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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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DYNAMIC BIOMARKERS FOR PREDICTING POST-TRAUMATIC EPILEPSY AFTER TRAUMATIC BRAIN INJURY

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Abstract.

Background: Post-traumatic epilepsy (PTE) is a delayed complication of traumatic brain injury (TBI), but its biological predictors remain insufficiently defined. This study evaluated the dynamic profile of serum biomarkers of neuroinflammation, neuroimmune activation and neuronal injury after moderate TBI, as well as their predictive value for late post-traumatic seizures and PTE during 12 months of follow-up.

Materials and Methods: This prospective observational study included 126 patients with moderate TBI, who were classified at 12 months into patients without late post-traumatic seizures (n = 113) and patients with late post-traumatic seizures or PTE-spectrum outcomes (n = 13). The biomarker panel included interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL) and high-mobility group box 1 (HMGB1), measured at 24-48 hours, 7-10 days, 30 ± 7 days and 3 months after injury. ROC analysis was used to determine clinically informative thresholds, and a composite BioIndex was calculated from IL-6, HMGB1 and NfL.

Results: Patients who developed late post-traumatic seizures or PTE-spectrum outcomes had significantly higher levels of inflammatory, neuroimmune and neuroinjury biomarkers throughout follow-up. IL-6, TNF-alpha and IL-1beta remained elevated from the acute phase to 3 months; GFAP and NfL indicated persistent astroglial and axonal injury; and HMGB1 reflected sustained neuroimmune activation. Clinically significant thresholds were IL-6 ≥ 10 pg/mL, IL-1beta ≥ 2.0 pg/mL, TNF-alpha ≥ 7.5 pg/mL, HMGB1 ≥ 6.0 ng/mL and NfL ≥ 35 pg/mL. BioIndex ≥ 2 was associated with an increased 12-month risk of late seizures or PTE-spectrum outcomes.

Conclusion: Late post-traumatic seizures and PTE after moderate TBI are associated with a persistent biomarker signature of neuroinflammation, neuroimmune activation and glial-axonal injury. A simple BioIndex based on IL-6, HMGB1 and NfL may serve as a practical laboratory tool for early risk stratification and individualized follow-up after moderate TBI.

Key words. Traumatic brain injury, post-traumatic epilepsy, neuroinflammation, biomarkers, IL-6, HMGB1, neurofilament light chain, risk stratification.

Introduction.

Post-traumatic epilepsy (PTE) is one of the most serious delayed consequences of traumatic brain injury (TBI), as confirmed by clinical reviews and meta-analyses of PTE risk factors. In contrast to early post-traumatic seizures, which occur in the acute phase, late post-traumatic seizures reflect the formation of a more stable epileptogenic state and are consistent with contemporary concepts of the clinical definition of epilepsy and seizure classification [1,2]. This process develops gradually and may remain clinically silent for several weeks or months [3,4]. Therefore, the absence of early biological indicators capable of identifying high-risk patients before recurrent seizures develop remains one of the key clinical problems [3,4].

The pathogenesis of post-traumatic epileptogenesis is not limited to mechanical damage. After TBI, the brain undergoes a cascade of secondary processes, including neuroinflammation, activation of innate immune signaling pathways, blood-brain barrier dysfunction, astroglial response, axonal injury and remodeling of neuronal networks [5-10]. These processes may lower the seizure threshold and support the formation of epileptogenic circuits [5-7]. In this context, blood biomarkers can provide clinically important information that is not fully captured by neurological examination, magnetic resonance imaging (MRI) or electroencephalography (EEG) alone [8-10].

Among inflammatory markers, interleukin-6 (IL-6) reflects systemic and neuroinflammatory activation, tumor necrosis factor-alpha (TNF-alpha) indicates activation of proinflammatory pathways, and interleukin-1beta (IL-1beta) is closely associated with inflammasome activity and increased neuronal excitability [5-7]. Glial fibrillary acidic protein (GFAP) is a marker of astrocyte injury and reactive gliosis, whereas neurofilament light chain (NfL) reflects axonal injury [9,10]. High-mobility group box 1 (HMGB1) is a damage-associated molecule involved in neuroimmune activation and may reflect blood-brain barrier disruption and persistent post-traumatic injury signaling [8].

However, isolated biomarkers are often difficult to use in routine clinical decision-making, especially when they are not integrated into an understandable prognostic model [11,12]. A practical approach is to combine several biologically meaningful markers into a simple index that links laboratory data with individualized risk stratification [11]. In the present study, we evaluated serial changes in IL-6, TNF-alpha, IL-1beta, GFAP, NfL and HMGB1 after moderate TBI and developed a composite BioIndex based on the most informative markers. The aim of the study was to determine whether a dynamic

biomarker profile can predict late post-traumatic seizures/PTE-spectrum outcomes during 12 months of follow-up.

Materials and Methods.

Study design:

This was a prospective observational study with serial biomarker monitoring after moderate TBI. The study was designed to assess biological predictors of late post-traumatic seizures and PTE during 12 months of follow-up. The design, analysis and reporting of prognostic results were oriented toward the TRIPOD reporting requirements for individual prognosis models [11].

The primary endpoint was the development of late post-traumatic seizures/PTE-spectrum outcomes, defined as at least one seizure occurring later than 7 days after TBI, which corresponds to the commonly accepted distinction between early and late post-traumatic seizures [1-4]. Clinically confirmed PTE, including recurrent late seizures and/or an established clinical diagnosis of PTE, was considered a more clinically expressed form of the same adverse outcome [1,2].

Study Population:

The study included 126 patients with moderate TBI. Moderate TBI was defined using clinical and neuroimaging criteria, including a Glasgow Coma Scale (GCS) score of 9-12 points within the first 24 hours, loss of consciousness, post-traumatic amnesia, focal neurological signs and structural evidence of brain injury; the choice of this patient category was based on the known association between TBI severity, structural injury and the risk of late post-traumatic seizures [3,4].

Patients were divided into two groups according to the 12-month outcome: group 1 included patients without late post-traumatic seizures (n = 113), and group 2 included patients with late post-traumatic seizures/PTE-spectrum outcomes (n = 13). Among patients in group 2, 4 had a single late post-traumatic seizure and 9 had clinically confirmed PTE.

Inclusion and Exclusion Criteria:

The inclusion criteria were age 23-40 years, confirmed moderate TBI, availability of serial biomarker assessment, ability to complete 12-month follow-up and written informed consent. The age range of 23-40 years was chosen as a methodological strategy to reduce the influence of age-related factors on baseline biomarker concentrations, primarily NfL, as well as GFAP and inflammatory/neuroimmune markers. This approach allowed us to form a more homogeneous adult cohort, reduce the likelihood of confounding between the effect of trauma and age-related changes in laboratory indicators, and improve the internal validity of the assessment of the association between biomarker dynamics and 12-month PTE risk [9,10]. At the same time, this decision was initially considered a limitation of external applicability, because the obtained thresholds require separate validation in patients younger than 23 years, older than 40 years, children and elderly individuals. Patients were excluded if they had epilepsy or unprovoked seizures before trauma, active neuroinfection, brain tumor, acute metabolic or toxic causes of seizures, severe somatic decompensation, inability to complete the protocol, loss to follow-up or refusal to participate.

Biomarker Panel:

The biomarker panel included six serum/plasma markers grouped according to their biological meaning: neuroinflammation markers - IL-6, TNF-alpha and IL-1beta; glial and axonal injury markers - GFAP and NfL; and a neuroimmune activation marker - HMGB1. This marker selection was based on evidence on the role of inflammation, HMGB1-mediated neuroimmune activation, GFAP and NfL in brain injury and post-traumatic epileptogenesis [5-10].

IL-6, TNF-alpha, IL-1beta and HMGB1 were determined using enzyme-linked immunosorbent assay. GFAP and NfL were determined using high-sensitivity immunoassays. Biomarker concentrations were expressed in pg/mL for IL-6, TNF-alpha, IL-1beta and NfL, and in ng/mL for GFAP and HMGB1.

Timing of Biomarker Assessment:

Biomarkers were assessed at four standardized time points: T1 - 24-48 hours after TBI; T2 - 7-10 days after TBI; T3 - 30 ± 7 days after TBI; and T4 - 3 months after TBI. Subsequent visits at 6 and 12 months were used for clinical monitoring and final outcome registration.

BioIndex Calculation:

To improve clinical applicability, a composite BioIndex was created from three markers representing different pathogenetic pathways: IL-6 as a marker of persistent neuroinflammation, HMGB1 as a marker of neuroimmune activation and damage-associated molecular signaling, and NfL as a marker of continuing axonal injury [5,8,9]. The creation of the index followed the logic of building an interpretable prognostic model recommended for clinical predictors of individual risk [11].

Each marker was converted into a binary variable according to ROC-derived thresholds: IL-6 $\geq$ 10 pg/mL = 1 point, HMGB1 $\geq$ 6.0 ng/mL = 1 point and NfL $\geq$ 35 pg/mL = 1 point. This approach made it possible to translate continuous laboratory indicators into clinically interpretable risk categories [11]. BioIndex was calculated as follows: BioIndex = IL-6 (bin) + HMGB1(bin) + NfL(bin)

The total score ranged from 0 to 3 points. BioIndex $\geq$ 2 indicated simultaneous activation of at least two biological pathways and was considered clinically significant.

Statistical Analysis and Methodological Reporting:

Continuous variables were presented as mean ± standard error (SE) or median with interquartile range, depending on the distribution. Categorical variables were presented as n and percentage. Between-group comparisons were performed using appropriate parametric or non-parametric tests. ROC analysis was used to determine optimal thresholds and assess prognostic accuracy. Logistic regression was used to calculate odds ratios (ORs). Correlation analysis was used to evaluate associations between biomarkers and 12-month seizure outcome. Reporting of prognostic models, threshold values, odds ratios and AUC increments was performed in accordance with the TRIPOD recommendations for individual prognosis studies [11]. Methodological limitations, model applicability and the need for external validation were interpreted according to PROBAST principles [12]. A p value < 0.05 was considered statistically significant.

Results.

Frequency of Late Post-Traumatic Seizures and PTE:

During 12 months of follow-up, late post-traumatic seizures/PTE-spectrum outcomes developed in 13 of 126 patients (10.3%). Clinically confirmed PTE was registered in 9 patients (7.1%). The remaining 113 patients did not develop late post-traumatic seizures.

Dynamic Profile of Neuroinflammatory Biomarkers:

Patients with late post-traumatic seizures/PTE-spectrum outcomes had a more pronounced inflammatory response that began in the acute period and persisted throughout follow-up. IL-6, TNF-alpha and IL-1beta were significantly higher in the PTE-spectrum group at all time points. IL-6 showed an early peak followed by incomplete normalization. At T1, IL-6 was 52.0 ± 6.10 pg/mL in the PTE-spectrum group versus 28.0 ± 1.41 pg/mL in patients without PTE ($p = 0.002$). At T2, IL-6 remained higher in the PTE-spectrum group: 34.0 ± 4.16 pg/mL versus 16.0 ± 0.85 pg/mL ($p = 0.001$). Differences persisted at T3 and T4. TNF-alpha also remained significantly elevated in patients with adverse outcomes. At T2, TNF-alpha was 10.8 ± 1.00 pg/mL in the PTE-spectrum group versus 7.2 ± 0.26 pg/mL in the group without PTE ($p = 0.004$). IL-1beta demonstrated the most stable inflammatory profile, with higher values in the PTE-spectrum group at all observation points.

For a sequential assessment of the inflammatory block at different stages of follow-up, Table 1 presents IL-6, TNF-alpha and IL-1beta levels in the groups without PTE and with PTE-spectrum outcomes. This comparison makes it possible to trace not only the intensity of the acute response, but also the persistence of inflammatory activity during the subacute and early recovery periods.

As shown in Table 1, all three inflammatory markers were significantly higher in patients with PTE-spectrum outcomes at all observation times. The most pronounced differences were noted at T1-T2, confirming a more intense and more prolonged neuroinflammatory response in patients who subsequently developed late seizures.

Threshold Values and Univariable Prognostic Characteristics of Biomarkers:

ROC analysis identified clinically meaningful thresholds not only for inflammatory cytokines but also for structural injury markers. Among the inflammatory markers, IL-1beta ≥ 2.0 pg/mL had the highest OR. In the glial-axonal block, GFAP ≥ 0.35 ng/mL at T1, GFAP ≥ 0.25 ng/mL at T2 and NfL ≥ 35 pg/mL at T3 were prognostically significant. The strongest effect among structural markers was demonstrated by NfL at T3: OR = 4.6; 95% CI 1.5-14.1; $p = 0.008$; AUC = 0.82. These data support the inclusion of NfL in BioIndex as an independent marker of continuing axonal injury.

To assess the clinical significance of the identified differences, ROC analysis followed by univariable logistic modeling was performed. Table 2 summarizes biomarker thresholds, ORs, 95% CIs, p values and AUCs, allowing comparison of the strength of the prognostic contribution of each indicator.

The data in Table 2 show that prognostic significance was observed not only for inflammatory cytokines but also for markers of glial-axonal injury. NfL at T3 was particularly important: the threshold NfL ≥ 35 pg/mL demonstrated the highest AUC among structural markers and statistically justified its inclusion in BioIndex.

GFAP and NfL as Markers of Glial and Axonal Injury:

GFAP and NfL were analyzed as markers of structural brain injury. GFAP reflected astrocyte injury and reactive gliosis, whereas NfL reflected axonal injury. Patients with PTE-spectrum outcomes had higher GFAP and NfL levels throughout follow-up, indicating that the risk of late seizures was associated not only with inflammation but also with persistent glial-axonal injury.

GFAP was highest in the acute period and then gradually decreased, but its values remained higher in the PTE-spectrum group. At T1, GFAP was 0.46 ± 0.061 ng/mL in the PTE-spectrum group versus 0.24 ± 0.013 ng/mL in the group without PTE ($p = 0.001$). At T2, GFAP was 0.30 ± 0.044 ng/mL versus 0.16 ± 0.009 ng/mL ($p = 0.002$).

NfL had particular prognostic importance because it reflected continuing axonal injury in the subacute and later phases. In univariable logistic regression, the threshold NfL ≥ 35 pg/mL at T3 was associated with a 4.6-fold increase in the risk of late post-traumatic seizures/PTE-spectrum outcomes (95% CI 1.5-14.1; $p = 0.008$), with an AUC of 0.82; therefore, this indicator was included in BioIndex. GFAP also demonstrated independent prognostic significance: GFAP ≥ 0.35 ng/mL at T1 (OR = 3.9; 95% CI 1.3-12.0; $p = 0.018$; AUC = 0.79) and GFAP ≥ 0.25 ng/mL at T2 (OR = 3.2; 95% CI 1.1-9.5; $p = 0.038$; AUC = 0.76).

Because structural and neuroimmune markers have independent value for understanding post-traumatic epileptogenesis, their dynamics are presented separately in Table 3. This format makes the changes in GFAP, NfL and HMGB1 visible at all follow-up stages from T1 to T4.

As shown in Table 3, differences between groups persisted for GFAP, NfL and HMGB1 at all observation stages. The T2-T3 period was the most informative, when signs of neuroimmune activation and continuing axonal injury persisted, strengthening the pathogenetic interpretation of BioIndex.

HMGB1 as a Marker of Neuroimmune Activation:

HMGB1 was significantly higher in patients with late post-traumatic seizures/PTE-spectrum outcomes at all observation points. The most clinically useful time point was the subacute period, because it allows early risk stratification before the 12-month outcome becomes evident. HMGB1 ≥ 6.0 ng/mL was associated with increased risk at both T2 and T3.

To more precisely assess the role of HMGB1 as a marker of neuroimmune activation, Table 4 presents its main prognostic characteristics at the most informative time points, T2 and T3.

According to Table 4, HMGB1 ≥ 6.0 ng/mL retained prognostic significance at both T2 and T3, with AUCs of 0.83 and 0.80, respectively. This confirms that HMGB1 reflects not only the acute response to injury but also sustained neuroimmune activation associated with the formation of post-traumatic epileptogenesis.

BioIndex and 12-Month Risk of Late Seizures/PTE:

BioIndex was created to integrate three biologically complementary pathways: inflammation, neuroimmune

Table 1. *Dynamic profile of neuroinflammatory biomarkers (values are presented as mean ± SE).*

Biomarker	Time point	No PTE (n = 113) Mean ± SE	PTE-spectrum (n = 13) Mean ± SE	p	Interpretation
IL-6 (pg/mL)	T1	28.0 ± 1.41	52.0 ± 6.10	0.002	Early inflammatory peak
	T2	16.0 ± 0.85	34.0 ± 4.16	0.001	Persistent subacute inflammation
	T3	6.2 ± 0.29	10.4 ± 1.16	0.004	Incomplete normalization
	T4	4.1 ± 0.21	7.0 ± 0.83	0.006	Residual inflammatory activity
TNF-alpha (pg/mL)	T1	9.0 ± 0.33	13.2 ± 1.14	0.006	More pronounced proinflammatory activation
	T2	7.2 ± 0.26	10.8 ± 1.00	0.004	Sustained proinflammatory cascade
	T3	5.4 ± 0.19	7.8 ± 0.72	0.012	Persistent activity
	T4	4.6 ± 0.17	6.2 ± 0.58	0.032	Residual difference at 3 months
IL-1beta (pg/mL)	T1	2.1 ± 0.09	3.9 ± 0.39	0.001	Inflammasome-associated activation
	T2	1.8 ± 0.08	3.1 ± 0.31	0.001	High subacute activity
	T3	1.3 ± 0.06	2.2 ± 0.25	0.002	Persistent neuroinflammation
	T4	1.1 ± 0.05	1.8 ± 0.22	0.004	Incomplete normalization

Table 2. *Threshold values and univariable prognostic characteristics of biomarkers for 12-month risk prediction.*

Predictor	Threshold value	OR	95% CI	p value	AUC
IL-6	≥ 10 pg/mL	3.7	1.2-11.4	0.024	0.76
IL-1beta	≥ 2.0 pg/mL	4.2	1.3-13.5	0.016	0.78
TNF-alpha	≥ 7.5 pg/mL	2.9	1.0-8.8	0.049	0.70
GFAP (T1)	≥ 0.35 ng/mL	3.9	1.3-12.0	0.018	0.79
GFAP (T2)	≥ 0.25 ng/mL	3.2	1.1-9.5	0.038	0.76
NfL (T3)	≥ 35 pg/mL	4.6	1.5-14.1	0.008	0.82

Table 3. *Dynamic profile of structural injury and neuroimmune activation markers (values are presented as mean ± SE).*

Biomarker	Time point	No PTE (n = 113) Mean ± SE	PTE-spectrum (n = 13) Mean ± SE	p value
GFAP (ng/mL)	T1	0.24 ± 0.013	0.46 ± 0.061	0.001
	T2	0.16 ± 0.009	0.30 ± 0.044	0.002
	T3	0.10 ± 0.006	0.18 ± 0.026	0.006
	T4	0.07 ± 0.004	0.12 ± 0.018	0.041
NfL (pg/mL)	T1	24.8 ± 1.10	38.5 ± 4.20	0.004
	T2	28.6 ± 1.28	45.2 ± 5.10	0.003
	T3	27.4 ± 1.20	49.0 ± 5.60	0.001
	T4	21.2 ± 0.95	35.8 ± 4.40	0.006
HMGB1 (ng/mL)	T1	4.2 ± 0.21	6.8 ± 0.73	0.003
	T2	4.7 ± 0.22	7.6 ± 0.78	0.001
	T3	3.8 ± 0.18	6.4 ± 0.69	0.002
	T4	3.1 ± 0.15	5.2 ± 0.58	0.011

Table 4. *Prognostic characteristics of HMGB1.*

Metric	HMGB1 at T2	HMGB1 at T3
Cut-off	≥ 6.0 ng/mL	≥ 6.0 ng/mL
OR	4.1	3.6
95% CI	1.4-12.1	1.2-10.6
p value	0.011	0.021
AUC	0.83	0.80
Sensitivity/specificity	77%/78%	69%/78%

Table 5. *BioIndex components.*

Component	Time point	Threshold value	Pathogenetic meaning	Score
IL-6	T2	≥ 10 pg/mL	Persistent neuroinflammation	1
HMGB1	T2	≥ 6.0 ng/mL	Neuroimmune activation/DAMP signaling	1
NfL	T3	≥ 35 pg/mL	Continuing axonal injury	1
BioIndex	Calculated	≥ 2 points	Activation of at least two pathways	Clinically significant

Table 6. *Incremental predictive value of BioIndex.*

Model comparison	AUC before BioIndex	AUC after BioIndex	Delta AUC	p value	Interpretation
Clinical + MRI -> + BioIndex	0.82	0.90	+0.08	0.03	Biomarkers improve structural-clinical prediction
Clinical + EEG -> + BioIndex	0.88	0.93	+0.05	0.04	BioIndex adds value even to a strong EEG model
EEG -> + BioIndex	0.83	0.91	+0.08	0.02	Strong gain from adding biological activity

activation and axonal injury. The index included IL-6, HMGB1 and NfL. BioIndex ≥ 2 indicated activity of at least two pathways.

The inclusion of NfL specifically at T3 was based on its marked between-group difference in the early recovery period (27.4 ± 1.20 pg/mL versus 49.0 ± 5.60 pg/mL; p = 0.001) and the independent prognostic significance of the threshold NfL ≥ 35 pg/mL (OR = 4.6; 95% CI 1.5-14.1; p = 0.008; AUC = 0.82), reflecting continuing axonal injury in patients who subsequently developed PTE-spectrum outcomes.

The composition of BioIndex is presented in Table 5, because this score translates individual laboratory indicators into a simple clinical tool for risk stratification. The table shows which markers, time points and threshold values form the final score.

Table 5 shows that BioIndex combines three biologically different but complementary mechanisms - inflammation, neuroimmune activation and axonal injury. BioIndex ≥ 2 significantly correlated with the 12-month outcome (r = 0.38; p < 0.001) and, in the logistic model, was associated with an increased risk of late post-traumatic seizures/PTE-spectrum outcomes (univariable OR 6.2; p < 0.001; adjusted OR 3.9; 95% CI 1.1-13.6; p = 0.031).

Incremental Predictive Value of BioIndex:

Adding BioIndex improved the prognostic performance of different clinical-instrumental models. When BioIndex was added to the clinical + MRI model, AUC increased from 0.82 to 0.90. When added to the clinical + EEG model, AUC increased from 0.88 to 0.93. When added to the EEG-only model, AUC increased from 0.83 to 0.91.

To test the incremental value of the biomarker block, Table 6 compares models before and after the addition of BioIndex. This approach shows how much laboratory information strengthens clinical, MRI and EEG data in predicting 12-month risk.

The results in Table 6 show that BioIndex does not duplicate clinical, MRI or EEG data, but adds an independent biological layer of prediction. The increase in AUC across all models confirms that accounting for the activity of the post-traumatic biological cascade improves the accuracy of 12-month risk stratification.

Discussion.

This study demonstrates that patients who develop late post-traumatic seizures/PTE-spectrum outcomes after moderate TBI have a distinct biomarker profile characterized by persistent neuroinflammation, neuroimmune activation and glial-axonal injury. This biological pattern is consistent with current concepts regarding the role of inflammation, HMGB1-mediated immune signaling, GFAP and NfL in post-traumatic brain injury and epileptogenesis [5-10]. The most important feature was not a single isolated increase in the acute period, but the persistence of abnormal biomarker levels during the subacute phase and early recovery period.

The age range of the sample is of particular importance. Restricting the age to 23-40 years was a deliberate methodological decision aimed at reducing the influence of age-related changes in biomarker background, especially NfL, which may increase with age and thereby distort the interpretation of post-traumatic axonal injury [9,10]. Therefore, the narrow age range strengthens the internal validity of the study and makes group comparisons cleaner in terms of biomarker dynamics. At the same time, this strategy limits the transferability of the results to adolescents, young adults younger than 23 years, patients older than 40 years and elderly individuals; these categories require separate age-specific thresholds and external validation.

The inflammatory block was closely associated with adverse seizure outcomes. IL-6 showed an early peak and remained elevated during follow-up, indicating prolonged inflammatory activation [5-7]. TNF-alpha reflected sustained proinflammatory signaling, whereas IL-1beta showed a particularly stable epileptogenic profile [5-7]. The high OR for IL-1beta ≥ 2.0 pg/mL supports the role of inflammasome-associated mechanisms in post-traumatic epileptogenesis, as previously described in studies on inflammation and epilepsy [5-7].

The glial-axonal injury block provided additional information. GFAP reflected astrocyte injury and reactive gliosis, which may contribute to pathological remodeling of neuronal networks [10]. In the present study, GFAP thresholds at T1 and T2 had independent prognostic significance (OR = 3.9 and OR = 3.2, respectively), emphasizing the role of the early astroglial response. NfL reflected axonal injury and was especially useful as a delayed marker: NfL ≥ 35 pg/mL at T3 showed OR = 4.6, p = 0.008 and AUC = 0.82. Therefore, the inclusion of NfL in BioIndex was biologically and statistically justified [9,10].

HMGB1 was another important marker because it links tissue damage with immune activation [8]. Its persistent increase in patients with PTE-spectrum outcomes suggests that damage-associated molecular signaling may persist beyond the acute phase of TBI [8]. The prognostic performance of HMGB1 at T2 and T3 indicates that this marker may be clinically useful for identifying patients who require closer neurological and EEG monitoring [8].

The main practical result of this study was the development of BioIndex. In routine practice, clinicians need simple and interpretable tools, and transparency of structure, clinical applicability and the possibility of external validation are particularly important for prognostic models [11,12]. BioIndex transforms a complex biomarker panel into a 0–3-point scale. A value ≥ 2 identifies patients with simultaneous activation

of at least two pathobiological pathways. This is important because post-traumatic epileptogenesis is unlikely to be driven by only one mechanism. Instead, it appears to arise from the convergence of inflammation, immune activation and structural neural injury [5-10].

The incremental value analysis showed that BioIndex improves prediction when added to clinical, MRI and EEG models. This supports the concept that laboratory markers provide a separate layer of information that should be evaluated within a transparent prognostic framework [11]. MRI shows structural damage, EEG shows functional excitability, and biomarkers show whether an active pathological biological process persists [8-10]. This explains the increase in AUC after adding BioIndex to different models.

Clinical Implications.

The proposed biomarker approach can be used for early risk stratification after moderate TBI, taking into account known PTE risk factors and principles of clinical interpretation of prognostic models [3,4,11,12]. Patients with elevated IL-6, HMGB1 and NfL, especially those with BioIndex ≥ 2, should be considered as a group requiring closer follow-up, earlier repeat EEG, stronger counseling regarding seizure triggers and more careful monitoring for paroxysmal events. BioIndex should not be interpreted as an isolated diagnostic test for epilepsy. Rather, it should be used as a biological risk signal that complements clinical assessment, MRI and EEG [11,12].

Strengths and Limitations.

The strengths of this study include prospective follow-up, standardized biomarker assessment time points, analysis of several biologically distinct marker groups and creation of a simple composite index. The study also links biomarker dynamics with clinically relevant 12-month outcomes and presents the prognostic block in line with TRIPOD requirements [11].

The main limitation is the relatively small number of patients with late seizures/PTE-spectrum outcomes, which limits the number of variables that can be included in multivariable models. Biomarker thresholds require external validation in larger cohorts and across different laboratory platforms, in accordance with TRIPOD recommendations and PROBAST principles for assessing applicability of prognostic models [11,12]. In addition, the study included adults with moderate TBI aged 23-40 years; therefore, the results should be validated separately in children, elderly patients, and in mild and severe TBI [12].

Thus, the age range of 23-40 years should be regarded simultaneously as a strength and as a limitation of the study: it reduces age-related confounding in the assessment of NfL and other biomarkers, but does not allow automatic extrapolation of the proposed BioIndex thresholds to younger, elderly or pediatric populations without additional validation.

Conclusion.

Late post-traumatic seizures and PTE after moderate TBI are associated with a persistent biomarker signature involving neuroinflammation, neuroimmune activation and glial-axonal injury. IL-6, IL-1beta, TNF-alpha, GFAP, NfL and HMGB1 were higher in patients who developed late seizure outcomes. A practical BioIndex based on IL-6, HMGB1 and NfL was significantly associated with 12-month risk and improved prediction when added to clinical-instrumental models, consistent with the logic of clinically interpretable prognostic models. This biomarker-based approach may help identify patients who require intensified monitoring and individualized follow-up after moderate TBI.

Institutional Review Board Statement: The study was approved by the local ethics committee. All procedures were conducted in accordance with the principles of the Declaration of Helsinki (Protocol No. 10 dated January 15, 2022). All participants provided written informed consent.

Conflicts of Interest: The authors declare no conflicts of interest.

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