the body's ability to defend against infections, including viral disorders like COVID-19.

Conclusion.

This study demonstrates that high-dose intravenous vitamin C can stimulate CD4+ and CD8+ T cell production in healthy adults, suggesting an immunostimulatory effect in the absence of active infection. Although the results are encouraging, they should be interpreted with caution due to the small sample size, lack of a control group, and lack of measurement of immunological functional parameters.

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Original contribution and importance of the article: Studying how vitamin C affects T cell levels helps us understand whether it has a positive impact on the immune response to respiratory diseases using flow cytometry. Although there is limited and controversial evidence regarding the direct benefits of vitamin C in the prevention or treatment of viral diseases, more focused research can provide more robust data and guidance on its use. This study will pave the way for larger studies and the consideration of vitamin C as part of additional therapeutic or preventative protocols. Research like this contributes to expanding our knowledge of how simple interventions, such as vitamin C supplementation, could influence immune health and enhance the response to viral diseases like SARS-CoV-2.

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