Observational cohort study of blood transcriptomics and proteomics information as biomarkers of traumatic encephalopathy syndrome

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Organization Committee

Tianjin Medical University General hospital

Contents

Protocol Abstract

Title	Observational cohort study of blood transcriptomics and proteomics information as biomarkers of traumatic encephalopathy syndrome
Ethics Committee (Grant Number)	The ethics committee of Tianjin Medical University General Hospital (IRB2021-YX-056-01)
Principal Investigator	Ping Lei (Ph.D.), Xintong Ge (M.D.)
Sponsor	Tianjin Medical University General Hospital
Fundings	Natural Science Foundation of China (Grant No. 82071394, 82072166)
Execute Time	05/01/2021-11/01/2022
Recruiting Time	05/01/2021-11/01/2022
Objective	Create a novel set of CTE/TES molecular diagnostic signatures to open a new avenue of clinical diagnosis of the disease and future research on the therapeutic strategy
Study Type	Observational
Enrollment	120 participants
Biospecimen	Description: Serum Retention: Samples with DNA
Sampling Method	Non-probability sample (invitation to volunteer)
Condition	Chronic Traumatic Encephalopathy (CTE) / Traumatic Encephalopathy Syndrome (TES)
Study Groups/Cohorts	1) 50 active or retired athletes from the Weightlifting, Wrestling, Judo, Boxing and Taekwondo Sports Management Center of Tianjin Sports Bureau. 2) 50 patients with multiple (≥2 times) exposure to brain trauma attending Tianjin Medical Insurance designated hospitals such as Tianjin Medical University General Hospital. 3) 20 healthy volunteers.
Screening Criteria	1. Athletes and patients with traumatic brain injury 1) Age ≥ 18 and ≤ 80 years old with independent behavior ability or authorized legal representative. 2) Have a clear history of repetitive mild TBI, concussion or subconcussion. 3) The most recent head injury occurred 3 months ago. 2. Healthy Volunteers 1) Age ≥ 18 and ≤ 80 years old with independent behavior ability. 2) No history of repetitive mild TBI, concussion or subconcussion.

	3) Fully understands the nature of the study, and voluntarily participates and signs the informed consent.
Inclusion Criteria	On the basis of meeting the screening criteria, the participants need to fully understand the nature of the study, and sign the informed consent form.
Exclusion Criteria	1. Athletes and patients with traumatic brain injury 1) Pregnant or lactating women. 2) History of other neurological diseases. 3) History of tumors, hematological diseases, severe cardiopulmonary diseases, hepatic failure or renal failure. 4) Have participated in clinical trials in the past four weeks. 5) The investigator believes that not appropriate for inclusion. 2. Healthy Volunteers 1) Pregnant or lactating women. 2) History of TBI or other neurological diseases. 3) History of tumors, hematological diseases, severe cardiopulmonary diseases, hepatic failure or renal failure. 4) Have participated in clinical trials in the past four weeks. 5) The investigator believe that not appropriate for inclusion.
Outcome Measures	1. Blood tests (30-ml venous blood) for the following items. 1) Transcriptomics and proteomics high-throughput detection and quantitative verification; 2) Exosomal transcriptomics and proteomics high-throughput detection and quantitative verification; 3) Quantitative detection for classical biomarkers of traumatic brain injury, including S100B, GFAP, UCH-L1, NFL, T-Tau and p-Tau181. 2. Cognitive function tests, including RPQ, MMSE and MoCA. 3. Possible head MRI (plain scan and DTI sequence) examination and head PET (FDG-PET, Tau-PET and Amyloid-PET) examination
Reference standard	Clinical Approach to the Diagnosis of TES (Reams N., JAMA Neurology, 2016)
Statistical Analysis	All statistical analyses were performed using Stata/MP Version 14.0 (Stata Corp., College Station, TX, USA) and PASW Stastics Version 18.0 (IBM, Armonk, NY, USA). A two-tailed p value of less than 0.05 was considered to be statistically significant.

1. Background and objective of the study

Chronic traumatic encephalopathy (CTE) is a distinct neurodegenerative disease associated with traumatic brain injury (TBI). It is characterized by the clinical manifestations of progressive cognitive and neurological dysfunction, and the pathological changes of abnormal deposition of phosphorylated tau protein in neurons, astroglia and synapses around small blood vessels at the depths of cortical sulci. The occurrence of the disease is closely related to a history of repetitive mild TBI, concussion and sub-concussion, so that it is susceptible in boxers and other contact sports athletes, military veterans, elderly people with mobility impairments and victims of domestic violence.

The clinical symptoms of CTE mainly include cognitive decline, behavioral change, emotional dysregulation and motor disturbance. The cognitive decline typically affects more than one domain of neurological deficits including executive, visuospatial, memory and language. Patients with mainly behavioral change may exhibit violence, poor impulse control, socially inappropriate behavior, avolition, apathy, change in personality and comorbid substance abuse. The emotional dysregulation includes depression, anxiety, agitation, aggression, paranoid ideation, deterioration of interpersonal relationships and suicidality, while the motor disturbance contains bradykinesia, tremor, rigidity, gait instability, dysarthria, dysphagia, ataxia and gaze disturbance. From this, **CTE can seriously influence the quality of life. Consequently, early diagnosis and treatment for the disease has important clinical significance.**

The traditional diagnostic criteria of CTE are based on neuropathology, which causes most patients to be diagnosed only by autopsy. In order to develop a clinical diagnostic criterion of CTE, so that the patients could receive effective treatment in time, the concept of Traumatic Encephalopathy Syndrome (TES) was proposed. Specifically, potential patients with CTE are divided into three categories: probable TES, possible TES and unlikely TES according to the likelihood of illness. The classification is based on the number of head traumas, clinical features and progressive courses. **This diagnostic framework of TES provides a practical approach for clinical evaluation of CTE.**

However, the diagnostic criteria of CTE and TES that are based solely on clinical symptoms still lack sufficient specificity and sensitivity, while biomarkers could be an important aid to fill the deficiency and further improve diagnosis level of the disease. Blood biomarkers have always been a research hotspot in the field of neurotrauma due to their easy access to specimens and convenience

for detection. In recent years, many researches have shown that protein biomarkers including S100B, GFAP, UCH-L1, NFL, T-Tau and p-Tau181 could have diagnostic value in mild TBI and cognitive dysfunction of chronic TBI. However, these researches are not special study on CTE and TES, and are limited by the research design (such as small sample size without high-throughput screening). In addition, because of the incompatibility (low sensitivity or reproducibility) of clinical examination platform, only S100B (with a dedicated Elecsys-Roche testing platform) from the above biomarkers has been applied in clinical auxiliary diagnosis of mild TBI till now. Therefore, **further exploration of new biomarkers for CTE/TES that can contribute to clinical diagnosis has great application value.**

Recently, with the development of molecular biology and bioinformatics, the potential value of transcriptomic information (including mRNA, miRNA, lncRNA, circRNA, etc.) as disease biomarkers has aroused wide attention. For clinical blood samples, the RNA detection method (RT-PCR) is mature with the advantage of high specificity and reproducibility. It is a same commonly used clinical testing technique as protein detection. Exosomes are small vesicles secreted by living cells into the extracellular space that function as important carriers of information transferred among cells. They contain a large number of specific proteins and functional nucleic acids, participate in many pathophysiological processes, and correlated with the occurrence and progression of many diseases. Because the membrane structure of exosomes can protect their internal RNA from RNase degradation, exosomal RNA is more stable and has higher concentration than free RNA in serum or plasma. In addition, in view of the maturity of the isolation and purification technology for exosomes (a large number of assay kits have been used in clinical examination and scientific research), exosomal protein and RNA have been regarded as an important source of clinical biomarkers. Consequently, **exploring exosomal diagnostic biomarkers for CTE/TES would be a breakthrough point to solve the problem of early diagnosis of the disease.**

In this study, we will use high-throughput screening and multi-omics (transcriptomics and proteomics) joint analysis technology to explore potential CTE/TES biomarkers (RNA and protein) in blood and its exosomes. We will also combine them with the reported TBI biomarkers to **create a novel set of CTE/TES molecular diagnostic signatures, in order to open a new avenue of the clinical diagnosis of the disease and the future research on its therapeutic strategy.**

2. Study design

2.1 Overall design

This project is an observational cohort study.

2.2 Estimated number of subjects

Estimated by statistical analysis, 70 subjects are expected to take part in this study, including 30 active or retired athletes from the Weightlifting, Wrestling, Judo, Boxing and Taekwondo Sports Management Center of Tianjin Sports Bureau, 30 patients with multiple (≥2 times) exposure to brain trauma attending Tianjin Medical Insurance designated hospitals such as Tianjin Medical University General Hospital, and 10 healthy volunteers.

3. Screening, Inclusion, Exclusion and Withdrawal Criteria

3.1 Athletes and Patients with TBI

Screening Criteria

1) Age ⩾ 18 and ⩽ 80 years old with independent behavior ability or authorized legal representative.

2) Have a clear history of repetitive mild TBI, concussion or subconcussion.

3) The most recent head injury occurred 3 months ago.

Inclusion Criteria

On the basis of meeting the screening criteria, the participants need to fully understand the nature of the study, and voluntarily participate in the study and sign the informed consent form.

Exclusion Criteria

1) Pregnant or lactating women.

2) History of other neurological diseases.

3) History of tumors, hematological diseases, severe cardiopulmonary diseases, hepatic failure or renal failure.

4) Have participated in clinical trials in the past four weeks.

5) The investigator believe that not appropriate for inclusion.

Withdrawal criteria

The subject is considered as withdrawal if he/she could not complete the trial for following reasons:

1) The subject decides to withdraw from the study.

2) Lost to follow-up.

3) The doctor or the researcher asks the subject to withdraw from the study (e.g. poor compliance).

3.2 Healthy Volunteers

Screening Criteria

1) Age ⩾ 18 and ⩽ 80 years old with independent behavior ability.

2) No history of repetitive mild TBI, concussion or subconcussion.

3) Fully understands the nature of the study, and voluntarily participates and signs the informed consent.

Inclusion Criteria

On the basis of meeting the screening criteria, the healthy volunteers need to fully understand the nature of the study, and sign the informed consent form.

Exclusion Criteria

1) Pregnant or lactating women.

2) History of TBI or other neurological diseases.

3) History of tumors, hematological diseases, severe cardiopulmonary diseases, hepatic failure or renal failure.

4) Have participated in clinical trials in the past four weeks.

5) The investigator believes that not appropriate for inclusion.

Withdrawal criteria

The subject is considered as withdrawal if he/she could not complete the trial for following reasons:

1) The subject decides to withdraw from the study.

2) Lost to follow-up.

3) The doctor or the researcher asks the subject to withdraw from the study (e.g. poor compliance).

4. Study procedure

Researchers initiate the study according to enrolled order after verification of the inclusion/exclusion criteria and collection of the informed consent.

4.1 Screening assessment

Researchers should clearly evaluate each inclusion and exclusion criteria for the subjects. The following works need to be done during screening:

1) Filling general information, such as date of birth, gender, occupation, et al.

2) Medical history collection: History of head injuries (number, degree, time, et al.), past medical history, et al.

3) Basic physical examination.

4.2 Included subjects

1) Signing the informed consent form.

2) Blood test

- Transcriptomics and proteomics high-throughput detection and quantitative verification;
- Exosomal transcriptomics and proteomics high-throughput detection and quantitative verification;
- Quantitative detection for classical biomarkers of traumatic brain injury, including S100B, GFAP, UCH-L1, NFL, T-Tau and p-Tau181.

3) Cognitive function tests, including RPQ, MMSE and MoCA.

4) Neuro-imaging examinations (if eligible): head MRI (plain scan and DTI sequence) and head PET (FDG-PET, Tau-PET and Amyloid-PET) examination

5. Evaluation Parameters

5.1 Primary outcome parameters

1) Blood level of novel protein biomarkers for CTE/TES screened out by multi-omics high-throughput detection, and further verified using ELISA assay.

2) Blood level of novel RNA biomarkers for CTE/TES screened out by multi-omics high-throughput detection, and further verified using RT-PCR.

5.2 Secondary outcome parameters

1) Blood level of classical biomarkers for CTE/TES, including S100B, GFAP, UCH-L1, NFL, T-Tau and p-Tau181 detected by ELISA assay.

2) Cognitive function tests, including RPQ, MMSE and MoCA.

3) Head MRI (plain scan and DTI sequence) examination using the Discovery MR750 3.0T system (GE Healthcare, Chicago, IL, USA)

4) PET examination using the Discovery 710 PET-CT system (GE Healthcare). The FDG PET ([18F]-FDG) scan, tau PET scan ([18F]-T807) and β-amyloid PET scan ([18F]-florbetapir) will be arranged on consecutive days.

6. Withdrawal of the subject

The subject has the right to withdraw from this trial at any time for any reasons. The researcher should have a thorough understanding on the reasons for withdrawal. Doctors and researchers also have the right to stop the subject from continuing to participate in the trial at any time during the research. In addition, subjects with early withdrawal should not be replaced by others.

As too many subjects withdrawing from the trial will lead to unreliable results, unnecessary withdrawal should be avoided. The falling rate of the trial should be controlled within 20%.

7. Trial Management

7.1 Ethics

To obtain the approval documents of the clinical trial, researchers should submit the trial protocol and a copy of the research documents including the informed consent form to the ethics committee.

The approval documents from the ethics committee should be accompanied by the name list of ethics committee members and their respective responsibilities. These documents will be delivered to the researchers in written form before the start of the study. In addition, a copy of the approval documents needs to be provided to the sponsor by the researchers.

Any safety-related issues must be promptly reported to the ethics committee, which includes revision on the trial protocol and modification on the subject information page. The end or early termination of the trial should also be reported.

7.2 Informed consent and data protection agreement

It is the responsibility of the researchers to explain the objectives, study procedures, benefits and potential risks of the trial for each subject. The researchers should also receive the informed

consent in written form signed by the subject before starting the trial. For the subject who can not sign the informed consent for any reason, the informed consent form should be signed by their parents, legal guardians or protectors. The subject should also permit researchers and health survey organizations to check his or her original data in the trial. Thus, the reliability of the research findings could be ensured.

The information of each subject, such as address and telephone number, should be collected in detail. And the researcher/doctor should give the subject his or her phone number, so that the patient can contact with him or her when needed. This is also helpful for the research and medical care.

The informed consent form with the signature and the date should be kept properly by the researchers.

7.3 Withdraw from the trial

The researchers have the right to ask the subject to withdraw from the clinical trial. But whenever the subject withdraws, the researcher should explain and record the reasons.

7.4 Subjects privacy

The researchers should protect the privacy of the subject. All documents submitted to the sponsor can only be identified using the subject's number instead of the name or admission number. In addition, the grouped table that records the correspondence between the subject's name and number can only be kept by the researcher, and could not be submitted to the sponsor

7.5 Modification of the trial protocol

The trial protocol is determined by researchers and the sponsor together, and approved by the ethics committee before starting the trial. During the research, any proposed modification on the trial protocol should be submitted to the principal investigator and the sponsor. The revised proposal will be then reviewed, and submitted to the ethics committee. The clinical research can only be resumed after the re-approval of the ethics committee.

7.6 Original records certification

Researchers should ensure that the subject's privacy is protected when collecting and organizing data. In addition, the data manager is authorized to review the original records in order to confirm the accuracy of the original data and to know the real-time progress of the trial.

7.7 Documents on file

According to relevant laws and regulations, researchers should keep the original records properly. All research duplicates should be retained for at least 5 years from the end of the study.

7.8 Quality control

The sponsor and the relevant medical administration has the authority to review the study procedure, in order to ensure that the trial is carried out as predetermined and that the research data be veritably recorded.

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