Prognostic significance of translocator protein in the brain tissue following traumatic brain injury

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Abstract

Background

The study aimed to measure the expression of translocator protein (TSPO) in brain tissue following traumatic brain injury (TBI) and to determine whether TSPO can predict outcomes.

Methods

TBI patients requiring emergent craniectomy and removing of intracranial hematoma were recruited from Wujin Hospital Affiliated with Jiangsu University between January 2018 and May 2020. TBI patients were divided into unfavorable and favorable groups according to GOS score. The TSPO in brain samples was analyzed by western blot and immunocytochemistry.

Results

The western blot and immunocytochemistry showed that the TSPO in the unfavorable group was higher than that in the favorable group. Double immunofluorescence staining exhibited that the percentage of TSPO positive cells in IBA1 and GFAP positive cells was 45.2 ± 3.1% and 3.5 ± 0.6% respectively. After adjusting for age, sex, CT, ICP and GCS, we found each 1-unit increase in TSPO was associated with 40% higher occurrence of unfavorable outcome (OR = 1.4, 95% CI 0.4–5.6). The area under the receiver operating characteristic curve (AUC), specificity, and sensitivity of TSPO was 0.87, 76.7%, 88.2% respectively.

Conclusion

Our study demonstrated that higher TSPO was associated with higher occurrence of unfavorable outcomes.

Introduction

Traumatic brain injury (TBI) remains one of the most complex diseases and the primary reason for mortality in persons below 45 years worldwide[6,8,25]. Consequently, a large number of studies focused on the mechanism of TBI. Previous studies showed that oxidative stress, neuroinflammatory response, apoptosis, and inflammatory activities may play important roles in the secondary injury of TBI. Several biomarkers, including inflammatory and apoptosis factors, in blood samples and cerebrospinal fluid (CSF) were proved associated with the mortality in TBI patients[5,8,9,11,12]. With the progress of the TBI mechanism study, more novel and reliable markers for predicting of the outcome of TBI were excavated.

The expression of translocator protein 18 kDa (TSPO) is very low in the brain and restricted to glial cells under normal physiological status. Previous studies demonstrated that the TSPO expression was linked with brain injury, neuroinflammation and neurodegenerative diseases[3,20]. Multiple researchers applied TSPO as a marker of brain injury using Positron Emission Tomography (PET) in experimental animals and humans[18,21,24]. Recently, it was reported that the serum TSPO was associated with outcome of patients with traumatic brain injury and acute ischemic stroke and the use of TSPO-specific ligands may have therapeutic implications in brain injury[3,13].

However, there was little study focusing on the expression of TSPO in TBI patients' brain tissue. The aim of this study was to measure the expression of TSPO in TBI patients' brain tissue using western blot and immunofluorescence and to determine whether TSPO can predict outcomes of TBI.

Materials And Methods

Subject

Patients with TBI were recruited at the Department of Neurosurgery, Wujin Hospital Affiliated with Jiangsu University between January 2018 and May 2020. The diagnosis of TBI required a clear history of injuries and positive cranial CT scan. The patients received medical treatment by the Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition (Neurosurgery. 2016 Sep 20)[2]. Patients firstly underwent implantation of an intraparenchymal ICP monitor in the operation room before any further surgical intervention. For all TBI subjects, inclusion criteria were: (1) patient age between 16 and 70 years, (2) emergent craniectomy and removed of intracranial hematoma were undergone and brain tissues were achieved, (3) no current or history of neurologic disease, or bleeding disorder, (4) signed consent from next-of-kin.

As control, the normal brain tissues were achieved around the operation channel when biopsy surgeries were undergone for the cerebral tumors. For all control subjects, inclusion criteria were: (1) patient age between 16 and 70 years, (2) no current or history of brain injury, neurologic disease, or bleeding disorder, (3) signed consent from next-of-kin or themselves.

Ultimately, 60 patients with TBI and 15 controls were included. The study was approved by the ethical committee of the Wujin Hospital Affiliated with Jiangsu University. A written informed consent was obtained from each participant or their relations.

Demographic and Clinical Injury Variables

Independent variables included sex, age, initial GCS, ICP and the initial CT classification. The duration from trauma occurring to surgery was calculated and defined as time to operating room. The GCS was taken within 8 hours of injury to limit the influence of alcohol, sedatives, or paralytics. The level of brain injury was determined according to the Marshall classification on initial CT findings.

Outcome variables

The GOS scores of subjects were assessed at 6 months after injury. Patients were assigned good recovery (5), moderate disability (4), severe disability (3), persistent vegetative state (2), or death (1). For this study, all TBI patients were divided into unfavorable outcome group (1/2/3) and favorable outcome group (4/5).

Samples

Brain samples were obtained from TBI patients requiring emergency craniotomy for mass effect in our hospital. Brain samples were obtained from surgically resected areas of contusions. As control, the normal brain samples were achieved around the operation channel when biopsy surgery were underwent. All the brain samples were stored in liquid nitrogen.

Western Blot

The TSPO in brain samples was detected using the Western Blot. 20μg samples were homogenized in a chilled lysis buffer and centrifuged at 4500 × g for 20 minutes. The supernatant was collected to analyze proteins using the Bio-Rad protein assay (Bio-Rad). Using a wet electro-blotting system, we transferred the separated proteins to PVDF membrane (Millipore Corporation, Billerica, MA, USA) for 2 hours, then blocked in TBST (50 mM Tris–HCl, 0.1%Tween 20, 154 mM NaCl, pH = 7.3) for 2 hours. The primary antibodies (rabbit anti-PBR, 1:1000, ab109497, Abcam) were used to incubate samples overnight in TBST. The secondary antibodies (goat anti-rabbit or donkey anti-goat coupled to horseradish peroxidase, 1:3000 dilution) were used to incubate the PVDF membranes for 2 hours at room temperature. The immunoreactive bands were visualized by an ECL kit (Thermo, USA). The β-actin was used to normalize all cytosol protein bands. Immunoblots were scanned by the densitometer and the gray value was analyzed by Quantity One software (BioRad, USA)[10].

Immunocytochemistry

The samples were soaked in 4% paraformaldehyde and 30% sucrose for 24 hours respectively. The samples were frozen and 10 μm thick serial coronal sections were cut using a cryostat. Tissue sections were placed in phosphate buffered saline (0.01 M PBS) for 30 minutes, blocked by 5% normal goat serum, then placed in 0.2% TritonX-100 for 90 minutes. The rabbit anti-PBR (ab109497, Abcam) at 1:100 was used to incubate the sections over two nights at 4°C. After the primary antibody incubation, the tissue sections were washed three times in 0.01 M PBS. Double immunostaining was performed by means of incubating with a mixture of anti-PBR and goat anti-IBA1 polyclonal antibody (Abcam, ab107159, 1:100) or rabbit anti-GFAP polyclonal antibody (Sigma, G9269, 1:1000) overnight at 4°C. All the above sections were treated by a mixture of FITC- and Cy3-conjugated secondary antibodies for 1 hour at room temperature. The sections were rinsed by the 0.01 M PBS for three times, then mounted on a gelatin-coated slide and dried in air. Images of the stained sections were captured by the fluorescence microscope attached to a CCD spot camera (LEICA DFC350FX/DMIRB, Germany). Then, the images were processed with LEICA IM50 software (Germany)[10].

Quantification and Statistical analysis

The percentage of positive area of brain samples were measured using LEICA Qwin V3 digital image processing system (Germany). An average percentage of area of TSPO-IR relative to the total area of the sections was obtained for each sample.

Those measurement data are expressed as mean ± SD, and those enumeration data are expressed as percentage. Differences between the groups are statistically analyzed using the t-test or the $\chi 2$ test depending on different variables. A univariate analysis was used to determine the significance of the association between TSPO and the outcome as well as the other independent variables. Multivariate logistic regression was employed to determine the independent association between TSPO and the outcome of TBI. The results were shown as odds ratios (ORs), 95% confidence intervals (95% CI) and p value. A receiver operating characteristic curve (ROC) analysis and area under the ROC curve (AUC) were applied to evaluate the reliability of TSPO for predicting patient outcomes. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. The p value less than 0.05 is regarded as statistically significant.

Results

Clinical Characteristics of Patients with TBI

Ultimately, 60 TBI patients were recruited into our study. According to the outcome of patients, 43 and 17 cases were included into favorable and unfavorable outcome group respectively. There was no significant difference between two groups in age, sex, and initial GCS ($p > 0.05$). While, the ICP and time to operating room was higher in unfavorable outcome group than that in favorable outcome group ($p < 0.05$). There was significant difference in the Marshall CT classification between two groups. The cases with IV, V and VI classification in the unfavorable outcome group were more than that in favorable outcome group, which implied that the brain injury in the unfavorable outcome group was more serious ($p < 0.05$) (Table 1).

Table 1
Clinical Summary of Patients' Data

Items	Favorable outcome group(N = 43)	Unfavorable outcome group(N = 17)	P
Age (year)	35.35 ± 16.64	26.82 ± 9.24	0.076
Sex			0.567
Male	25 (58.14%)	8 (47.06%)	
Female	18 (41.86%)	9 (52.94%)	
CT			< 0.001
I	0	0	
II	19 (44.19%)	1 (5.88%)	
III	17 (39.53%)	2 (11.76%)	
IV	6 (13.95%)	6 (35.29%)	
V	0 (0.00%)	5 (29.41%)	
VI	1 (2.33%)	3 (17.65%)	
ICP (mmHg)	19.42 ± 7.26	28.53 ± 5.94	< 0.001
TSPO	2.59 ± 0.97	4.12 ± 0.94	< 0.001
Time to operating room	2.0 ± 0.9	3.4 ± 1.2	< 0.001
GCS			0.170
<=8	37 (86.05%)	17 (100.00%)	
> 8	6 (13.95%)	0 (0.00%)	
ICP: intracranial pressure; TSPO: translocator protein; GCS: Glasgow Coma Score			

Expression of TSPO

The western blot showed that the relative TSPO protein in the unfavorable outcome group was 4.12 ± 0.94, which was higher than that in the favorable outcome group (2.59 ± 0.97) ($p < 0.05$). The immunocytochemistry showed that the percentage of TSPO positive cells in unfavorable outcome group was 41.3 ± 9.8%, which was higher than that in the favorable outcome group (13.5 ± 4.5) ($p < 0.05$) (Table 1, Fig. 1). Double immunofluorescence staining exhibited that TSPO co-localized with IBA1 and GFAP. Moreover, the percentage of TSPO positive cells in IBA1 and GFAP positive cells was 45.2 ± 3.1%

and 3.5 ± 0.6% respectively, which meant the TSPO expressed mainly in microglia and macrophages (Fig. 2)

Relationship between TSPO and outcome

Univariate regression analysis was performed to determine the relationships between clinical parameters and outcome. As shown in Table 2, we observed a significant correlation between CT classification, ICP, TSPO, time to operating room and the outcome of TBI ($P < 0.05$). The odds ratio (OR) of TSPO for outcome were 3.9, which meant, with increasing of TSPO, the rate of unfavorable outcome increased (Table 2).

Table 2
Univariate analysis for outcome

Items	OR (95%CI)	p
Age (year)	1.0 (0.9, 1.0)	0.063
sex		
Male	Reference	
Female	1.6 (0.5, 4.8)	0.438
CT		
II	Reference	
III	2.2 (0.2, 26.9)	0.526
IV	19.0 (1.9, 190.9)	0.012
V	inf. (0.0, Inf)	0.991
VI	57.0 (2.8, 1176.8)	0.009
ICP (mmHg)	1.2 (1.1, 1.3)	< 0.001
TSPO	3.9 (2.0, 7.8)	< 0.001
Time to operating room	1.8 (1.2, 2.7)	0.002
GCS		
<=8	Reference	
> 8	0.0 (0.0, Inf)	0.992
Abbreviations: CI, confidence interval. OR, odds ratio.		

Multivariate logistic regression was employed to determine the independent association between TSPO and the outcome of TBI. In the model I (adjusting for age and sex), we found each 1-unit increase in

TSPO was associated with 4 times higher occurrence of unfavorable outcome with adjusting for age and sex (OR = 5.0, 95% CI 2.1–11.7; P < 0.001). We also converted TSPO from a continuous variable to a binary categorical variable depending on the mean level of TSPO. Compared to TSPO level < 3.0, TSPO level > = 3.0 was associated with higher occurrence of unfavorable outcome (OR = 26.2, 95% CI 4.6-150.2, p < 0.001). In fully adjusted model (model II, adjusting for age; sex, CT, ICP, time to operating room and GCS), we found each 1-unit increase in TSPO was associated with 40% higher occurrence of unfavorable outcome (OR = 1.4, 95% CI 0.4–5.6, p = 0.019). Compared to TSPO level < 3.0, TSPO level > = 3.0 was associated with higher occurrence of unfavorable outcome (OR = 2.4, 95% CI 0.2–28.0, p = 0.047) (Table 3).

Table 3
Relationship between TSPO and outcome

Outcome	Crude model		Model I		Model II	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
TSPO	3.9 (2.0, 7.8)	< 0.001	5.0 (2.1, 11.7)	< 0.001	1.4 (0.4, 5.6)	0.019
TSPO group						
< 3.0	Reference		Reference		Reference	
>=3.0	19.4 (3.8, 97.8)	< 0.001	26.2 (4.6, 150.2)	< 0.001	2.4 (0.2, 28.0)	0.047
Crude model adjust for: None						
Model I adjust for: age; sex						
Model II adjust for: age; sex; CT; ICP; operation duration; GCS						

In order to determine whether TSPO is reliable biomarkers for the prognosis of TBI, a receiver operating characteristic curve (ROC) analysis was applied. The results showed that the AUC, specificity, and sensitivity of TSPO was 0.87, 76.7%, 88.2% respectively, which indicated that TSPO was a reliable biomarker for the prognosis of TBI (Table 4) (Fig. 3).

Table 4
ROC analysis results

Items	AUC	P	Specificity (%)	Sensitivity (%)
TSPO	0.87	< 0.001	76.7	88.2

Discussion

The TBI is one of the main causes of disability and mortality. The mechanism of TBI was complicated because the it is heterogeneous. With the progress of TBI research, it is demonstrated that the pathophysiology of TBI is associated with not only the primary injury, but also the secondary injury caused by neuroinflammatory response, apoptosis, and development of edema[8,14,17,27]. Consequently, it is difficult to predict the prognosis of TBI. Previous studies showed there were differences of the ICP, GCS, and CT classification in TBI cases with different outcome[1,22]. In our study, we also observed a significant correlation between CT classification, ICP, time to operating room and the TBI outcome by univariate regression analysis. While, as previously proved, the factors above were not reliable enough to predict the outcome of TBI [1].

There are increasing studies showing that several biomarkers may predict the outcome of TBI. In the latest decades, several studies proved a series of biomarkers, including phospho-Tau, GFAP, S100B, NSE, which represent post-injury neurodegeneration, astroglial injury or neuronal cell body injury[8,17,27]. While, it was a slow process to validate of the prognostic utilities of biomarker. Consequently, it is necessary to discover more biomarker for predicting the outcome. Recently, it was reported that the serum TSPO was associated with outcome of patients with TBI and acute ischemic stroke[4,13]. However, there was little study focusing on the expression of TSPO in TBI patients' brain tissue. In our study, we found the expression of TSPO in the unfavorable outcome group was higher than that in the favorable outcome group. Double immunofluorescence staining exhibited that the TSPO expressed mainly in microglia, which was consistent with previous studies. The results above implied that the TSPO may be a biomarker for the prognosis of TBI.

The TSPO takes part in multifarious cellular functions, including mitochondrial respiration, apoptosis, and cell proliferation[3,15]. The positron emission tomography (PET) has been used to exhibit TSPO expression in the animal and human brain. As a result, the PET showed that TSPO expression was low in the healthy brain and upregulated in the injury brain. The increased expression of TSPO in microglia demonstrated that TSPO may be a factor of influencing the progression of TBI [16,28]. Previous studies showed that the TSPO ligands, PK-11195 and Ro5-4864, are important to TBI treatment through anti-apoptotic[7,19,23,26]. Soustiel et al. recently showed that the Ro5-4864 increased the number of surviving neurons significantly by reducing activation of caspase 3 in TBI rat models[19,23]. Previous studies implied that the TSPO played an important role in TBI and may be a biomarker for the outcome. In our study, we found each 1-unit increase in TSPO was associated with 40% higher occurrence of unfavorable outcome after adjusting all the confounding factors such as initial GCS, ICP, time to operating room and Marshall CT classification. Moreover, the ROC also proved that TSPO was a reliable biomarker for the prognosis of TBI.

The results of the present study are only preliminary. The main limitation of this study is its small sample size, consequently the predictive effect was susceptible to bias. Secondly, we just gathered the brain samples in serious TBI cases who needed surgery treatment, which may affect the prognosis assessment. Therefore, in further studies, multiple brain samples should be collected to check the role of TSPO in predicting the prognosis of TBI. Moreover, as previous studies showed, multitudinous factors

influenced the outcome of TBI. Consequently, in the new biomarker for prediction of the outcome of TBI, we would adjust more variables to improve the reliability.

Conclusion

In our study, we established models for predicting the TBI outcome and found higher TSPO was associated with higher occurrence of unfavorable outcome. Moreover, the ROC also proved that TSPO was a reliable biomarker for the prognosis of TBI.

Declarations

Acknowledgements

None

Statement of Ethics

This clinical study followed the Declaration of Helsinki and was approved by the Medical Ethics Committee of Wujin Hospital Affiliated with Jiangsu University (NO. 2018-07). A written informed consent was obtained from each participant or their relations.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author contributions

Wenqing Jiang conducted the study and drafted the manuscript. Peng Jin, Wenfeng Wei Qing Bao, Xuesong Yuan and Xiaoxing Bian participated in the design of the study and performed statistical analyses. All the authors read and approved the final manuscript.

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Figures

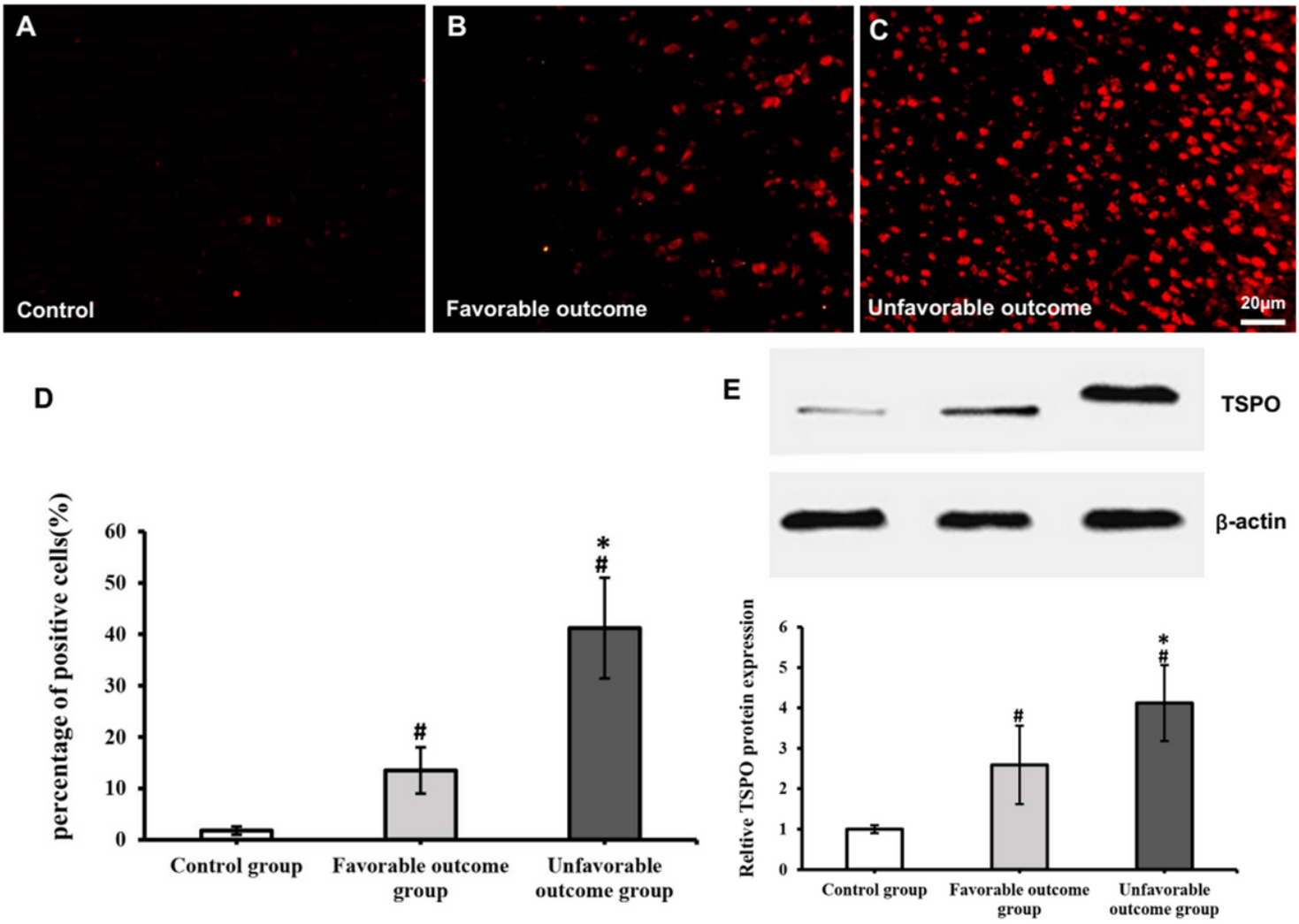

Figure 1

The expression of TSPO in the brain tissue The western blot showed that the relative TSPO protein in the unfavorable outcome group was 4.12 ± 0.94, which was higher than that in the favorable outcome group (2.59 ± 0.97). The immunocytochemistry showed that the percentage of TSPO positive cells in unfavorable outcome group was 41.3 ± 9.8%, which was higher than that in the favorable outcome group (13.5 ± 4.5). #p<0.05 versus favorable outcome group. *p<0.05 vs. control group.

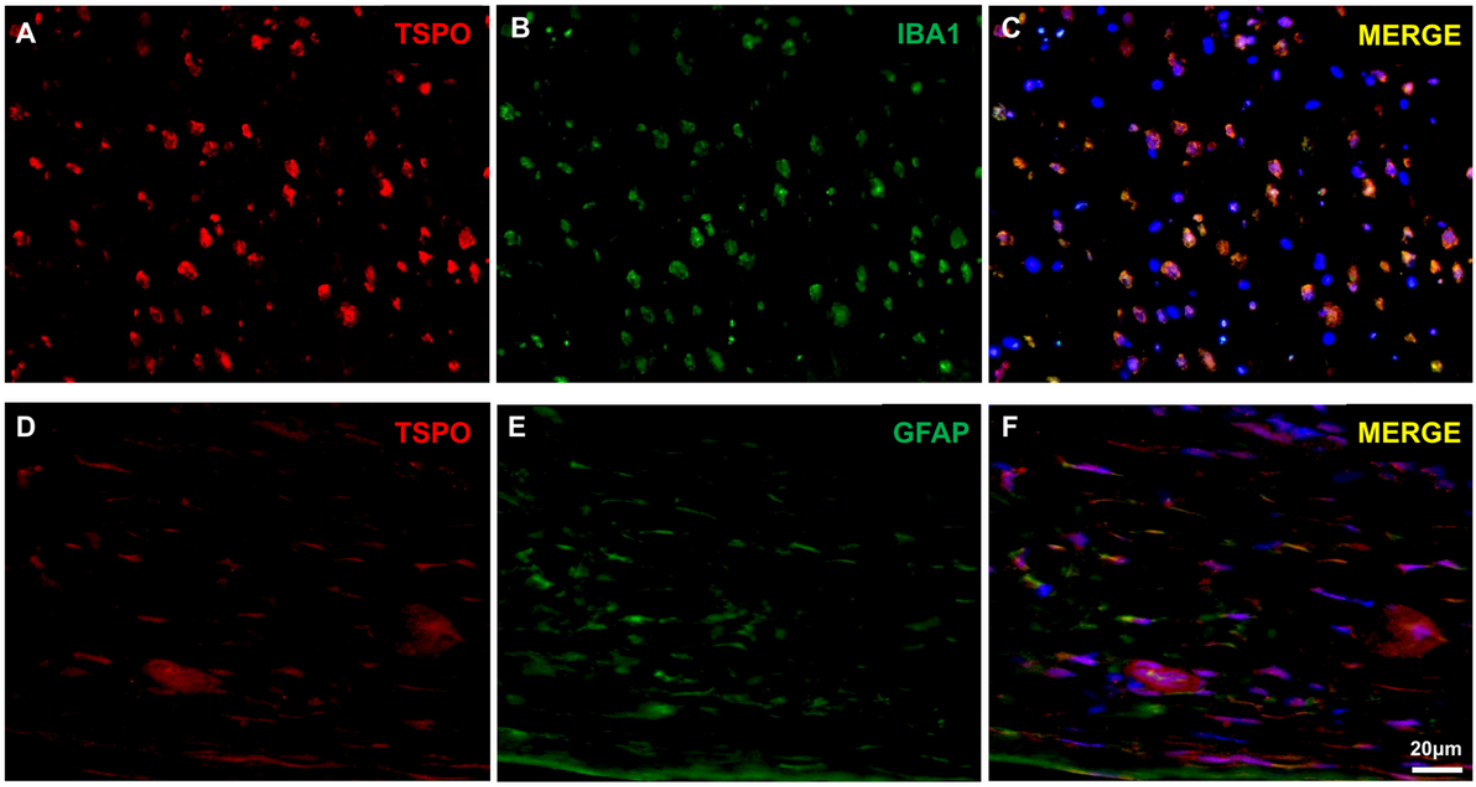

Figure 2

Double immunofluorescence staining in the brain tissue Double immunofluorescence staining exhibited that TSPO co-localized with IBA1 and GFAP. Moreover, the percentage of TSPO positive cells in IBA1 and GFAP positive cells was 45.2± 3.1% and 3.5±0.6% respectively, which meant the TSPO expressed mainly in microglia.

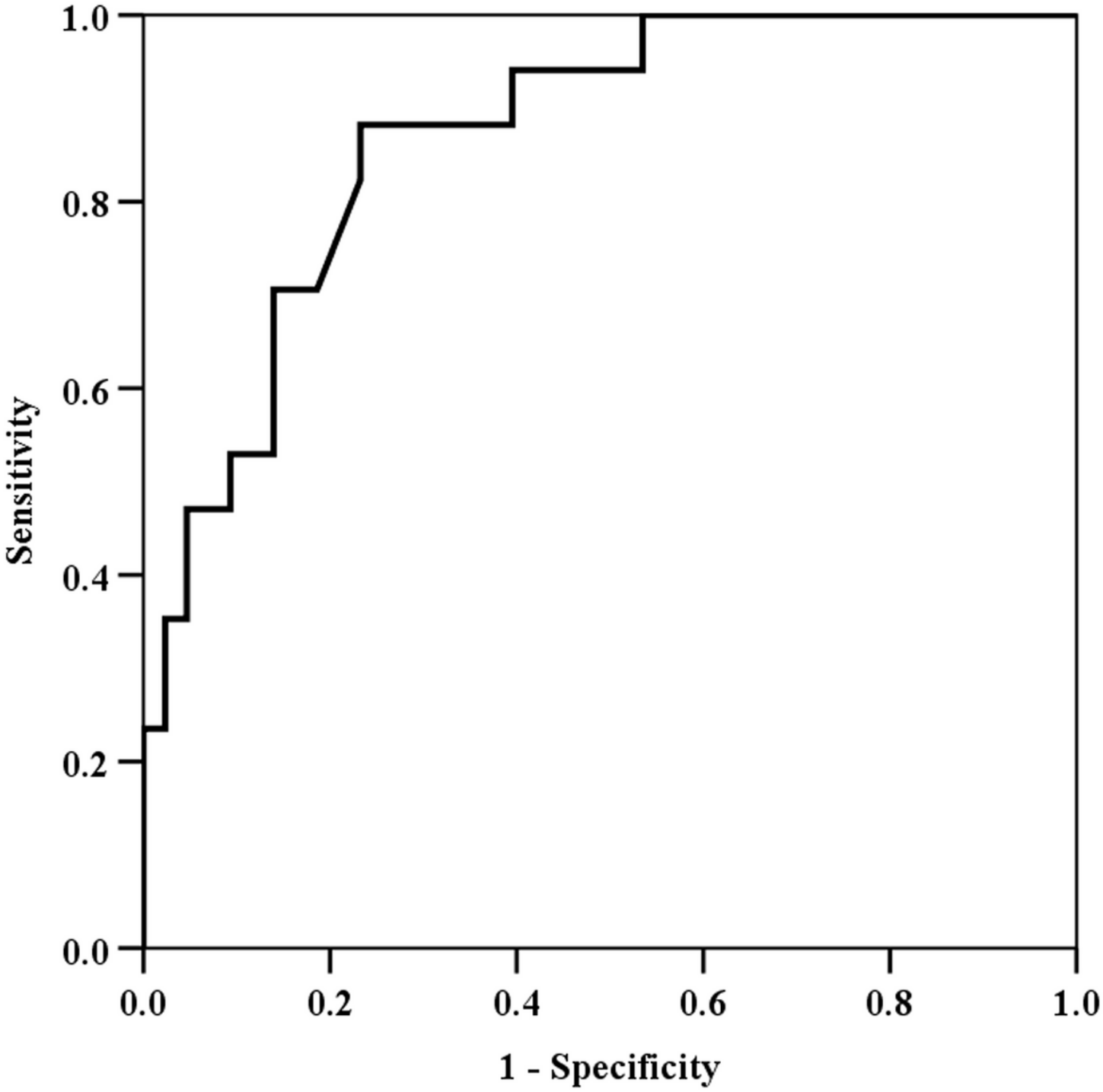

Figure 3

The ROC analysis of TSPO for the prognosis of TBI The ROC analysis showed that the AUC, specificity, and sensitivity of TSPO was 0.87, 76.7%, 88.2%.