Relationship between acute lung injury and pulmonary hyaluronic acid deposition after subarachnoid hemorrhage

Version: 1.0

Date: September 30th, 2024

Ethics Committee

Tianjin Medical University General hospital

Protocol Abstract

Title	Relationship between acute lung injury and pulmonary hyaluronic acid deposition after subarachnoid hemorrhage
Ethics Committee (Grant Number)	The ethics committee of Tianjin Medical University General Hospital (IRB2024-YX-425-01)
Project Executor	Xintong Ge (M.D. & Ph.D.)
Sponsor	Tianjin Medical University General Hospital
Participant	Tianjin Huanhu Hospital
Principal Investigator	Ye Tian (Ph.D.) - Tianjin Medical University General Hospital Yadan Li (M.D.) - Tianjin Huanhu Hospital
Fundings	High-Level Talent Project of Tianjin Health Research Program (Grant No. TJWJ2024RC002)
Execute Time	10/01/2024-09/30/2025
Recruiting Time	10/01/2024-09/30/2025
Objective	Clarify the correlation between pulmonary inflammatory injury and hyaluoronic acid deposition after subarachnoid hemorrhage
Study Type	Observational
Time Perspective	Prospective
Enrollment	24 participants
Biospecimen	Description: bronchoalveolar lavage fluid and serum Retention: Samples without DNA
Condition	Subarachnoid Hemorrhage
Inclusion Criteria	1) Age between 18 and 80 years old with independent behavior ability or authorized legal representative. 2) A documented diagnosis of SAH within 5 days. 3) A Hunt-Hess scale of IV or V. 4) Absence of clinical and etiological evidence of pulmonary infection.
Exclusion Criteria	1) Pregnant or lactating women. 2) Present history of traumatic brain injury or intracranial hemorrhage.

	3) Past history of neurological disorders, lung infection within the past six months, cancer, chronic cardiopulmonary diseases, hematological diseases or renal failure. 4) Have participated in clinical trials in the past 4 weeks. 5) The investigator considers that not appropriate for inclusion.
Withdrawal Criteria	1) Clinical or etiological evidence of pulmonary infection after recruitment. 2) Failure to complete tracheoscopy treatments within the time frame specified in the study. 3) Voluntary withdrawal by the subject. 4) Withdrawal requested by the physician or researcher due to issues such as poor compliance.
Outcome Measures	Hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels in bronchoalveolar lavage fluid and serum at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) of patients with SAH.
Reference standard	Hyaluronic acid is associated with organ dysfunction in acute respiratory distress syndrome. Critical Care. 2017, 21(1): 304.
Statistical Analysis	All statistical analyses were performed using SPSS Statistics Version 27.0 (IBM, Armonk, NY, USA). A two-tailed p value of less than 0.05 was considered to be statistically significant.

1. Research Background and Objectives

Acute lung injury is a common complication of subarachnoid hematoma (SAH), and a significant risk factor for death in patients with SAH. Unlike neurogenic pulmonary edema and pneumonia following brain injury, the clinical causes of pulmonary injury after SAH are not intracranial hypertension or pulmonary infection. Its occurrence is influenced by the release of catecholamines, the regulatory function of the hypothalamic-pituitary-adrenal (HPA) axis and systemic inflammatory response, but the specific mechanisms are still unclear. Therefore, delving into the pathological mechanisms of SAH-induced lung injury and developing therapeutic strategies based on the findings is of great importance to improve the prognosis of patients.

Abnormal accumulation of hyaluronic acid in the lungs has been reported to be closely related to the pathological progression of various pulmonary injury diseases, such as chest trauma, pulmonary infection and chronic obstructive pulmonary disease. From this, the present research is aimed to explore the levels and dynamic changes of hyaluronic acid in the bronchoalveolar lavage fluid and blood of patients with acute lung injury following SAH, and to analyze its correlation with the prognosis of pulmonary complications, thereby providing assistance for the clinical diagnosis and treatment of SAH.

2. Study design

2.1 Overall design

This research is an observational prospective cohort study.

2.2 Estimated number of subjects

Estimated by statistical analysis, a total of 24 subjects were included from patients admitted to the neurosurgical intensive care unit of Tianjin Medical University General Hospital and the intensive care unit of Tianjin Huanhu Hospital due to aneurysmal SAH.

3. Inclusion, Exclusion and Withdrawal Criteria

3.1 Inclusion Criteria

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

2) A documented diagnosis of SAH within 5 days.

3) A Hunt-Hess scale of IV or V.

4) Absence of clinical and etiological evidence of pulmonary infection.

3.2 Exclusion Criteria

1) Pregnant or lactating women.

2) Present history of traumatic brain injury or intracranial hemorrhage.

3) Past history of neurological disorders, lung infection within the past six months, cancer, chronic cardiopulmonary diseases, hematological diseases or renal failure.

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

5) The investigator considers that not appropriate for inclusion.

3.3 Withdrawal criteria

1) Clinical or etiological evidence of pulmonary infection after recruitment.

2) Failure to complete tracheoscopy treatments within the time frame specified in the study.

3) Voluntary withdrawal by the subject.

4) Withdrawal requested by the physician or researcher due to issues such as poor compliance.

4. Study procedure

1) Researchers initiate the study according to the inclusion/exclusion criteria. After confirming the subject has signed the informed consent, doctors will record the basic information, GCS at admission, Hunt-Hess scale and past medical history. Besides, researchers will track the patient's surgical status during hospitalization and GOSE at discharge.

2) The patient undergoes bronchoalveolar lavage treatment under bronchoscopy at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) of SAH. For each session, 5 ml of bronchoalveolar lavage fluid is collected to quantify the levels of hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10). At the same timepoints, 5 ml of venous blood is drawn to assess serum hyaluronic acid and inflammatory factors (IL-1β, TNF-α and IL-10) levels.

3) The patient undergoes a chest CT scan 14 days after the onset of SAH.

5. Evaluation Parameters

3.1 Primary outcome parameters

Hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels in bronchoalveolar lavage fluid at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) of patients with SAH.

3.2 Secondary outcome parameters

Serum hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) of SAH patients.

6. Withdrawal of the subject

The subject has the right to withdraw from this trial at any time for any reason. The researcher should have a thorough understanding and a record of 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. Besides, 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 10%.

7. Statistical analysis

1) The difference between hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels in bronchoalveolar lavage fluid at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) was compared using one-way ANOVA followed by LSD post-hoc test.

2) The difference between serum hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) was compared using one-way ANOVA followed by LSD post-hoc test.

3) Pearson correlation coefficient was used to evaluate the correlation between hyaluronic acid levels in bronchoalveolar lavage fluid and serum, hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels in bronchoalveolar lavage fluid, as well as serum hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10) levels.

8. Trial Management

8.1 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 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.

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.

8.2 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 receive the informed consent signed by the subject before starting the trial, and keep it properly. The subject should also permit researchers and the clinical trial management agency to check his or her original data. Thus, the reliability of the research findings could be ensured.

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

8.3 Subjects privacy

The researchers should protect the privacy of the subject. All research documents can only be identified using the subject's number instead of name or physical examination 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 any institution or individual.

8.4 Modification of the trial protocol

The trial protocol is approved by the ethics committee before starting the trial. During the research, any proposed modification on the trial protocol should be reported to the principal investigator, and then be submitted to the ethics committee for review. The clinical research can only be resumed after the re-approval of the ethics committee.

8.5 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.

8.6 Documents on file

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

8.7 Quality control

The clinical trial management agency has the authority to review the study progress, in order to ensure that the trial is carried out as predetermined and that the research data be veritably recorded.

Relationship between acute lung injury and pulmonary hyaluronic acid deposition after subarachnoid hemorrhage

Version: 1.0

Date: September 30th, 2024

Ethics Committee

Tianjin Medical University General hospital

Information Sheet of Informed Consent Form

Dear potential research participants,

We sincerely invite you to participate in a study titled **Relationship between acute lung injury and pulmonary hyaluronic acid deposition after subarachnoid hemorrhage**. This research is supported by **Department of Geriatrics and Department of Neurosurgery, Tianjin Medical University, as well as Department of Intensive Care Unit, Tianjin Huanhu Hospital**. The principal investigator is **Dr. Xintong Ge**.

The ethics committee of Tianjin Medical University General Hospital has reviewed the research plan of this study, and agreed to conduct the clinical research program (Grant No. **IRB2024-YX-425-01**, Approved starts on: **10/01/2024**, Approval expires on: **09/30/2025**). The study has been registered at the global clinical research authority database, **ClinicalTrials.gov (Identifier: NCT)**. The research program is funded by **High-Level Talent Project of Tianjin Health Research Program (Grant No. TJWJ2024RC002)**.

Please read the following text carefully as much as possible before deciding whether participate in this study. It can help you understand the purpose, content, procedures and duration of the study, and the possible benefits, risks and discomforts of being a participant. If you prefer, you can also discuss with your family and friends, or ask your doctor for explanations to help you make a decision. In addition, taking part in this study is entirely voluntary. You can refuse to participate, which will not affect your relationship with the doctor and the investigator. In addition, you will not be charged any additional fee.

1. Research Background and Objectives

Acute lung injury is a common complication of subarachnoid hematoma (SAH), and a significant risk factor for death in patients with SAH. Unlike neurogenic pulmonary edema and pneumonia following brain injury, the clinical causes of pulmonary injury after SAH are not intracranial hypertension or pulmonary infection. Its occurrence is influenced by the release of catecholamines, the regulatory function of the hypothalamic-pituitary-adrenal (HPA) axis and systemic inflammatory response, but the specific mechanisms are still unclear. Therefore, delving into the pathological mechanisms of SAH-induced lung injury and developing therapeutic strategies based on the findings is of great importance to improve the prognosis of patients.

Abnormal accumulation of hyaluronic acid in the lungs has been reported to be closely related to the pathological progression of various pulmonary injury diseases, such as chest trauma, pulmonary infection and chronic obstructive pulmonary disease. From this, the present research is aimed to explore the levels and dynamic changes of hyaluronic acid in the bronchoalveolar lavage fluid and blood of patients with acute lung injury following SAH, and to analyze its correlation with the prognosis of pulmonary complications, thereby providing assistance for the clinical diagnosis and treatment of SAH.

2. Estimated Number of Subjects

A total of 24 subjects were included from patients admitted to the neurosurgical intensive care unit of Tianjin Medical University General Hospital and the intensive care unit of Tianjin Huanhu Hospital due to aneurysmal SAH.

3. Inclusion and Exclusion Criteria

3.1 Athletes and Patients with TBI

Inclusion Criteria

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

2) A documented diagnosis of SAH within 5 days.

3) A Hunt-Hess scale of IV or V.

4) Absence of clinical and etiological evidence of pulmonary infection.

Exclusion Criteria

1) Pregnant or lactating women.

2) Present history of traumatic brain injury or intracranial hemorrhage.

3) Past history of neurological disorders, lung infection within the past six months, cancer, chronic cardiopulmonary diseases, hematological diseases or renal failure.

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

5) The investigator considers that not appropriate for inclusion.

Withdrawal Criteria

1) Clinical or etiological evidence of pulmonary infection after recruitment.

2) Failure to complete tracheoscopy treatments within the time frame specified in the study.

3) Voluntary withdrawal by the subject.

4) Withdrawal requested by the physician or researcher due to issues such as poor compliance.

4. Study Procedures

4.1 Before you enroll in the study, the doctor will record your basic information, present medical history and past medical history.

If you are a qualified subject, you could voluntarily participate in the study, and sign the informed consent.

If you do not meet the inclusion criteria, we will suspend your participation in this study.

4.2 If you agree to participate in the study, you will undergo bronchoalveolar lavage treatment under bronchoscopy at the acute phase (1-5 days post-onset) and the chronic phase (10-14 days post-onset) of SAH. For each session, 5 ml of bronchoalveolar lavage fluid will be collected to quantify the levels of hyaluronic acid and inflammatory cytokines (IL-1β, TNF-α and IL-10). At the same timepoints, 5 ml of venous blood will be drawn to assess serum hyaluronic acid and inflammatory factors (IL-1β, TNF-α and IL-10) levels.

4.3 If you agree to participate in the study, you will undergo a chest CT scan 14 days after the onset of SAH.

The bronchoalveolar lavage fluid and blood samples collected during the study will be used only for this research.

5. Benefits

All examinations and treatments will help doctors monitor the changes in your condition and adjust the diagnosis and treatment plan.

6. Risks, Discomforts and Inconveniences

During the research, you will undergo alveolar lavage treatment, blood draws, and chest CT scans. These procedures are part of the routine diagnostic and treatment process for SAH, which may cause some discomfort. Apart from these, the study will not pose any additional risks or discomfort to you.

7. Financial Information

You will not be charged for participating in this study.

8. Privacy

Your research records will be kept by Tianjin Medical University General Hospital. The investigator and the clinical trial management agency will be permitted to assess your records. Any public report on the results of this study will not reveal your personal information. We will make every reasonable effort to protect the privacy of your personal research records.

According to medical research ethics, the research data will be available for public inquiry and sharing. The query and sharing will be limited to web-based electronic data, ensuring that no personal privacy information will be disclosed.

9. How to obtain more information about the study?

You can ask any questions about this research at any time and get answers. We will keep you informed if there is any critical new information during the research course that may affect your willingness to continue participating in the study.

10. Voluntary Participation and Withdrawal

Participating in this study is completely voluntary. You may refuse to take part in this research, or stop participation at any time that you wish without losing any of your rights.

For your best interests, the doctor and the investigator may stop your participation at any time during the study.

11. What need to do now?

Thanks for reading the materials. Before making a decision on whether to take part in this study, please ask the doctor or the investigator about the research as much as possible. If you decide to participate, please tell your research coordinator, he will arrange everything for your participation.

Please keep this form properly.

12. Sharing the Results

Your test reports can be accessed at any time during or after hospitalization. In addition, the results of this study will be published and shared through academic papers, professional academic conferences, internet, Wechat, etc., so that the practitioners in the medical field can learn.

13. Who can I contact about this study?

Project executor: Dr. Xintong Ge; E-mail: xge@tmu.edu.cn; Telephone: 022-60363844

Research Coordinator: Dr. Ye Tian; Telephone: 022-60814359

Research Coordinator: Dr. Yadan Li; Telephone: 022-60362237

Program Assignment No. IRB2024-YX-425-01

Certificate of Informed Consent Form

Study Title: Relationship between acute lung injury and pulmonary hyaluronic acid deposition after subarachnoid hemorrhage

Participating Organizations: Tianjin Medical University General Hospital, Tianjin Huanhu Hospital

Consent Statement:

I have read the above introduction to this study, and have the opportunity to discuss it with **Dr. Xintong Ge** (project executor), **Dr. Ye Tian** (research coordinator) or **Dr. Yadan Li** (research coordinator). All the questions I asked have been satisfactorily answered.

I know that participating in this study is completely voluntary. I understand the risks and benefits that may arise from taking part in this study. I confirm that I have enough time to consider, and realize that:

- I can ask the doctor and the investigator for more information about the study at any time.
- I can withdraw from the study at any time without discrimination or retaliation, and my rights will not suffer any loss.
- If I have more questions or concerns about this research, I can contact with **Dr. Xintong Ge**.

I agree that the investigator and the ethics committee could review my research materials.

I will receive a copy of this form with my signature and the date.

Finally, I decide to agree to participate in this study, and promise to do my best to follow the instructions from the doctor and the investigator.

Name of participant: _____ Telephone: _____ Date: _____

(If applicable) **The informed consent form of _____ (participant) can not be signed in person due to his/her illness. _____ is granted as the legal representative to represent the patient's informed consent and all of his/her rights in the study.**

_____ (representative) agree with _____ (participant) on participating in the study.

Name of representative: _____ Telephone: _____ Date: _____

Relationship between the participant and representative: _____

(If applicable) I have witnessed the authorization of the participant to the legal representative, and the participant volunteering to participate in this study.

Name of witness: _____ Telephone: _____ Date: _____

I confirm that the details of this study have been stated to the participant, including his/her power, potential benefits and risks. The participant has been given opportunities to put questions about the study, and all the questions have been answered accurately. I confirm that the participant was not coerced into giving consent, and a copy of this form has been provided to him/her.

Name of Doctor: _____ Date: _____