Inflammation biomarkers IL-6 and IL-10 may improve the diagnostic and prognostic accuracy of currently authorized traumatic brain injury tools

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Abstract. Traumatic brain injury (TBI) is currently one of the leading causes of mortality and disability worldwide. At present, no reliable inflammatory or specific molecular neurobiomarker exists in any of the standard models proposed for TBI classification or prognostication. Therefore, the present study was designed to assess the value of a group of inflammatory mediators for evaluating acute TBI, in combination with clinical, laboratory and radiological indices and prognostic clinical scales. In the present single-centre, prospective observational study, 109 adult patients with TBI, 20 adult healthy controls and a pilot group of 17 paediatric patients with TBI from a Neurosurgical Department and two intensive care units of University General Hospital of Heraklion, Greece were recruited. Blood measurements using the ELISA method, of cytokines IL-6, IL-8 and IL-10, ubiquitin C-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein, were performed. Compared with those in healthy control individuals, elevated IL-6 and IL-10 but reduced levels of IL-8 were found on day 1 in adult patients with TBI. In terms of TBI severity classifications, higher levels of IL-6 (P=0.001) and IL-10 (P=0.009) on day 1 in the adult group were found to be associated with more severe TBI according to widely used clinical and functional scales. Moreover, elevated IL-6 and IL-10 in adults were found to be associated with more serious brain imaging findings (r_s<0.442; P<0.007). Subsequent multivariate logistic regression analysis in adults revealed that early-measured (day 1) IL-6 [odds ratio (OR)=0.987; P=0.025] and UCH-L1 (OR=0.993; P=0.032) are significant independent predictors of an unfavourable outcome. In conclusion, results from the present study suggest that inflammatory molecular biomarkers may prove to be valuable diagnostic and prognostic tools for TBI.

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Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; CRASH, corticosteroid randomisation after significant head injury; CT, computed tomography; ECOG/WHO score, Eastern Cooperative Oncology Group/WHO score; GCS, Glasgow Coma Scale; GFAP, glial fibrillary acidic protein; GOS, Glasgow Outcome Scale; GOS-E, Glasgow Outcome Scale-Extended; ICP, intracranial pressure; ICU, intensive care unit; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; ISS, injury severity score; KPS, Karnofsky Performance Scale; mGCS, motor component of Glasgow Coma Scale; MRS, Modified Rankin Scale; ROC, receiver operating characteristic; TBI, traumatic brain injury; TNF, tumour necrosis factor; UCH-L1, ubiquitin C-terminal hydrolase L1

Key words: interleukin 6, interleukin 8, interleukin 10, traumatic brain injury, biomarkers, neuroinflammation

Introduction

Traumatic brain injury (TBI) is becoming a major public health concern, due to a steadily rising annual incidence of ~50 million individuals (1-4). TBI involves a heterogeneous set of functional, anatomical and histological alterations that are induced by external physical forces exerting excessive forces to the brain (5-9). This in turn ultimately leads to neuronal or glial cell apoptotic and necrotic damage, blood vessel rupture, thrombosis, disruption of the blood-brain barrier, skull fractures and/or meningeal tears (3,5-9). This type of sudden primary injury in TBI can present as a number of pathophysiological characteristics, including macroscopic focal or diffuse lesions, hematomas, haemorrhages, cerebral contusions and/or diffuse axonal injuries, which may not be treatable (3,9,10). In addition, delayed neuronal damage may be inflicted by secondary insults involving several molecular, biochemical and neuroinflammatory disturbances, which can last from several minutes to even months after the first mechanical insult (9,11). The profile of primary or secondary pathology is dependent on the injury mechanism, existence

of concurrent injuries and comorbidities and treatment effectiveness (12,13). These aforementioned events are generally accompanied with robust local and/or systemic immune activation (14). Therefore, targeting certain immunological pathways may prove beneficial for developing future TBI treatment strategies (14).

The primary brain injury may involve damage to the intracranial contents, volume-occupying effects, in addition to neuronal, glial and/or cerebral vascular dysregulation (3,8). By contrast, secondary brain injury depends on the activation status of several interconnected pathophysiological pathways (10). The cell populations that will either undergo apoptotic cell death or sustain the significant functional impairments are determined by the degree of activation of these complex pathways and processes (8,10).

A variety of metabolic and/or molecular cascades can be activated in TBI, ultimately leading to elevated intracellular concentrations of calcium and sodium, mitochondrial dysfunction, free radical production, oxidative phosphorylation impairment, apoptosis activation, cumulative release of neurotransmitters, and in energy expenditure (11,15,16).

Pro-inflammatory, anti-inflammatory cytokines and chemokines can be secreted by neurons, glial cells and systemic immune cells, which can also serve a significant role in intracellular pathological signalling (8,11).

Mechanical processes such as volume-occupying traumatic lesions can cause cerebral oedema, ischaemia, recurrent haemorrhage, impaired cerebral autoregulation, decreased cerebral perfusion pressure, increased intracranial pressure and herniation syndromes (17).

Systemic processes can result in a variety of conditions, including reduced cerebral blood flow, electrolyte disorders, hyperglycaemia, hypoglycaemia, hypoxia, anaemia, hypo- or hypercapnia, acid-base disorders and seizures (2).

On a molecular level, mechanical energy transfer can disrupt neurotransmitter circuits and homeostasis, even if there is no anatomical damage on cellular or macroscopic levels (16).

Neuroinflammation is normally orchestrated through a coordinated web of neuronal and microglial signalling (18-20). It possesses a diverse array of both beneficial and neurotoxic components (18-20). There is also emerging evidence that an isolated incidence of brain injury can lead to complex immunological alterations in the levels of circulating leukocytes, complement proteins, pro- or anti-inflammatory cytokines and coagulation factors (18-20). Subsequently, pro-inflammatory cytokines activate M_1 macrophages to repair damage, whereas anti-inflammatory cytokines can stimulate M_2 macrophages, which serves to regenerate the neural tissue (11,12).

TBI severity is commonly classified as severe, moderate or mild (Table SI). In addition, certain biomarkers, CT imaging prognostic scales and multidimensional computer prognostic models are also currently used to determine the severity of TBI (13,15,21). In particular, because of its simplicity, low cost, speed, ability to reveal osseous structures and surgical lesions, head CT scan remains to be the gold standard for the initial evaluation of an injured patient with a TBI (15). In terms of molecular biomarkers, ubiquitin C-terminal hydrolase L1 (UCH-L1) is considered to be a protein biomarker for neuronal cell body injury, whereas glial fibrillary acidic protein (GFAP) is generally considered as a biomarker for astro-glial injury (10,22). UCH-L1 is a deubiquitinating neuronal enzyme, whilst GFAP is a monomer of intermediate filaments forming the astrocytic cytoskeleton (23-25). The i-STAT TBI Plasma test, which measures both UCH-L1 and GFAP, has been approved by the U.S. Food and Drug Administration for the detection of possible candidates for CT scan, between patients with mild TBI discrimination in adults (26). Apart from GFAP, astroglial calcium-binding protein B (S100B) is also one of the most extensively studied TBI biomarkers (27-33). Scandinavian Guidelines for Initial Management of Minimal, Mild and Moderate Head Injuries in Adults have already incorporated S100B, to triage patients with mild TBI for brain imaging (34). Furthermore, two large clinical trials (INTREPID used GFAP and UCH-L1 and Bio-ProTECT used GFAP, S100B and UCH-L1) have incorporated these three biomarkers for evaluating treatment efficacy (10).

IL-6 is a member of the IL-6 cytokine family (35). Although it is generally considered to be a pro-inflammatory cytokine, a recent study has revealed that it can also possess anti-inflammatory properties (36). A large group of different immune, epithelial, neuronal or astroglial cells can release IL-6, which in turn triggers a multitude of biological cascades (35,37,38). Amongst the list of reported metabolic and neurotrophic functions, an essential role in neuronal survival during TBI-induced neuroinflammation has also been ascribed to this important family of cytokines (36,39,40). By contrast, IL-8 is a chemoattractant cytokine that is produced by various cell types, including monocytes, neutrophils, fibroblasts, endothelium, epithelial cells and cancer cells (41). Unlike other cytokines, IL-8 has a distinct specificity for attracting neutrophils towards inflammatory regions, in addition to recruiting epithelial cells for angiogenesis and tissue healing (41-44). It has been extensively studied in a variety of inflammatory responses, including fever, sepsis and carcinogenesis (41,45). However, its role in the pathophysiology of the neuroinflammation following TBI remains unclear. IL-10 is considered to be an anti-inflammatory cytokine that can inhibit the expression of pro-inflammatory factors, such as IL-1β, TNF-α, IL-1α, IL-6 and IL-8 (46-50). In terms of its neurological role, IL-10 can appear to mediate the recovery process following TBI-induced neuroinflammation (48). Additionally, IL-10 has been reported to facilitate cytokine storm resolution, to prevent prolonged secondary brain damage (51). Monocytes and lymphocytes can both secrete IL-10, which stimulates the IL-10/Janus kinase 1/STAT3 pathway to suppress these damaging inflammatory processes (47,49,50).

A number of studies have previously examined the potential changes in the serum levels of pro- or anti-inflammatory cytokines during the acute phase following TBI in animal models and humans (Tables SII-SVI). Although results remain inconclusive, they generally suggest the upregulation of IL-6, IL-8 and IL-10 following TBI compared with that in healthy controls. However, studies examining the association of cytokine levels, measured at the time of injury and/or soon after, with injury severity in human patients remain elusive. This is compounded by the potential value of applying cytokine levels for disease prognosis receiving next to no attention. To the best of our knowledge, there is insufficient information on the association of IL-6, IL-8 and IL-10 with the extant set of clinical,

imaging, laboratory indices of injury severity and clinical prognosis in TBI. Instead, the vast majority of previous studies tended to focus on the association of each interleukin with a limited set of clinical parameters.

Therefore, present study attempted a more holistic evaluation of the possible relationship among some of the inflammatory serum biomarkers and TBI severity and prognosis. Numerous variables, including epidemiological, clinical, laboratory, imaging, specific neurobiomarkers, complications and functional outcomes were recorded and analysed. The present study had the following two primary objectives: i) To assess the value of IL-6, IL-8 and IL-10 and cell injury markers (such as UCH-L1 and GFAP) as potential complementary TBI severity classification indices upon admission, in combination with standardised existing clinical, imaging variables and scoring systems; and ii) To assess the possible prognostic value of ILs, as assessed using validated functional outcome scoring scales.

Patients and methods

Patient recruitment. In the present single-centre, prospective observational study, adult and paediatric patients from a Neurosurgical Department and two Intensive Care Units (ICU) of University General Hospital of Heraklion, in eastern Crete, Greece were recruited between 2019 and 2022. Adult (>16 years old) patients who were consecutively admitted with mild, moderate or severe TBI, as described in the introduction section, were eligible for enrolment. A smaller pilot group of paediatric patients (aged 1-16 years) was also enrolled for comparison, to clarify whether paediatric patients with TBI show similar neuroinflammatory responses to adults. A group of healthy adult individuals served as the control group for comparisons. Inclusion criteria: i) Patients with mild, moderate or severe TBI, with any type of haemorrhagic traumatic brain imaging findings; ii) Patients or patients' legal representatives were able or willing to provide written informed consent; and iii) Blood sampling feasible within the first 12 h after TBI; iv) Blood sampling feasible before any surgical intervention. Exclusion criteria: i) Previous history of neurological disease, or CNS malignancy, ii) Concurrent acute infectious, neoplastic, inflammatory or immunological disease; iii) Previous TBI or CNS surgery; and iv) Patients with blast or penetrative injuries.

Data acquisition. Demographic and clinical data, co-occurring injuries and comorbidities, imaging findings, injury severity scoring systems, surgical interventions and clinical outcomes were recorded. Additionally, the occurrence of possible related complications was recorded throughout the first 7 days and at 6 months following the injury.

Variables collected

Clinical diagnostic variables. Signs and symptoms, types of injury, co-occurrence of multiple traumatic injuries, Glasgow Coma Scale (GCS; Table SI) score, motor component of the GCS (mGCS) score (Table SVII), Karnofsky Performance Scale (KPS) score (Table SVIII), Modified Rankin Scale score (Table SIX), Eastern Cooperative Oncology Group/WHO (ECOG/WHO) score (Table SX), pupil size and reactivity and vital signs (hypotension and hypoxia) were obtained upon admission. The Injury Severity Score (ISS) was also calculated (Table SXI). GCS was used as the primary clinical parameter for TBI severity definition, whilst the systematic traumatic injuries of patients were assessed through ISS.

Brain imaging diagnostic variables. The different types of traumatic lesions found on brain CT scanning (General Electric Revolution CT-GSI) upon admission, the presence and types of skull fractures, the patency of basal cisterns (Table SXII), the presence of midline shift (Table SXIII) and the volume of space-occupying haemorrhagic lesions (Table SXIV) were all recorded. The scores for the following TBI imaging scales were calculated: i) Rotterdam CT; ii) Marshall CT Classification; iii) Stockholm CT; and iv) Helsinki CT (Table SXV).

Outcome scales. The KPS and Glasgow Outcome Scale (GOS) (Table SXVI) scores on day 7 post-injury and Glasgow Outcome Scale-Extended (GOS-E; Table SXVII) and mortality at 6 months post-injury were used as outcome variables.

Prognostic models. The Corticosteroid Randomisation After Head Injury (CRASH) predictive model was also calculated (Table SXV).

Assays. Blood samples were obtained within 12 h from patients with TBI and on day 7 after the TBI, before the serum was stored at -80˚C until further quantification analysis. IL-6 (Cat. no. 430504; BioLegend, Inc.), IL-8 (Cat. no. 431504; BioLegend, Inc.) and IL-10 (Cat. no. 430604; BioLegend, Inc.), GFAP (cat. no. E-EL-H6093; Elabscience Biotechnology, Inc.) and UCH-L1 (cat. no. E-EL-H2377; Elabscience Biotechnology Inc.) were measured through ELISA according to manufacturer's protocols. All specimens were assayed in duplicate. The sensitivities of the assays were 4 pg/ml for IL-6, 8 pg/ml for IL-8, 2 pg/ml for IL-10, 9.38 pg/ml for GFAP and 46.88 pg/ml for UCH-L1. The detection range was 7.8-500 pg/ml for IL-6, 15.6-1,000 pg/ml for IL-8, 3.9-250 pg/ml for IL-10, 15.63-1,000 pg/ml for GFAP and 78.13-5,000 pg/ml for UCH-L1.

Statistical analysis

Univariate analyses. Descriptive statistics of serum biomarkers are presented for the three study groups (adult or paediatric TBI patients, and healthy adults). Categorical variables are described as absolute values and frequencies. The Kolmogorov-Smirnov and Shapiro-Wilk analyses were used to determine whether a normal distribution model fitted the observations as appropriate. Based on this test of normality, quantitative clinical variables are expressed as the mean ± standard deviation (parametric analyses), or as median and interquartile range (non-parametric analyses). Non-parametric tests were used to handle serum biomolecule data, given significant deviations from normality. Spearman's correlation coefficient was used for correlations between two continuous variables and χ^2 test for categorical variables, respectively. Non-parametric group differences were examined using the Mann-Whitney U-test, or the Kruskal-Wallis independent samples test with post hoc Dunn's pairwise tests, in the event that an independent variable consisted of > two groups. Receiver Operating Characteristic Curves (ROC) were used to examine the response function of potential biomolecule predictors toward specific outcome variables.

Table I. Patient demographic and clinical characteristics.

Variable	Adults (n=109)	Children (n=17)	P-value
Sex, n (%)			0.289
Males	72 (66.1)	13 (76.5)	
Females	37 (33.9)	4 (23.5)	
Age, mean (SD)	62.37 (22)	10.2 (4.5)	-
GCS score, median (IQR)	14 (6-15)	14 (10-15)	0.642
ISS, median (IQR)	14.5 (9-25)	9 (6-21)	0.312
LOS (days), median (IQR)	15 (8-30)	7 (4-14)	0.191
WBC x10^3 (cells/μl), median (IQR)	12 (8.6-15.6)	13 (10-19.2)	0.078
CRP (mg/dl), median (IQR)	1.2 (0.3-2.6)	0.19 (0.06-0.76)	0.002
Marshall CT Classification score, n (%)			0.918
I + II	60 (55)	11 (64.7)	
III + IV	4 (3.7)	6 (35.3)	
V + VI	45 (41.3)	0 (0)	
Rotterdam CT score, median (IQR)	3 (2-3)	2 (2-3)	0.013
Stockholm CT score, median (IQR)	1.7 (1-2.5)	1 (0.7-1.5)	0.034
Helsinki CT score, median (IQR)	2.5 (2-6)	1 (0-2)	0.039

IQR, interquartile range; GCS, Glasgow Coma Scale; LOS, length of stay; WBC, white blood cells; CRP, C-reactive protein; ISS, injury severity score.

The 'optimal' cut off point for the best sensitivity-specificity combination of the selected discriminators was calculated by the Youden index (J), and confirmed by the Closest to (0,1) Criteria (52).

Multivariate analyses. Selected variables that displayed statistically significant associations with specific outcomes were entered into a multivariate logistic regression model to identify parameters that were independently associated with an adverse outcome. Nagelkerke R2 goodness of fit value was used to evaluate the best-fitting model.

Statistical analyses were performed using the SPSS software for Windows (version 25; IBM Corp.). A P-value <0.05 was considered as an indicator of statistically significant differences.

Results

The subsequent analyses refer to the adult population, unless differently specified.

Patient characteristics. In total, 109 adult cases were eligible for inclusion into the present study (66.1% males and 33.9% females; mean age, 62.37±22 years). Mechanisms of brain injury included falls from <1 m (44%) or >1 m (16.8%), road vehicle accidents (22.4%), pedestrian involved accidents (6.4%), bicycle or skating accidents (4%), assaults (4%) or object percussion injuries (2.4%). The control group consisted of 20 healthy adult volunteers (55% males and 45% females; mean age, 39.0±9.6 years). A smaller paediatric pilot group of 17 patients was also enrolled (age range, 1-16 years; mean age, 10.2±4.5 years). Demographic, imaging and clinical characteristics of the patients are summarized in Table I.

The majority of adult patients exhibited clinical presentations consistent with mild TBI (66.9%) upon admission, whereas 16.5% patients suffered from moderate TBI and 16.6% from severe TBI. The 6-month mortality rate in the adult TBI patient sample was 23.5%. By contrast, the majority of children exhibited clinical presentation consistent with mild TBI (66.7%) upon admission, whereas 14.3% patients suffered from moderate TBI and 19% with severe TBI. The 6-month mortality rate was 5.6% for the paediatric TBI patient sample.

Group differences. Group differences of the studied protein levels are presented in Table II. Serum levels of IL-6 and IL-10 on day 1 were found to be significantly elevated in adult patients with TBI compared with those in healthy individuals (P=0.001 and 0.015 respectively) (Fig. 1). No significant differences were recorded among the adult and paediatric patient groups regarding the day 1 serum levels of IL-6, IL-8 and IL-10. On day 1, adult patients with TBI displayed significantly lower IL-8 (P=0.004) and significantly higher UCH-L1 levels (P=0.001) compared with those in the control group. Consecutive measurements of inflammatory markers revealed significant reduction for IL-6 and IL-10, or elevation for UCH-L1 serum levels for adult TBI cases (P<0.021 for all) (Fig. 2). It should be noted that the IL data were not included in subsequent longitudinal analyses in cases of missing values (which explains the differences in medians among Table II and Fig. 2) or if a given patient underwent any surgical procedures during the first 7 days following the TBI. These measures were taken to avoid confounding measurements of IL on day 7.

Associations of inflammatory indices with TBI severity in adults. On day 1, adult patients who suffered from severe TBI (as indicated by GCS<9) displayed significantly higher levels

Table II. Serum levels of the biomolecules measured in patients with TBI compared with healthy controls.

Biomolecules	Adult TBI patients	Paediatric TBI patients	Healthy adults
IL-6 Day1	63 (34-179)[a]	52.6 (19.2-272)	21.1 (18.2-23)
IL-6 Day7	37.2 (18.7-78)	17.7 (13-40.7)	-
IL-8 Day1	9.5 (6.6-16.9)[a]	7.35 (6-13.2)	17.6 (15.6-19)
IL-8 Day7	10.6 (7-16.2)	8.9 (7.3-15)	-
IL-10 Day1	25.2 (15-44.5)[a]	29.8 (12.1-45)	17.4 (15.8-20)
IL-10 Day7	15.3 (11-24.4)	14.5 (10.3-16.7)	-
UCH-L1 Day1	207.2 (110-472)[a]	190 (140-540)	54 (46-105)
UCH-L1 Day7	323 (136-556.3)	279 (210-393.4)	-
GFAP Day1	55.7 (27.3-94)	46.2 (15.5-81)	43.7 (24.5-71)
GFAP Day7	64 (35-88.5)	29.4 (14.6-58.6)	-

[a]P<0.05 between adult TBI patients and healthy controls (Kruskal-Wallis test). IQR, Interquartile Range; IL, interleukins; UCH-L1, ubiquitin C-terminal hydrolase L1; GFAP, glial fibrillary acidic protein. Values are median (IQR) in pg/ml.

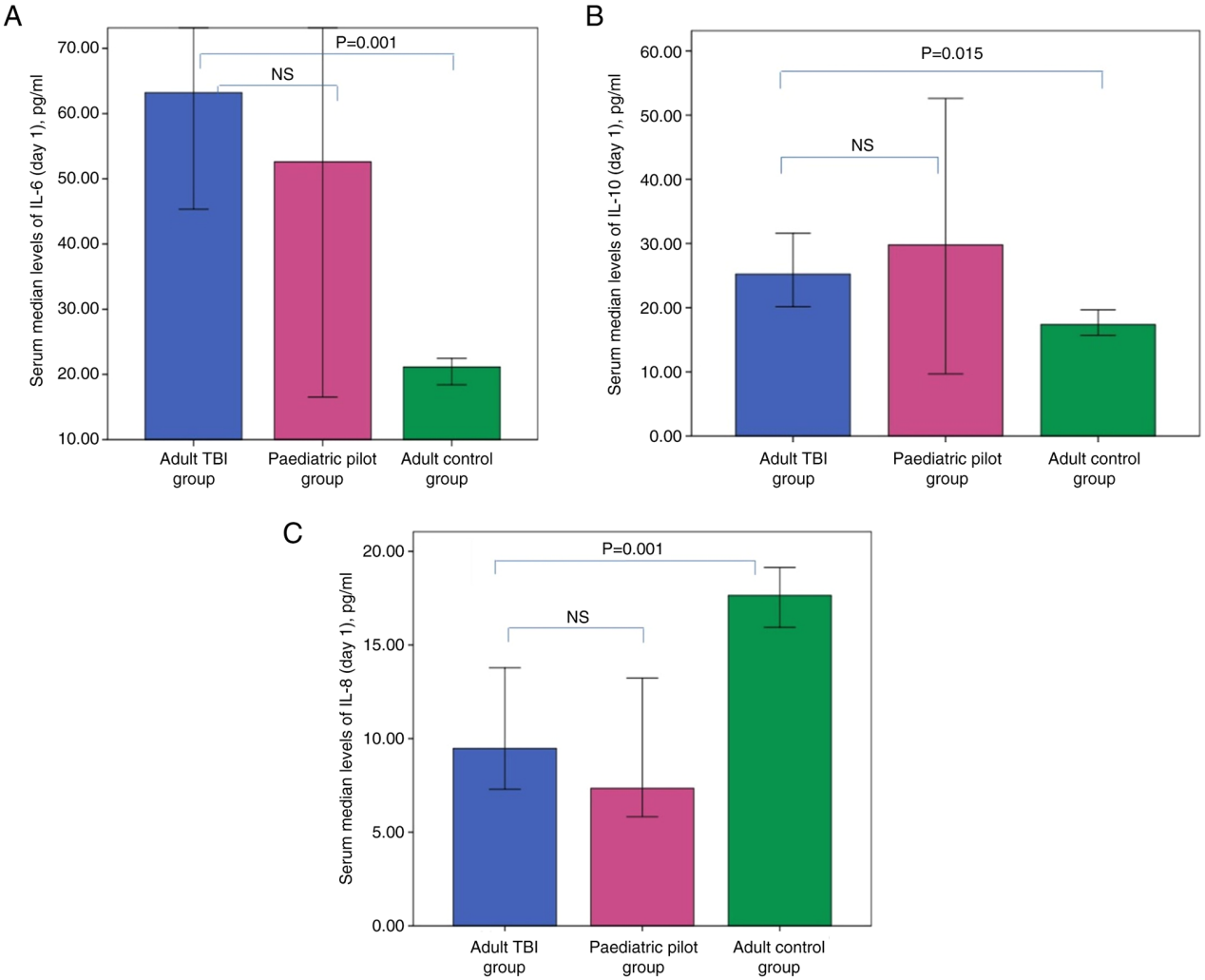

Figure 1. Significantly elevated early (day 1) serum levels of (A) IL-6 and (B) IL-10, but (C) lower IL-8 for adult patients with TBI, compared with healthy adults (controls). No significant differences between adult and paediatric TBI patients were found. IL, interleukin; TBI, traumatic brain injury.

of IL-6 (P=0.001), IL-10 (P=0.009), and GFAP (P=0.001) compared with those in patients with mild or moderate TBI. Similarly, patients who had lower mGCS on day 1(as indicated by mGCS≤3) showed significantly higher levels of IL-6 (P=0.001), IL-10 (P=0.035) and GFAP (P=0.028). In addition, patients who suffered from severe injuries throughout

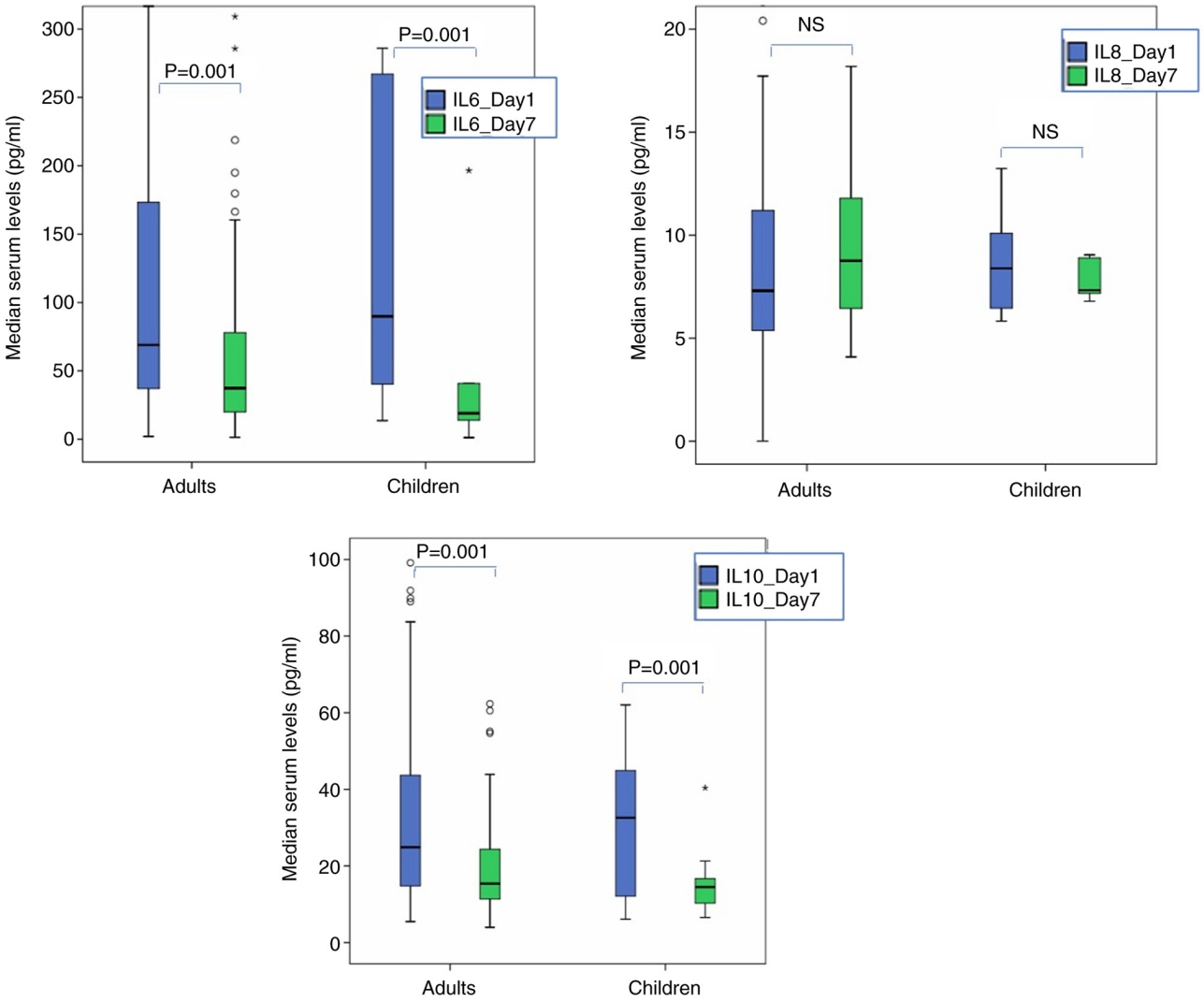

Figure 2. Significant changes of IL-6 and IL-10 serum levels on days 1 and 7 for adult and paediatric patients with TBI. IL, interleukin; TBI, traumatic brain injury.

the body as indexed by scores of ISS >24 displayed significantly elevated IL-6 (P=0.006), IL-10 (P=0.047) and GFAP (P=0.006) levels on day 1, compared with those in the remaining patients (ISS≤24). Patients who scored <50 points according to KPS also had significantly higher levels of IL-6 and IL-10 (P=0.004 for both) on day 1, compared with those who scored >40. Similarly, patients who scored >3 according to MRS classification displayed significantly elevated IL-6 (P=0.005), IL-10 (P<0.001) and UCH-L1 (P=0.037) levels on day 1, compared with those who scored <4. However, patients who scored >2 according to ECOG/WHO classification displayed significantly elevated IL-6 (P=0.017) and IL-10 (P<0.001) levels, but significantly lower UCH-L1 (P=0.022) levels on day 1, compared with those who scored <3.

Inflammation biomarkers and imaging findings upon admission (day 1) in adult TBI patients. Higher levels of IL-6 and IL-10 on day 1 were found to be connected with increased risk according to the imaging indices of TBI severity, such as basal cistern compression (P<0.007), midline shift >5 mm (P<0.003) and larger total lesion volume (P<0.006). The association between inflammatory markers and the finding of traumatic intraventricular haemorrhage or traumatic subarachnoid haemorrhage was found to be negligible. Severe cases, found according to higher scores on the Stockholm and Rotterdam CT scales, were correlated with elevated IL-6 (r_s=0.4 and 0.274, respectively; P<0.009 for all), IL-10 (r_s=0.323 and 0.305, respectively; P<0.003 for all) and GFAP (r_s=0.203 and 0.213, respectively; P<0.045 for all) on day 1. In addition, severe cases according to higher scores on the Marshall CT Classification and Helsinki CT scales were correlated with elevated IL-6 (r_s=0.386 and 0.446, respectively; P<0.001 for all) and IL-10 (r_s=0.272 and 0.335, respectively; P<0.009 for all) on day 1. However, the association between UCH-L1 and none of the severity CT scores used reached significance. The CRASH head injury prognostic score (14-day mortality risk or 6-month mortality and severe disability risk) was found to be positively correlated with IL-6, GFAP and UCH-L1 (r_s=0.372, 0.357 and 0.369 respectively; P<0.031 for all) on day 1.

Inflammation biomarker-independent associations with functional outcomes and 6-month mortality. Adult patients who did not survive during the first 6 months following TBI (n=32) had significantly elevated IL-6, IL-10 (on day 1) and

UCH-L1 levels (on days 1 and 7; P<0.018) compared with those in survivors. In particular, adult patients who exhibited an unfavourable outcome on day 7 (n=22 as indicated by a score <4 according to GOS) displayed significantly elevated IL-6 (P=0.003) and IL-10 (P=0.007) levels on day 1. Similarly, patients who had an unfavourable outcome at 6 months (n=45 as indicated by a score <5 according to GOS-E) exhibited significantly elevated IL-6 (P=0.045), IL-10 (P=0.048) and UCHL-1 (P=0.042) on day 1. Significantly elevated IL-6 and IL-10 levels on day 1 were also recorded for patients with more severe functional state according to KPS (as indicated by a score <50) of day 7 compared with those who scored >40 (P<0.004 for all).

A multivariate predictive logistic regression analysis was conducted to distinguish adult patients with TBI at risk of an unfavourable outcome based on GOS-E. The following variables were included into the model: IL-6, IL-10 and UCH-L1 levels on days 1 and 7; age; serum glucose levels; GCS; MRS; and KPS upon admission. The final model was associated with acceptable fit to the data (χ^2=11.28, P=0.004, Nagelkerke R^2=0.458) with the following significant predictors: IL-6 on day 1 [Exp (B)=0.987; P=0.025] and UCH-L1 of day 1 [Exp (B)=0.993; P=0.032], along with age (P=0.014) and GCS (P=0.045; Table III).

ROC analysis in adults revealed that without considering other potential predictors, age, IL-6 and UCH-L1 on day 1 were marginally acceptable, independent predictors of 6-month severe disability based on GOS-E (Fig. 3; Table IV). The optimal cut-off values according to the Youden index (J) were 55.51 pg/ml (sensitivity 73% and specificity 55%) for IL-6 and 204 pg/ml for UCH-L1 (sensitivity 65% and specificity 59%).

Other associations in adult patients with TBI. GFAP levels on day 1 were positively correlated with IL-6 and IL-10 (r_s=0.335 and 0.357, respectively; P=0.001 for both; Figs. 4 and 5), whilst a significant negative correlation was found between UCH-L1 and IL-8 levels on day 1 (r_s=-0.302; P=0.001). Additionally, elevated IL-6 and IL-10 on days 1 and 7 were correlated with increased risk for ICU admission (r_s=0.329 and 0.510 respectively; P<0.005 for all). Among the common laboratory markers, IL-6 and IL-10 levels on day 1 were positively correlated with white blood cell and neutrophil counts (r_s=0.271 and 0.282 respectively; P<0.05 for both), glucose (r_s=0.265 and 0.313 respectively; P<0.025 for both), troponin (r_s=0.285; P=0.001 only for IL-6) and creatine phosphokinase (r_s <0.218; P<0.041 for both). GFAP levels on day 1 were positively correlated with glucose (r_s=0.199; P=0.044) and troponin (r_s=0.3; P=0.002). No significant correlations among these common laboratory markers and IL-8 or UCH-L1 could be found.

In terms of paediatric patients with TBI, a significant positive correlation between GFAP and IL-10 levels (r_s=0.555; P=0.029) on day 1 was found, whilst a significant negative correlation between UCH-L1 and IL-8 (r_s=-0.554; P=0.04) was recorded.

Table III. Multivariate logistic regression of potential predictors of an unfavourable outcome based on GOS-E.

Variable	Odds ratio	95% CI	P-value
IL-6 Day 1	0.987	0.975-0.998	0.025[a]
IL-10 Day 1	1.009	0.925-1.100	0.843
UCH-L1 Day 1	0.993	0.987-0.999	0.032[a]
Age	0.907	0.840-0.980	0.014[a]
Glucose	1.029	0.997-1.062	0.077
GCS	1.768	1.013-3.084	0.045[a]
MRS	0.542	0.263-1.119	0.098
KPS	0.978	0.911-1.044	0.468

[a]P<0.05. CI, 95% confidence intervals; MRS, Modified Rankin Scale; KPS, Karnofsky Performance Scale; UCH-L1, ubiquitin C-terminal hydrolase L1.

Discussion

In the present study, it was found that the levels of IL-6, IL-10 and UCH-L1 are significantly elevated on admission in adult patients with TBI compared with healthy individuals, whilst the levels of IL-6 and IL-10 tended to decrease over the first week post-injury. In addition, relatively higher serum levels of IL-6 and UCH-L1 were found to be predictive of increased mortality and poorer functional outcome at 6 months post-injury, even after controlling for other demographic parameters (such as age), common laboratory parameters (such as glucose) and clinical indices of injury severity (such as KPS and MRS scores). By contrast, the vast majority of previous studies focused on associations of separate IL with a limited set of parameters.

UCH-L1 has been previously proposed to be an important index of TBI classification and prognostication (23,53), whilst other studies also support their significant associations with outcome (54). However, they did not highlight their potential predictive power in TBI prognostic models (54). IL-6 and IL-10 are important inflammatory cytokines with primary protective roles against pathogenic, traumatic or stressful insults that are regularly present in relatively low levels, even in healthy individuals (36,37,39,49). Results from the present study support the notion that post-traumatic inflammatory and anti-inflammatory mechanisms start concurrently during the early stages of TBI to maintain the ideal inflammatory equilibrium (55).

Previously reported findings are in accordance with multiple human studies, which showed the statistically significant upregulation of IL-6 concentrations in patients with TBI compared with those in controls (56-73). However, it should be noted that two large-scale studies (N_{total}=245) failed to detect significant differences (71). In particular, three studies have reported a significant negative correlation between IL-6 and admission GCS (72,74,75), but other studies failed to find such a relationship (67,76,77). In addition, four studies have discovered a statistically significant positive correlation between IL-6 and severe imaging findings (such as size of lesions and traumatic subarachnoid haemorrhage) (78-81). However, other previous studies have reported that IL-6 concentration is significantly associated with the concentration of various TBI-specific neurological biomarkers (such as nerve growth factor, S100 calcium-binding protein B and neuron-specific enolase) (58,80). Furthermore, several studies consistently

Table IV. Data for ROC curve showing the independent discriminators of an unfavourable outcome for patients with TBI based on GOS-E.

				Asymptotic 95% confidence interval	
Prognostic discriminators	Area	Std. error	Asymptotic sig.	Lower bound	Upper bound
IL-6 day 1 (pg/ml)	0.632	0.057	0.045	0.515	0.740
UCH-L1 day 1 (pg/ml)	0.640	0.062	0.033	0.519	0.761
Age (years)	0.758	0.048	0.001	0.716	0.904
GCS day 1	0.366	0.063	0.041	0.243	0.489
MRS	0.673	0.058	0.008	0.560	0.787
KPS	0.337	0.058	0.013	0.223	0.451
ECOG/WHO	0.644	0.058	0.028	0.526	0.762

IL, interleukin; UCH-L1, ubiquitin C-terminal hydrolase L1; GCS, Glasgow Coma Scale; MRS, Modified Rankin Scale; KPS, Karnofsky Performance Scale; ECOG/WHO score, Eastern Cooperative Oncology Group/WHO score.

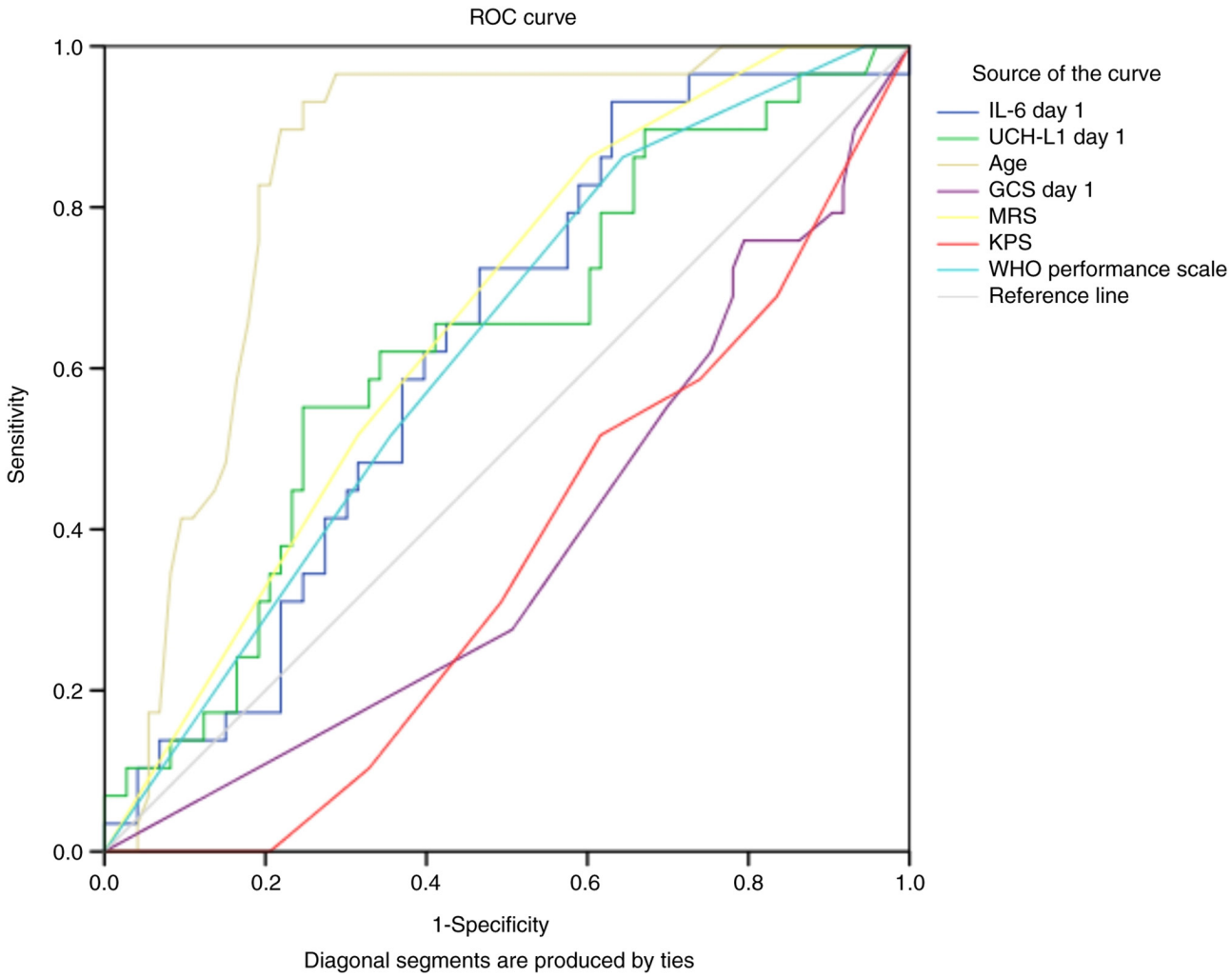

Figure 3. ROC curve showing the independent discriminators of an unfavourable outcome for patients with TBI based on Glasgow Outcome Scale-extended. ROC, receiver operating characteristic.

highlighted the predictive value of IL-6 for mortality or functional outcome (47,67,76,77,81-93). Clinical prognosis in TBI may depend on both local and systemic components, since nerve tissue damage stimulates neuroinflammation through both local and systemic inflammatory cells (12). In the present report, a significant association of IL-6 with several indices of

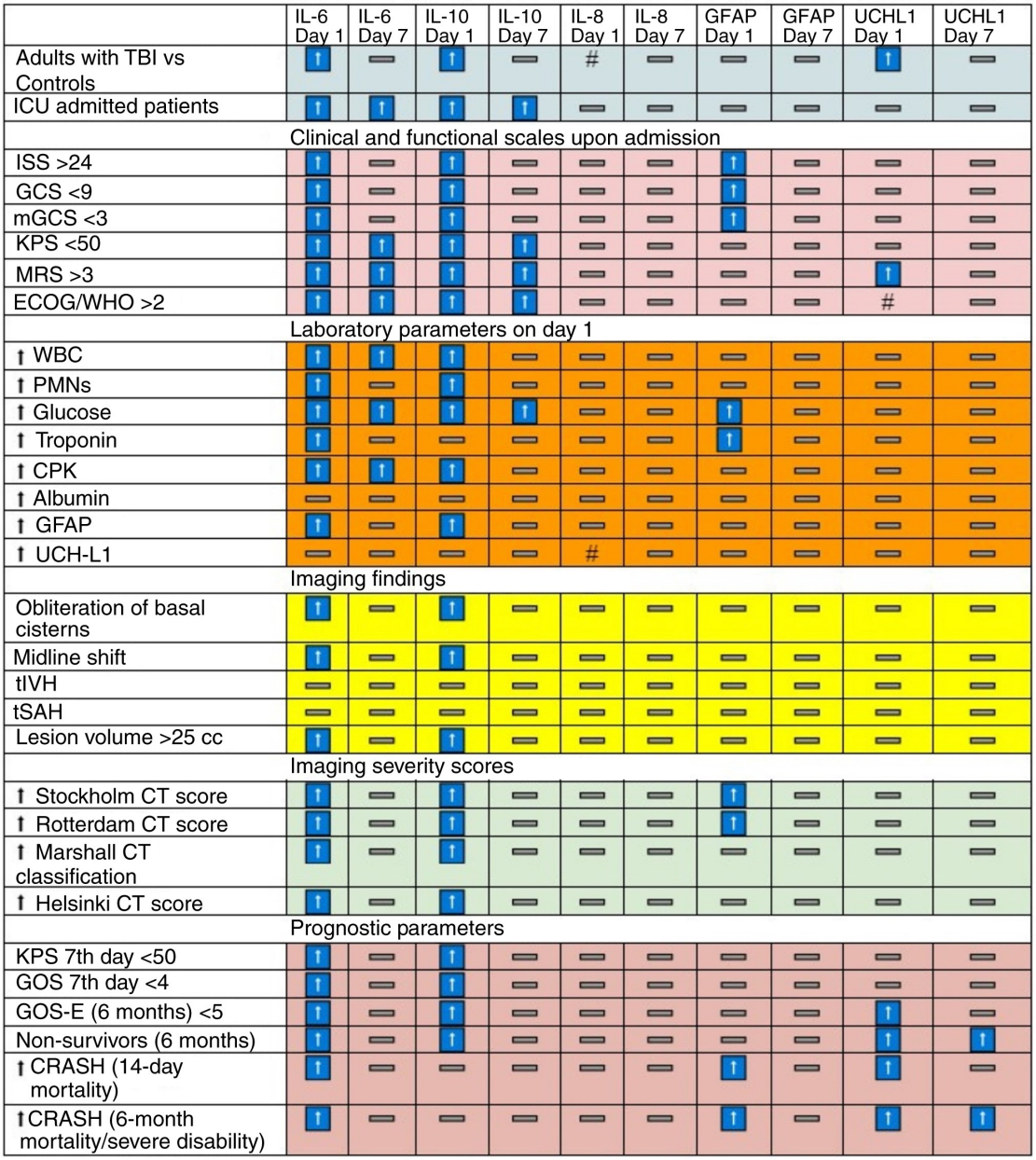

Figure 4. Significant associations of biomarkers with the clinical, diagnostic and prognostic parameters for the adult population of the study. Arrows indicate significant positive (↑) or negative (#) associations of interleukins. CRASH, corticosteroid randomisation after significant head injury; CPK, creatine phosphokinase; ECOG/WHO score, Eastern Cooperative Oncology Group score/WHO score; GCS, Glasgow Coma Scale; GFAP, glial fibrillary acidic protein; GOS, Glasgow Outcome Score; GOS-E, Glasgow Outcome Scale-Extended; ICU, Intensive Care Unit; ISS, Injury Severity Score; KPS, Karnofsky Performance Scale; LOS, length of stay; mGCS, Motor Component of Glasgow Coma Scale; MRS, Modified Rankin Scale; PMNs, neutrophils, tIVH, traumatic intraventricular haemorrhage; tSAH, traumatic subarachnoid haemorrhage; UCH-L1, ubiquitin C-terminal hydrolase L1; WBC, white blood cells.

diagnostic or prognostic classification of TBI was documented, highlighting the potential value of IL-6 as a complementary index of both localised neural tissue damage and multisystem pathology. This is complementary to other TBI-specific neurobiomarkers, such as GFAP and UCH-L1.

The present analysis demonstrated consistent associations among IL-10 and multiple variables associated with the diagnostic and prognostic classification of TBI. Increased values of IL-6 and IL-10 have both been connected with an increased risk for ICU admission, lower scores in GCS, lower scores in mGCS and lower scores in KPS, higher scores in ECOG/WHO and ISS upon admission, higher blood levels of multiple common laboratory markers (such as white blood cells, polymorphonuclear leukocytes, glucose, creatine phosphokinase) , higher blood levels of GFAP and with positive imaging findings (midline shift >5 mm, obliteration of basal cisterns, larger volume of lesions, higher values in Stockholm, Rotterdam, Marshall and Helsinki CT scales). The role of IL-10 as an index of injury severity and prognosis of TBI has previously been highlighted in the literature (47,49,57,94-96), although a negative finding has also been found (97). Therefore, existing evidence appears to be stronger regarding the association of elevated IL-10 with mortality or with unfavourable functional outcomes as indexed by GOS or GOS-E (47,57,89,94,98-101). The relationship of IL-10 with admission GCS, complications or other biomarker levels has not been sufficiently examined to date.

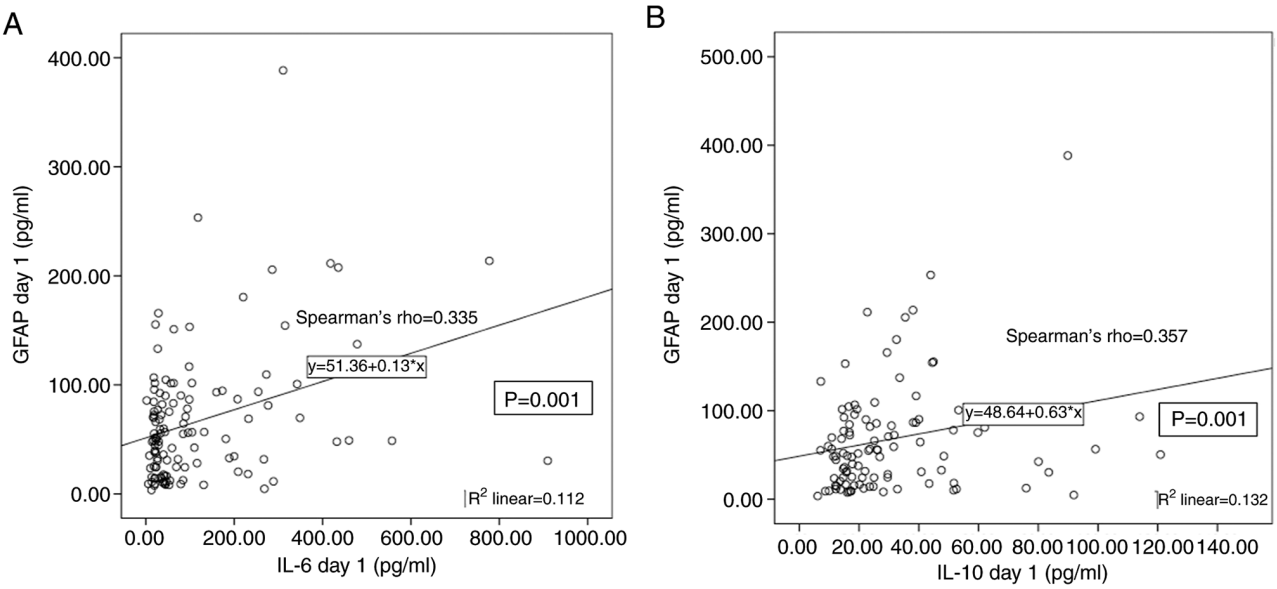

Figure 5. Significant positive correlations between early measured (day 1) GFAP and inflammation biomarkers (A) IL-6, and (B) IL-10. IL, interleukin; GFAP, glial fibrillary acidic protein.

Serum levels of IL-8 were found to be significantly lower in patients with TBI compared with those in healthy adult controls with relatively stable levels throughout the week 1 post-injury. Due to the neutrophil chemoattractant properties that have been attributed to IL-8 (102), this finding may not be surprising. Systemic serum IL-8 levels could be suppressed during TBI, due to its valuable role locally as a neuroinflammation danger signal. In addition, it has been reported that IL-8 is involved in angiogenesis, which is hypothesised to promote neurodegeneration (42,103). However, early and sustained downregulation of IL-8 may confer a protective response against neurodegeneration. This possibility warrants further investigation (104). Therefore, the present results do not support the diagnostic (67,74,103,105-108) or prognostic significance of IL-8 in TBI (67,87,89,93,108-111).

A potential novelty of the present study is that a pilot group of paediatric patients was also included, given the scarcity of paediatric TBI data. However, the present study has certain limitations. A sample of consecutive patients with TBI was enrolled, whose age varied widely. By contrast, the control group consisted of significantly younger healthy volunteers. Moreover, the largest part of the present study was conducted during the COVID-19 pandemic, which could have influenced the demographic and TBI mechanism and severity characteristics of the patients. Further studies will be needed to clarify the preliminary results of the present study with regards to inflammatory biomarkers. Another limitation concerns the pilot paediatric population, which consisted of only a small number of children, offering little information in drawing remarkable conclusions. This was compounded by the lack of control paediatric individuals in the present study.

In conclusion, patients with TBI may have multiple injuries and/or complications. The prognosis of a patient with TBI is dependent on the presence of both local and systemic responses, in addition to that of damage to other tissues. Therefore, an adequate TBI prognostic model should take into consideration all aspects of systematic inflammation and local neuroinflammation, which is generated by both localised neural tissue damage and systemic immune responses. At present, a significant number of CNS-specific or non-specific inflammation biomarkers are being studied for clinical use. However, to the best of our knowledge, no reliable biomarker or group of biomarkers with adequate sensitivity or specificity for TBI severity classification or prognostication have been found to date. In the present study, three systemic biomarkers associated with inflammatory processes, IL-6, IL-8 and IL-10, in addition to two specific biomarkers associated with nerve tissue injury, UCH-L1 and GFAP, were examined. The present study indicated that IL-6 and IL-10, but not IL-8, may serve to be independent TBI severity discriminators in neurocritical patients with TBI, based on a holistic comparison with already authorised TBI neurological biomarkers (UCH-L1 and GFAP), clinical and imaging tools. Furthermore, IL-6 and UCH-L1 seemed to act as viable prognostic TBI biomarkers. Therefore, the incorporation of inflammation biomarkers IL-6 and IL-10, alongside TBI-specific neurological biomarkers (such as UCH-L1) into diagnostic and prognostic models may optimise the guidance of and enhance current existing clinical decisions and practices, to facilitate outcome prognostication.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CT, AV, PS and MV conceptualized the study. CT, MM, EP, SL, NM, KN and AT were in charge of data curation, investigation and formal analysis. The methodology of the study was designed by CT, MM, SL, NM, KN, AT, SI, AV, PS and MV. CT, SL, NM, KN, AT, SI, AV, PS and MV performed data validation. Data visualization was carried out by CT and MM. CT, SL, NM, KN, AT, SI, AV, PS and MV confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Institutional (University of Crete) ethics committee approval was obtained for the present study [approval nos. 198/14.11.2019 (first) and 132/07.09.2022 (revised); Heraklion, Crete, Greece]. Approval was acquired for both participation and publication of the study's findings. All data were de-identified.

Patient consent for publication

Written informed consent was obtained from the patients or patients' representatives (surrogate decision makers) before inclusion into the present study.

Competing interests

The authors declare that they have no competing interests.

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