
IL6 in Combination with Either NfL, NTproBNP, or GFAP Can Safely Rule Out Intracranial Injuries in Children with Mild Traumatic Brain Injury

[Anne-Cécile Chiollaz](#)*, Virginie Pouillard, [Michelle Seiler](#), Céline Habre, [Fabrizio Romano](#), Céline Ritter Schenk, [Fabian Spigariol](#), Christian Korff, Fabienne Maréchal, Verena Wyss, Lyssia Gruaz, Joan Montaner, Sergio Manzano, [Jean-Charles Sanchez](#)

Posted Date: 5 February 2025

doi: 10.20944/preprints202502.0270.v1

Keywords: biomarker; combination; mTBI; pediatric; rule out; children; emergency



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

IL6 in Combination with Either NfL, NTproBNP, or GFAP Can Safely Rule Out Intracranial Injuries in Children with Mild Traumatic Brain Injury

Anne-Cécile Chiollaz ^{1,*}, Virginie Pouillard ², Michelle Seiler ³, Céline Habre ⁴, Fabrizio Romano ⁵, Céline Ritter Schenk ⁶, Fabian Spigariol ⁷, Christian Korff ², Fabienne Maréchal ⁸, Verena Wyss ⁵, Lyssia Gruaz ¹, Joan Montaner ⁹, Sergio Manzano ^{10,†} and Jean-Charles Sanchez ^{1,†}, On behalf of t-BIOMAP Investigators and Participants

¹ Department of Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland

² Pediatric Neurology Unit, Woman, Child and Adolescent Department, Geneva University Hospitals, Geneva, Switzerland

³ Pediatric Emergency Department, University Children's Hospital Zurich, Zurich, Switzerland

⁴ Division of Radiology, University Hospitals of Geneva, Geneva, Switzerland

⁵ Division of Pediatric Emergency Medicine, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

⁶ Department of Pediatrics, Fribourg Hospital HFR, Fribourg, Switzerland

⁷ Pediatric Emergency Department, Neuchâtel Hospital (RHNE), Neuchâtel, Switzerland

⁸ Platform of Pediatric Clinical Research, Woman, Child and Adolescent Department, Geneva University Hospitals, Geneva, Switzerland

⁹ Neurovascular Research Group, Institute of Biomedicine of Seville, IBiS/Virgen Macarena University Hospital/CSIC/University of Seville, Seville, Spain,

¹⁰ Pediatric Emergency Department, Geneva University Hospitals and Faculty of Medicine, University of Geneva, Geneva, Switzerland

* Correspondence: anne-cecile.chiollaz@unige.ch. ORCID: 0009-0007-3928-8805

† Jean-Charles Sanchez and Sergio Manzano are co-last authors.

Abstract: Background: Mild traumatic brain injury (mTBI) in children is a public health concern resulting in one of the main causes of pediatric emergency department (PED) visits. However, the acute care of mTBI patients remains challenging due to the limited use of specific and safe diagnostic tools. **Objectives:** To evaluate the performances of combined blood biomarkers to safely rule out intracranial injuries in children with mTBI in the PED. **Methods:** This was a prospective multicenter cohort study of children aged 0-16 years who presented to the PED within 24 hours of sustaining mTBI. Blood was drawn at admission and levels of IL6, NfL, NTproBNP, GFAP, IL10, S100b, and HFABP were analyzed. Patients were dichotomized in two groups: 1) with intracranial injuries (ICI) on computed tomography scan (CT) (= CT+) and 2) without ICI on CT or kept in observation without CT (= CT- & Obs.). Biomarker age correlation was assessed in a healthy group of children aged 0-16 years. **Results:** 419 children with mTBI and 99 healthy children were enrolled. All the single and duplex combinations of blood-biomarkers were tested for their capacity to safely rule out intracranial injuries. IL6 was present in the three best combinations reaching 100% sensitivity (SE) and with the highest associated specificity (SP). IL6 + NfL yielded 61% SP, followed by IL6 + NTproBNP with 60% SP, and IL6 + GFAP with 57% SP. Neither IL6 nor NTproBNP were found to be age correlated. **Conclusions:** IL6 in combination with either NfL, NTproBNP, or GFAP could safely rule out 61% of children without ICI (corresponding to 33/79 unnecessary CT scans and 212/322 observation stays at PED). Blood panels incorporating IL6 show promise as decision-making tools for the acute management of children with mTBI. However, further external studies are required to validate these findings.

Keywords: biomarker; combination; mTBI; pediatric; rule out; children; emergency

1. Introduction

Mild traumatic brain injury (mTBI) is a major public health concern, particularly in children, representing 25% of all TBI cases in Europe[1], and one of the leading causes of pediatric emergency departments (PED) visits[2]. Children are particularly vulnerable to mTBI, and their developing brains may exhibit different injury mechanisms and recovery kinetics compared to adults. The management challenges in pediatric mTBI are distinct[3], as clinicians strive to avoid unnecessary radiation while ensuring accurate diagnosis.

Diagnosis of mTBI relies primarily on self-reported symptoms and signs collected during clinical assessments[4]. To detect potential intracranial injuries (ICI), imaging techniques such as Computer Tomography (CT) scans are employed[5,6]; however, their use is often limited in children due to concerns about radiation exposure[7]. Consequently, clinicians frequently opt for monitoring the child's symptoms over time rather than immediate imaging[3,8]. While this observational approach aims to minimize unnecessary risks, it often prolongs the child's stay in the PED, creating additional stress for both the patient and their family, and contributing to overcrowding. At present, clinicians lack reliable bedside tools to confidently discharge children with mTBI during the acute phase of management[9].

mTBI can affect multiple brain structures, enhancing neuroinflammatory cascade, and damaging the blood-brain barrier (BBB)[10]. All these injuries can lead to the leakage of proteins into the bloodstream[11,12]. Blood-based biomarkers have gained attention as a potential fast and cost-effective method for determining whether a patient with mTBI should undergo a head CT scan. Given the complexity and variability of mTBI, relying on a single biomarker for diagnosis may lack sufficient sensitivity and specificity. As a result, combining biomarkers into panels appeared to be more effective for better detecting diverse types of brain injuries[13,14]. While combinations of biomarkers have been investigated in adult mTBI[15,16], their performance in the pediatric population remains to be explored.

Glial Fibrillary Acidic Protein (GFAP) is a well-established protein released by astrocytes and has been extensively studied in the context of mTBI. Notably, its combination with Ubiquitin C-terminal hydrolase L1 (UCHL-1) has been explored to differentiate between patients with ICI on CT (CT+) and patients without (CT-) following mTBI. The combination of GFAP and UCHL1 showed a sensitivity of 97 %, a negative predictive value (NPV) of 99%, and a specificity of 36% in mTBI patients[16,17]. Another combination including GFAP has been tested in adult mTBI patients alongside Heart Fatty Acid Binding Protein (HFABP). HFABP, a capillary-binding protein indicative of vascular brain injury, has been found elevated in adult mTBI patients with CT+ results[18]. The GFAP and HFABP combination improved specificity to 46% while maintaining 100% sensitivity[15].

The S100 calcium-binding protein B (S100b) is another protein associated with astrocyte damage and has been extensively studied in the context of mTBI[19,20]. It has been included in both the Scandinavian[21] and French[22] guidelines for adults, based on its demonstrated ability to identify one third of patients for whom unnecessary CT scans could have been avoided[23,24]. In children, S100b is to date the most described blood biomarker in mTBI patients, also showing a reduction of one third of unnecessary CT scans[20,25,26]. However, S100b is age-related, with significantly higher physiological expression observed in the healthy pediatric population during the first three years of life[27].

Additional blood-based biomarkers that may be released from different brain cell components or cell types have also been investigated in mTBI[28]. They have been shown to help differentiate between CT+ and CT- patients[29,30]. In pediatric TBI patients, a recent study demonstrated altered cytokine profiles following mTBI, highlighting the effect of neuro-inflammation triggered by the trauma[31].

Based on these results, this study aimed to explore biomarker combinations to enhance diagnostic accuracy in safely ruling out children without ICI after mTBI.

2. Materials and Methods

2.1. Study Population

Children aged 0 to 16 years with a mTBI occurring within 24 hours prior to their presentation at one of the participating PED were included. Five hospitals in Switzerland were part of the study (Geneva University Hospitals, Fribourg Hospital HFR, Neuchâtel Hospital (RHNE), University Children's Hospital Zurich and Inselspital, Bern University Hospital). Institutional review board approval was received; the study was conducted in accordance with Good Clinical Practice guidelines and provisions of the Declaration of Helsinki; and was registered at www.clinicaltrials.gov: NCT06233851.

Informed consent was obtained from the parents or legal guardians of the children, as well as from the children themselves if they were 14 years of age or older. mTBI was defined by one of the following conditions: Head trauma with (1) a GCS score of 14; or (2) a GCS score of 15 with at least one of the following symptoms: loss of consciousness (LOC), post-traumatic amnesia (PTA), persistent headaches, irritability, three or more episodes of vomiting, confusion, dizziness or vertigo, post-traumatic seizure, or transient neurological abnormalities; or (3) evidence of a basilar skull fracture; or (4) severe mechanism of injury, such as a traffic accident or a fall from a height greater than 0.9 meters in children under 2 years old, or over 1.5 meters in children 2 years or older.

Exclusion criteria included: participation in another clinical study involving pharmacological treatment, recent alcohol consumption or psychoactive substance use, a traumatic brain injury (TBI) within the past month, seizures within the last month, a diagnosis of Down syndrome, acute encephalopathy, encephalitis, meningitis, or refusal to provide consent.

A group of healthy children was also recruited. Inclusion criteria were any child aged 16 or younger with a scheduled blood sample in the ambulatory care unit and without TBI. Exclusion criteria were the same as those defined for the TBI group.

2.2. Intervention and Data Collection

After informed consent was obtained, a blood sample was drawn as soon as possible, but no later than 24 hours after the trauma. The study did not interfere with any medical decision-making. Study data were collected and managed using REDCap electronic data capture tools hosted at Hôpitaux Universitaires de Genève (HUG)[32,33].

2.3. Blood-Based Biomarker Analysis

Serum samples were obtained by centrifugation and stored at -80°C . IL6, NfL, NTproBNP, GFAP, IL10, S100b, and HFABP concentrations were measured using enzyme-linked immunoassay (ELISA): R-plex Human NfL (F217X), NTproBNP (F214I), GFAP (F211M), S100b (F212E), and FABP3/HFABP (F214T) antibody sets; and V-plex Proinflammatory Panel 1 (Human) with IL6 and IL10 antibodies sets (Meso Scale Diagnostics, Rockville, MD, USA). Lower limit of detection (LLoD) was respectively 5.5 pg/mL with a calibration range of 12.21-50,000 pg/mL for NfL; 0.30 pg/mL and 0.12-500 pg/mL for NTproBNP; 63 pg/mL and 122-500,000 pg/mL for GFAP; 1.6 pg/mL and 1.22-5000 pg/mL for S100b; 90 pg/mL and 24.41-100,000 pg/mL for HFABP; 0.06 pg/mL and 0.06-488 pg/mL for IL6; and finally 0.04pg/mL and 0.04-233 pg/mL for IL10. The lower limit of quantification (LLoQ) was determined as the minimum concentration with a coefficient of variation (CV) under 20% and a recovery rate between 80% and 120%. Duplicate control serum samples were analyzed on each plate, ensuring that intra- and inter-plate coefficients of variation (CVs) remain below 20%. All kits were utilized following the manufacturers' guidelines.

2.4. Outcome Measures

The main outcome measure was the identification of ICI. To assess ICI, the study followed the PECARN criteria, which include intracranial hemorrhage or contusion, cerebral edema, traumatic infarction, diffuse axonal or shearing injuries, sigmoid sinus thrombosis, midline shift, signs of brain herniation, skull diastasis, pneumocephalus, or a skull fracture depressed by at least the thickness of the skull table. A single pediatric radiologist (CH), who was blinded to both clinical details and biomarker results, reviewed all CT scans. Cases with any of these criteria were classified as CT+, while those without were considered CT-. For non-scanned mTBI patients, they were considered negative for ICI in the absence of secondary presentation to PED.

2.5. Statistical Analysis

Statistical analysis was performed using R (<http://www.rproject.org>, version 4.4.1) in RStudio (<http://www.rstudio.com>, version 2024.09.0). Biomarker concentrations were normalized using their medians as correction factors. Patients were dichotomized into two groups: 1) patients with ICI on CT (= CT+); and 2) patients without ICI on CT or without CT scan but with in-hospital-observation (= CT- & Obs.). Differences between groups were established using the nonparametric Mann-Whitney U test, given that the Kolmogorov-Smirnov test revealed that all protein levels were non-normally distributed ($p < 0.05$). Chi-squared test was used for statistical analyses of the clinical data. Statistical significance was inferred at $p < 0.05$. The levels of biomarkers are presented using box- and dot-plots with a log₁₀ Y-scale. Biomarker's ability for classifying patients according to their group was evaluated using receiver operating characteristic (ROC) curves with the pROC package in R. Individuals' blood-biomarkers performances were already explored during intermediate analysis[26,34,35]. Biomarker combinations were analyzed with PanelomiX[36,37], which uses iterative permutation-response calculations. The cut-off values for each biomarker were adjusted iteratively by 2% increment quantiles. In each iteration, the optimal performance was identified by maximizing specificity (SP) while maintaining 100% sensitivity (SE). This strategy aimed to avoid any false negatives among mTBI patients (100% Negative Predictive Value (NPV)) and safely discharge the maximum of patients without ICI. Models were limited to a maximum of two biomarkers. ROC analysis was applied to assess model performance. Only patients with complete data for all tested biomarkers were included in the analysis. The correlation between biomarker concentrations and patient age were evaluated in the healthy population, by Spearman correlation coefficient and its p-value.

3. Results

3.1. Clinical parameters

A total of 419 mTBI children were included between October 2020 and February 2023. Among these, 18 (4%) patients had an ICI on CT scan (CT+), 79 patients (19%) had a CT scan without ICI (CT-), and the remaining 322 patients (77%) were kept for observation without imaging (in-hospital-observation). None of the last group developed a subsequent ICI. Both the CT- and in-hospital-observation without imaging patients were grouped together (CT- & Obs.), and their clinical parameters were compared to the CT+ group (Table 1).

The age of included children ranged from 1 month to 16 years in the CT- & Obs. group, and from 9 months to 15 years in the CT+ group. The mean age across both groups was 8 years. Most patients had a GCS of 15 with associated symptoms (86% and 66% for the CT- & Obs. and CT+ group, respectively). Signs of basilar skull fracture and severe mechanism of injury were reported in both groups, but significantly more in CT+ patients (33.3% and 77.8% respectively). There were no significant differences in the reported symptoms at admission between the compared groups. However, the two most prevalent associated symptoms were PTA (28.9%) and persistent headaches (26.2%) in the CT- & Obs. group; and persistent headaches (33.3%) and more than three episodes of

vomiting (22.2%) in the CT+ group (Table 1). Extracranial injuries (ECI) were significantly more reported in the CT+ group and mainly corresponded to fractures of the facial bones. The presence of simple skull fractures seen on CT was also significantly higher in the CT+ patient group ($p < 0.001$) (Table 1). No other significant differences in clinical parameters were observed within the CT- & Obs. group and the CT+ group.

Table 1. Clinical parameters of mTBI patients with or without CT scan.

N=419 mTBI	CT- (N=79) or Obs. (N=322) (N=401) 96% of total cohort	CT+ (N=18) 19% of CT- scanned 4% of total cohort	P- value
INCLUSION CRITERIA:			
Severity of injury, n (%)			
GCS14	54 (13.5%)	6 (33.3%)	0.044
GCS15*	347 (86.5%)	12 (66.7%)	
Sign of basilar skull fracture, n (%)	10 (2.5%)	6 (33.3%)	<0.001
Severe mechanism of injury\diamond, n (%)	175 (43.6%)	14 (77.8%)	0.009
*Symptoms at admission (within GCS 15), n (%)			
Loss of consciousness	85 (21.2%)	2 (11.1%)	0.772
Post-traumatic amnesia	116 (28.9%)	1 (5.6%)	0.127
Persistent headaches	105 (26.2%)	6 (33.3%)	0.268
More than three episodes of vomiting	64 (16.0%)	4 (22.2%)	0.367
Vertigo	37 (9.2%)	0 (0%)	0.471
Confusion	46 (11.5%)	1 (5.6%)	0.936
Convulsion	8 (2.0%)	0 (0%)	1
OTHER CLINICAL VARIABLES:			
Age (yo)			
Mean (SD)	8.8 (4.40)	7.8 (4.27)	0.338
Median [Min, Max]	9.1 [0.1, 16.0]	7.1 [0.9, 15.0]	
Sex, n (%)			
Boys	234 (58.4%)	12 (66.7%)	0.669
Girls	167 (41.6%)	6 (33.3%)	
Extracranial injuries (ECI), n (%)			
mTBI + either body/face fractures	13 CT- (16.5%); 13 Obs. (4%)	4 (22.2%)	0.031

Skull fracture (on CT), n (%)

Simple skull fracture (not PECARN criterion)	14 CT- (17.7%)	15 (83.3%)	<0.001
--	----------------	------------	--------

◇Severe mechanism of injury: such as a traffic accident or a fall from a height greater than 0.9 meters in children under 2 years old, or over 1.5 meters in children 2 years or older. CT-: negative result on CT scan; CT+: positive result on CT scan (defined by PECARN criteria); Obs.: patients kept in observation without CT scan.

3.2. Individuals' Biomarkers Performances

The mean with SD of all biomarkers, as well as the median with minimum and maximum values are reported in Table 2. The median time between head trauma and blood sampling was 8 hours for CT+ patients and 6 hours for the other mTBI patients, with no significant difference ($p = 0.298$).

Blood concentration of IL6, NfL, GFAP, and S100b was significantly increased in CT+ patients compared to CT- and in-hospital-observation patients (Table 2).

The diagnostic performance of each individual biomarker is presented with ROC curves and their AUC in Figure 1a. At 100% sensitivity, IL6 yielded the best specificity of 47.6% [95% IC: 42.9 – 52.4 %], followed by NfL with 29.4% [95% IC: 25.0 – 33.8 %], GFAP with 25.7% [95% IC: 21.5 – 29.9%], and NTproBNP with 24.4% [95% IC: 20.3 – 28.6%]. IL10, S100b, and HFABP were not able to reach more than 20% of specificity at 100% of sensitivity (Figure 1a and Table 3a).

Table 2. Biomarkers expression in mTBI patients with or without CT scan.

N=419 mTBI	CT- & Obs. (N=401)	CT+ (N=18)	P-value (Mann-Whitney U test)
TimelapsTBI-blood (hours)			
Mean (SD)	6.43 (4.98)	8.44 (7.11)	0.298
Median [Min, Max]	5.00 [0, 24.0]	6.50 [2.00, 24.0]	
IL6 (pg/ml)			
Mean (SD)	2.45 (7.66)	5.55 (8.20)	<0.001
Median [Min, Max]	1.05 [0.0385, 99.4]	2.64 [0.932, 34.2]	
NfL (pg/ml)			
Mean (SD)	23.2 (19.2)	49.8 (41.6)	<0.001
Median [Min, Max]	17.7 [0.119, 127]	31.3 [13.5, 141]	
NTproBNP (pg/ml)			
Mean (SD)	98.5 (90.6)	112 (96.0)	0.433
Median [Min, Max]	69.9 [0.471, 709]	67.8 [36.0, 337]	
GFAP (pg/ml)			
Mean (SD)	559 (910)	3140 (3450)	<0.001
Median [Min, Max]	208 [0.746, 8770]	2080 [75.7, 11200]	
IL10 (pg/ml)			

Mean (SD)	1.21 (5.16)	1.34 (2.09)	0.061
Median [Min, Max]	0.423 [0.0634, 94.9]	0.587 [0.211, 9.27]	
S100b (pg/ml)			
Mean (SD)	56.7 (66.5)	125 (137)	0.002
Median [Min, Max]	41.5 [0.108, 672]	74.6 [2.05, 582]	
HFABP (pg/ml)			
Mean (SD)	3390 (3250)	3810 (3210)	0.629
Median [Min, Max]	2480 [20.2, 32200]	2730 [1220, 13200]	

3.3. Combinations of Biomarkers Performances

Each possible duplex combination of biomarkers was tested for their capacity to rule out, setting the sensitivity at 100%. IL6 was present in each of the three best duplex combinations (Figure 1b and Table 3b). In association with either NfL, NTproBNP, or GFAP; IL6 was found to safely identify CT- and in-hospital-observation patients with more than 55% specificity. The duplex IL6 + NfL showed the best performance with a specificity reaching 61% [95% IC: 56-65%]; followed by IL6 + NTproBNP (60% [95% IC: 55-65%]), and IL6 + GFAP (57% [95% IC: 53-62%]). ROC curves of the three best duplex, compared to individual proteins are presented in Figure 1b. The other duplex combinations were below 50% specificity when sensitivity was set at 100%.

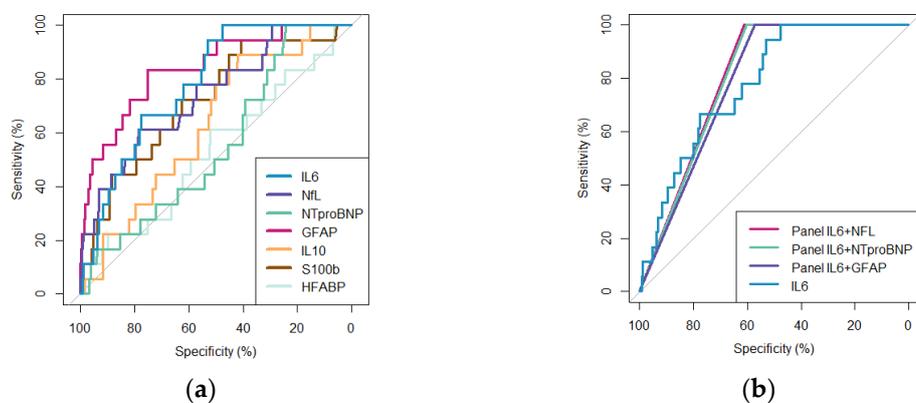


Figure 1. Biomarkers ROC curves in discriminating between mTBI patients with and without ICI. (CT+ versus CT- & Obs.). (a) Individuals' biomarkers; (b) The three best duplex combinations versus the best individual biomarker.

Table 3. Performances in discriminating between mTBI patients with and without ICI. (CT+ versus CT- & Obs.). (a) Individuals' biomarkers; (b) The three best duplex combinations; (c) Confusion matrix with IL6+NfL predictions.

(a)				
Individuals	Sensibility (%) (95%CI)	Specificity (%) (95%CI)	AUC (%) (95%CI)	Threshold (pg/ml)
IL6	100 (100 - 100)	47.6 (42.9 - 52.4)	78.1 (69.6 - 86.6)	> 0.93

NfL	100 (100 - 100)	29.4 (25.1 – 33.8)	73.9 (61.9 – 85.8)	> 13.53
NTproBNP	100 (100 - 100)	24.4 (20.3 – 28.6)	55.5 (43.2 – 67.7)	> 35.97
GFAP	100 (100 - 100)	25.7 (21.5 – 29.9)	83.9 (74.0 – 93.8)	> 75.36

(b)

Combinations (duplex)	Sensibility (%) (95%CI)	Specificity (%) (95%CI)	AUC (%) (95%CI)	Threshold (pg/ml)
IL6 + NfL	100 (100 - 100)	61,1 (56,4 – 65,8)	80.55 (78.2 – 83.0)	> 0.93 > 13.52
IL6 + NTproBNP	100 (100 - 100)	60,1 (55,4 – 64,8)	80.05 (77.7 – 82.5)	> 0.93 > 35.07
IL6 + GFAP	100 (100 - 100)	57,4 (52,6 – 62,1)	78.68 (76.3 – 81.1)	> 0.93 > 75.36

(c)

Confusion matrix	CT- & Obs.	CT+	Total
<i>Predicted</i> CT- & Obs.	245 (33 CT- and 212 Obs.) <i>True Negative : 61%</i>	0	245
<i>Predicted</i> CT+	156 (46 CT- and 110 Obs.)	18 <i>True Positive : 100%</i>	174
Total	401	18	419

A confusion matrix was built based on the prediction rules defined by the IL6 + NfL combinations (Table 3c) and allowed to truly identify all CT+ (100% NPV) and truly discharged 33 CT- (out of 79) and 212 (out of 322) observed without imaging patients.

3.4. Age-Correlation in the Healthy Population

The age-correlation of each biomarker was investigated in a healthy group of 99 children, aged 1 month to 16 years (mean age 8 years). Spearman correlation analysis revealed that GFAP, IL10, and S100b were negatively and significantly correlated with age. NfL and HFABP were also significantly age-correlated but with a low correlation coefficient (Spearman coef. of -0.22); and neither NTproBNP nor IL6 were age-correlated.

Table 4. Age-correlation in the healthy population (n=99).

Biomarkers	Spearman coefficient (r)	p-value
IL6	0.17	0.09

GFAP	-0.73	<0.001
NTproBNP	0.00	0.52
NfL	-0.22	0.028
HFABP	-0.22	0.029
S100b	-0.44	<0.001
IL10	-0.55	<0.001

4. Discussion

This study explored both the individual and combined performances of seven blood biomarkers in distinguishing children with and without ICI presenting in a PED with mTBI. Among the 419 children included, 23% underwent CT scans, of whom 19% were found to have an ICI (representing 4% of the total cohort). These proportions are consistent with reported prevalence in the literature[2,3,38–41] and underscore the relevance of finding reliable biomarkers to reduce unnecessary CT scans and minimize prolonged hospital stays for children without ICI.

The measurement of IL6 within 24 hours of trauma successfully identified 47% of children without ICI, while maintaining 100% sensitivity in detecting those with ICI. These results confirm prior findings from intermediate analyses in this cohort[34]. But it also highlights the limitation of using a single biomarker, due to the complex pathophysiology of mTBI.

Combining biomarkers aimed to increase diagnostic specificity while maintaining high sensitivity to safely discharge patients without ICI. Notably, the combination of IL6 with either NfL, NTproBNP, or GFAP significantly improved specificity, with the IL6 + NfL panel reaching 61% specificity and 100% sensitivity. Retrospectively, in our cohort, these combinations would have allowed the safe discharge of 33 out of 79 children who underwent CT scans without an ICI and 212 out of 322 children who were kept under observation for symptom monitoring in the PED, without missing any patients with an ICI. These findings represent a significant clinical benefit by reducing 42% of unnecessary CT scans and decreasing 67% of observations in PED, with a 100% NPV.

Among previously studied biomarkers, S100b has been shown to identify 34% of CT- patients with 100% sensitivity. Its short half-life in blood has led to recommendations for a maximum delay of 3–6 hours for sampling after head trauma[20,22,25,42]. In our study, S100b exhibited lower specificity (below 20%) when assessed across the full cohort. Even when selecting only patients sampled within 6 hours post-trauma, IL6 outperformed S100b, reinforcing IL6's potential as a more robust marker in both early and late trauma windows[34]. This suggests that IL6 could be particularly valuable in real-world settings, where many patients present to the hospital more than 6 hours after injury. In our cohort, patients arriving at the PED later than 6 hours after trauma represented one quarter and should not be ignored.

Importantly, IL6 also benefits from being independent of age, making it a versatile decision-making tool across pediatric populations. While both S100b and GFAP are known to be age-dependent, requiring adjustments for use in young children, IL6, along with NTproBNP, can be applied without age stratification, further enhancing its clinical utility.

Given that IL6 is a cytokine released during neuroinflammatory responses, its elevated level post-trauma is unsurprising, although cytokines are not currently used clinically in this context. Furthermore, its combination with brain-specific proteins such as NfL, GFAP, or NTproBNP enhances its specificity, suggesting that pairing systemic inflammatory markers with proteins reflecting brain injury could yield a powerful diagnostic tool[28]. In cases of multiple traumas, where IL6 might be elevated due to injuries outside the brain, combining it with brain-specific markers could help isolate brain trauma.

The three proteins IL6, NfL, and NTproBNP are already widely available in routine clinical analysis for other conditions, making them readily applicable in clinical practice once their utility in mTBI is validated. GFAP is also available on established diagnostic platforms, which could expedite the integration of these biomarker panels into clinical guidelines for mTBI management.

Strengths and Limitations

This study's key strengths include its large cohort size, making it one of the largest studies of pediatric mTBI biomarkers to date; and its comprehensive biomarker analysis within 24 hours of injury. The inclusion of both scanned and non-scanned patients enhances the generalizability of the findings, particularly in a pediatric population where radiation exposure is a critical concern and imaging rate is lower than in adult mTBI care.

Our study highlights the need to address not only CT scanned patients but also the high majority (77% in this cohort) of mTBI patients kept under observation without imaging. Reducing observation times for these patients could significantly alleviate stress for families and reduce hospital costs.

However, this study also has limitations. The cohort contained relatively few CT+ patients, reflecting the fact that most children with mTBI do not have ICI. While this is consistent with clinical practice, it limits the power of our analysis to fully explore the diagnostic performance for more severe injuries. Indeed, future mTBI biomarker studies should focus on detecting clinically important traumatic brain injuries rather than just ICI. To clearly incorporate blood-biomarker measurements into the widely used PECARN[3] decision-making algorithm, further studies should be based on the same inclusion criteria.

Additionally, we only evaluated combinations of two biomarkers due to statistical limitations related to sample size. This prevented us from exploring more complex multi-biomarkers or clinical panels that could potentially yield even higher specificity. However, given that IL6 and its combinations showed robust performance, expanding this work to include larger cohorts and more biomarkers should be a priority.

Lastly, while we used a research-based platform for measuring biomarker levels, routine clinical validation is needed to establish practical cutoff values for each biomarker. This limitation can be addressed in future studies, as the biomarkers assessed are already available on validated clinical platforms.

Ultimately, mTBI is not always mild. It presents in various ways, with diverse recovery trajectories. Researchers are working to refine its classification by identifying endophenotypes[43,44]. Additionally, combined blood biomarkers may contribute to a biological signature of the trauma.

5. Conclusions

This study confirms, in a larger pediatric cohort, the diagnostic potential of seven blood-based proteins to safely identify children without ICI after a mTBI. Among these, the combination of IL6 with either NfL, NTproBNP, or GFAP demonstrated strong diagnostic value, suggesting its potential integration as a clinical decision-making tool for the management of mTBI in children. Specifically, the combination of IL6 and NfL was able to safely rule out ICI in 61% of cases with 100% NPV, significantly reducing the need for unnecessary CT scans and shortening the length of stay in PEDs. These findings highlight the promising role of biomarker panels in optimizing pediatric mTBI management, though further validation in external cohorts is needed to confirm their broader applicability.

Author Contributions: Conceptualization, ACC, VP, JCS, SM, CK; data curation, ACC, VP; formal analysis, ACC; funding acquisition, JCS and SM; investigation, ACC, LG; methodology, ACC, VP, JCS, SM, CK; project administration: ACC, VP, JCS, SM, CK; resources, SM, FM, FS, FR, VW, MS, CRS, CH; JCS, VP; software, ACC; supervision, JCS and SM; validation, ACC, JCS, SM; writing – original draft preparation, ACC; writing – review and editing, all authors;

Funding: This research was funded by a private grant from Geneva University Hospitals (HUG) for its first year of recruitment.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Geneva (CCER-ID: 2020-01533 on the 12th of August 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We especially thank patients and their families for their participation in this study. We thank all the members of the t-BIOMAP study, clinicians, research nurses, radiologists, neuropsychologist, hospital laboratories and case managers. We thank the Platform of Pediatric Clinical Research of the HUG for their invaluable help.

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

Abbreviations

The following abbreviations are used in this manuscript:

AUC	Area Under the Curve
BBB	Blood-Brain Barrier
CT	Computed Tomography
CV	Coefficient of Variation
ECI	ExtraCranial Injuries
ELISA	Enzyme-Linked Immunoassay
GCS	Glasgow Coma Scale
GFAP	Glial Fibrillary Acidic Protein
HFABP	Heart Fatty-Acid-Binding Protein
HFR	Fribourg Hospital
HUG	Hopitaux Universitaires de Genève
IC	Confidence Interval
ICI	Intracranial injury
IL10	Interleukin 10
IL8	Interleukin 8
IL6	Interleukin 6
IQR	Inter Quartile Range
LLD	Lower Limit of Detection
LLOQ	Lower Limit of Quantification
LOC	Loss of Consciousness
Max	Maximum
Min	Minimum
mTBI	mild Traumatic Brain Injury
NfL	Neurofilament Light chain
NPV	Negative Predictive Value
NTproBNP	N-terminal prohormone of brain natriuretic peptide
PECARN	Pediatric Emergency Care Applied Research Network
PED	Pediatric Emergency Department
PTA	Post Traumatic Amnesia
RHNE	Neuchâtel Hospital
ROC	Receiver Operating Characteristic
S100b	S100 calcium-binding protein B
SE	Sensitivity
SD	Standard Deviation
SP	Specificity
TBI	Traumatic Brain Injury
UCHL-1	Ubiquitin Carboxy-terminal Hydrolase L1

References

1. Lumba-Brown A, Yeates KO, Sarmiento K, et al. Centers for Disease Control and Prevention Guideline on the Diagnosis and Management of Mild Traumatic Brain Injury Among Children. *JAMA Pediatr.* Nov 1 2018;172(11):e182853. doi:10.1001/jamapediatrics.2018.2853
2. Dewan MC, Mummareddy N, Wellons JC, 3rd, Bonfield CM. Epidemiology of Global Pediatric Traumatic Brain Injury: Qualitative Review. *World Neurosurg.* Jul 2016;91:497-509 e1. doi:10.1016/j.wneu.2016.03.045

3. Kuppermann N, Holmes JF, Dayan PS, et al. Identification of children at very low risk of clinically-important brain injuries after head trauma: a prospective cohort study. *Lancet*. Oct 3 2009;374(9696):1160-70. doi:10.1016/s0140-6736(09)61558-0
4. Babl FE, Borland ML, Phillips N, et al. Accuracy of PECARN, CATCH, and CHALICE head injury decision rules in children: a prospective cohort study. *Lancet*. Jun 17 2017;389(10087):2393-2402. doi:10.1016/S0140-6736(17)30555-X
5. Borg J, Holm L, Cassidy JD, et al. Diagnostic procedures in mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med*. Feb 2004;(43 Suppl):61-75. doi:10.1080/16501960410023822
6. Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PM. Indications for computed tomography in patients with minor head injury. *N Engl J Med*. Jul 13 2000;343(2):100-5. doi:10.1056/nejm200007133430204
7. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. *N Engl J Med*. Nov 29 2007;357(22):2277-84. doi:10.1056/NEJMra072149
8. Carroll LJ, Cassidy JD, Holm L, Kraus J, Coronado VG, Injury WHOCTFoMTB. Methodological issues and research recommendations for mild traumatic brain injury: the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med*. Feb 2004;(43 Suppl):113-25. doi:10.1080/16501960410023877
9. Levin HS, Diaz-Arrastia RR. Diagnosis, prognosis, and clinical management of mild traumatic brain injury. *Lancet Neurol*. May 2015;14(5):506-17. doi:10.1016/S1474-4422(15)00002-2
10. Jarrahi A, Braun M, Ahluwalia M, et al. Revisiting Traumatic Brain Injury: From Molecular Mechanisms to Therapeutic Interventions. *Biomedicines*. Sep 29 2020;8(10)doi:10.3390/biomedicines8100389
11. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nature Reviews Neurology*. Apr 2013;9(4):201-210. doi:10.1038/nrneuro.2013.9
12. Sapin V, Gaulmin R, Aubin R, Walrand S, Coste A, Abbot M. Blood biomarkers of mild traumatic brain injury: State of art. *Neurochirurgie*. May 2021;67(3):249-254. doi:10.1016/j.neuchi.2021.01.001
13. Bouvier D, Oris C, Brailova M, Durif J, Sapin V. Interest of blood biomarkers to predict lesions in medical imaging in the context of mild traumatic brain injury. *Clinical Biochemistry*. Nov 2020;85:5-11. doi:10.1016/j.clinbiochem.2020.08.001
14. Siman R, Toraskar N, Dang A, et al. A Panel of Neuron-Enriched Proteins as Markers for Traumatic Brain Injury in Humans. *Journal of Neurotrauma*. Nov 2009;26(11):1867-1877. doi:10.1089/neu.2009.0882
15. Lagerstedt L, Egea-Guerrero JJ, Bustamante A, et al. Combining H-FABP and GFAP increases the capacity to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. *PLoS One*. 2018;13(7):e0200394. doi:10.1371/journal.pone.0200394
16. Bazarian JJ, Welch RD, Caudle K, et al. Accuracy of a rapid glial fibrillary acidic protein/ubiquitin carboxyl-terminal hydrolase L1 test for the prediction of intracranial injuries on head computed tomography after mild traumatic brain injury. *Acad Emerg Med*. Nov 2021;28(11):1308-1317. doi:10.1111/acem.14366
17. Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurology*. Sep 2018;17(9):782-789. doi:10.1016/S1474-4422(18)30231-X
18. Lagerstedt L, Egea-Guerrero JJ, Bustamante A, et al. H-FABP: A new biomarker to differentiate between CT-positive and CT-negative patients with mild traumatic brain injury. *PLoS One*. 2017;12(4):e0175572. doi:10.1371/journal.pone.0175572
19. Oris C, Kahouadji S, Durif J, Bouvier D, Sapin V. S100B, Actor and Biomarker of Mild Traumatic Brain Injury. *Int J Mol Sci*. Apr 1 2023;24(7)doi:10.3390/ijms24076602
20. Oris C, Pereira B, Durif J, et al. The Biomarker S100B and Mild Traumatic Brain Injury: A Meta-analysis. *Pediatrics*. Jun 2018;141(6)doi:10.1542/peds.2018-0037
21. Uden J, Ingebrigtsen T, Romner B, Scandinavian Neurotrauma C. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. *BMC Med*. Feb 25 2013;11:50. doi:10.1186/1741-7015-11-50
22. Gil-Jardine C, Payen JF, Bernard R, et al. Management of patients suffering from mild traumatic brain injury 2023. *Anaesth Crit Care Pain Med*. Aug 2023;42(4):101260. doi:10.1016/j.accpm.2023.101260

23. Calcagnile O, Anell A, Undén J. The addition of S100B to guidelines for management of mild head injury is potentially cost saving. *Bmc Neurol.* 2016/10/20 2016;16(1):200. doi:10.1186/s12883-016-0723-z
24. Unden L, Calcagnile O, Unden J, Reinstrup P, Bazarian J. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. *BMC Med.* Dec 9 2015;13:292. doi:10.1186/s12916-015-0533-y
25. Manzano S, Holzinger IB, Kellenberger CJ, et al. Diagnostic performance of S100B protein serum measurement in detecting intracranial injury in children with mild head trauma. *Emerg Med J.* Jan 2016;33(1):42-6. doi:10.1136/emmermed-2014-204513
26. Chiollaz AC, Pouillard V, Spigariol F, et al. Management of Pediatric Mild Traumatic Brain Injury Patients: S100b, Glial Fibrillary Acidic Protein, and Heart Fatty-Acid-Binding Protein Promising Biomarkers. *Neurotrauma Rep.* May 1 2024;5(1):529-539. doi:10.1089/neur.2024.0027
27. Bouvier D, Castellani C, Fournier M, et al. Reference ranges for serum S100B protein during the first three years of life. *Clin Biochem.* Jul 2011;44(10-11):927-9. doi:10.1016/j.clinbiochem.2011.05.004
28. Reyes J, Spitz G, Major BP, et al. Utility of Acute and Subacute Blood Biomarkers to Assist Diagnosis in CT-Negative Isolated Mild Traumatic Brain Injury. *Neurology.* Nov 14 2023;101(20):E1992-E2004. doi:10.1212/Wnl.0000000000207881
29. Posti JP, Takala RSK, Lagerstedt L, et al. Correlation of Blood Biomarkers and Biomarker Panels with Traumatic Findings on Computed Tomography after Traumatic Brain Injury. *Journal of Neurotrauma.* Jul 15 2019;36(14):2178-2189. doi:10.1089/neu.2018.6254
30. Hossain I, Marklund N, Czeiter E, Hutchinson P, Buki A. Blood biomarkers for traumatic brain injury: A narrative review of current evidence. *Brain Spine.* 2024;4doi:ARTN 10273510.1016/j.bas.2023.102735
31. Ryan E, Kelly L, Stacey C, et al. Mild-to-severe traumatic brain injury in children: altered cytokines reflect severity. *J Neuroinflamm.* Feb 7 2022;19(1)doi:ARTN 3610.1186/s12974-022-02390-5
32. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform.* Jul 2019;95:103208. doi:10.1016/j.jbi.2019.103208
33. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* Apr 2009;42(2):377-81. doi:10.1016/j.jbi.2008.08.010
34. Chiollaz AC, Pouillard V, Habre C, et al. Diagnostic potential of IL6 and other blood-based inflammatory biomarkers in mild traumatic brain injury among children. *Front Neurol.* 2024;15:1432217. doi:10.3389/fneur.2024.1432217
35. Chiollaz AC, Pouillard V, Habre C, et al. Evaluating NfL and NTproBNP as predictive biomarkers of intracranial injuries after mild traumatic brain injury in children presenting to emergency departments. *Front Neurol.* 30 January 2025 2025;Volume 16 doi:DOI 10.3389/fneur.2025.1518776
36. Robin X. PanelomiX for the Combination of Biomarkers. *Methods Mol Biol.* 2019;1959:261-273. doi:10.1007/978-1-4939-9164-8_17
37. Robin X, Turck N, Hainard A, et al. PanelomiX: A threshold-based algorithm to create panels of biomarkers. *Translational Proteomics.* 2013/01/01/ 2013;1(1):57-64. doi:<https://doi.org/10.1016/j.trprot.2013.04.003>
38. Crowe L, Babl F, Anderson V, Catroppa C. The epidemiology of paediatric head injuries: Data from a referral centre in Victoria, Australia. *J Paediatr Child H.* Jun 2009;45(6):346-350. doi:10.1111/j.1440-1754.2009.01499.x
39. Sönnervqvist C, Brus O, Olivecrona M. Validation of the scandinavian guidelines for initial management of minor and moderate head trauma in children. *Eur J Trauma Emerg S.* Aug 2021;47(4):1163-1173. doi:10.1007/s00068-019-01288-x
40. Bressan S, Eapen N, Phillips N, et al. PECARN algorithms for minor head trauma: Risk stratification estimates from a prospective PREDICT cohort study. *Academic Emergency Medicine.* Oct 2021;28(10):1124-1133. doi:10.1111/acem.14308
41. Nigrovic LE, Kuppermann N. Children With Minor Blunt Head Trauma Presenting to the Emergency Department. *Pediatrics.* Dec 2019;144(6)doi:10.1542/peds.2019-1495

42. Uden J, Dalziel SR, Borland ML, et al. External validation of the Scandinavian guidelines for management of minimal, mild and moderate head injuries in children. *BMC Med.* Oct 12 2018;16(1):176. doi:10.1186/s12916-018-1166-8
43. Giza CC, Gioia G, Cook LJ, et al. CARE4Kids Study: Endophenotypes of Persistent Post-Concussive Symptoms in Adolescents: Study Rationale and Protocol. *J Neurotrauma.* Jan 2024;41(1-2):171-185. doi:10.1089/neu.2023.0073
44. Haber M, Amyot F, Kenney K, et al. Vascular Abnormalities within Normal Appearing Tissue in Chronic Traumatic Brain Injury. *Journal of Neurotrauma.* Oct 2018;35(19):2250-2258. doi:10.1089/neu.2018.5684

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.