Standardized operational protocol of human brain banking for amyotrophic lateral sclerosis

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease for which there is currently no reliable treatment. Existing clinical diagnostic methods include neurophysiological examination, neuroimaging, genetic testing, etc. In contrast, there is a relative insufficiency of data in neuropathology research, and the pathogenesis is still poorly understood. The neuropathological evaluation results obtained from autopsies of patients with ALS and various organ and tissue samples are crucial resources for the study of this disease. The ALS Human Brain Bank is urgently needed for basic and clinical research on this disease. However, currently, there is no standardized protocol for such an ALS Brain Bank. On the basis of the "Standardized Operational Protocol for the Human Brain Banking in China", this Standardized Operational Protocol is drafted to provide a guideline for the construction and operation of ALS Brain Bank to ensure the quality and homogeneity in China and worldwide. This article is focused on the collection of antemortem information, the donation process, the harvesting, preservation and pathological evaluation of the brain, spinal cord, and other organ/tissue samples of ALS donors in the human brain bank. The establishment of ALS Brain Banks may foster relevant research works and pave the way for the treatment of this disease.

Keywords: amyotrophic lateral sclerosis; human brain bank; neuropathology; brain; spinal cord.

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS), also named Lou Gehrig's disease, is a fatal neurodegenerative disease that attacks the brain cortex, brain stem, and spinal cord motor neurons. ALS is one of a group of conditions known as Motor Neuron Diseases (MNDs) that affect the motor neurons throughout the body, resulting in progressive skeletal muscle weakness, atrophy, muscle bundle fibrillation, bulbar paralysis, and pyramidal tract sign. Some patients may even develop frontotemporal dementia (Cui, 2022; Rosenbohm *et al.*, 2018). The prevalence rate of ALS is about 4.1–8.4 per 100 thousand people in Asia, which is lower than the Europe and America (Aktekin & Uysal, 2020). The male-to-female ratio of patients with ALS is approximately 1:2, and the average age of disease onset is between 51 and 66 years old. The average time between the appearance of the first symptom

and invasive ventilatory support or death is between 24 and 50 months (Talbott *et al.*, 2016). So far, ALS-related research has been mainly focused on clinical diagnosis and treatments, including body examinations, spinal cord imaging, electroencephalogram, electromyography, and genetic examinations (Cui, 2022; Li & Wu, 2016; Shefner *et al.*, 2020; Tao *et al.*, 2018). However, cadaver autopsy and pathological evaluations are vital resources for the final diagnosis and study of the pathogenesis of this disease (Nolan *et al.*, 2020; Riku *et al.*, 2021; Stoyanov *et al.*, 2021). Despite the rapid development of human brain bank worldwide, to our knowledge there has been no specific brain bank for patients with ALS, leaving a remarkable lack of brain and spinal cord tissue from ALS donors as compared to other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Liu *et al.*, 2022). To promote the translational research of ALS, it would be crucial to establish a dedicated human brain bank to systemically collect, process, and share the antemortem information and postmortem tissue (including the brain, spinal cord, and other organ and tissue samples) from patients with ALS. Such an *ALS Brain Bank* will pave the way to eventually reveal the underlying mechanisms and provide a solid basis for the development of effective preventive and therapeutic strategies for this disease. This Standardized Operational Protocol (SOP) is drafted to provide a guideline for the construction and operation of ALS Brain Bank to ensure the quality and homogeneity in China and worldwide. It is worthwhile to notice that the SOP for ALS Brain Bank is based on the established SOP of Human Brain Banking as published previously (Piao *et al.*, 2003; Qiu *et al.*, 2017, 2019; Xue *et al.*, 2022).

1. AIMS AND DONORS OF ALS BRAIN BANK

The primary purpose of the ALS brain bank is to systemically collect and process the postmortem brain, spinal cord, and other tissue samples together with the antemortem information from donors with or without ALS based on the SOP, and share with qualified investigators to foster the research in this field and eventually benefit the treatment of this disease. ALS brain bank belongs to a special type of human brain tissue resource bank, and could be constructed as part of an established human brain bank.

Since ALS is a rare disease only discovered in human beings, the brain, spinal cord and other tissue samples from patients with ALS are extremely important in the study of this disease. Efforts should be taken in broadcasting the value of the ALS brain bank and motivating patients with ALS to register for willed donations to the brain bank. It is important for brain banks and related institutions to actively approach and collaborate with organizations and personnel that may potentially help to facilitate this process. This may include hospitals and neurologists with expertise in ALS or motor neuron disorders, charity organizations such as the Red Cross, Red Crescent and foundations that may support the care and research of ALS, patient support groups such as the "Mutual Aid Home for ALS" (founded by Mr. Cai Lei, a famous patient with ALS who co-initiate the ALS Brain Banking Project in China). The ethical review process and legal basis for ALS Brain Bank are the same as the regular human brain tissue bank (Piao *et al.*, 2010; Qiu *et al.*, 2017, 2019; Xue *et al.*, 2022).

2. CLINICAL DATA COLLECTION FROM ALS DONORS

The collection of clinical data for ALS donors is mainly based on clinical examinations and diagnoses from clinical specialists. On the other hand, descriptions from the donors and their family members are also important sources for information collection. A list of critical clinical information for the ALS brain bank is included in Table 1.

3. SAMPLING PROCEDURE OF SPINAL CORD AND RELATED TISSUE FOR ALS BRAIN BANK

In addition to brain and related tissue that is required to be collected in a regular human brain bank, the ALS brain bank should harvest spinal cord and related nerve roots that are of particular importance to the research of ALS and other MNDs. It is worthwhile to notice that the spinal cord and related tissues from donors without ALS are also important to the establishment of the ALS brain bank as valuable control samples.

After the donor passed away, the family member (next-of-kin) or assignee will notify the staff of the brain bank or willed body donation station, and complete the donation procedures such as the donation agreement and informed consent. Extra

Age of onset: □□ years old
Course of illness: □ years □ months
Initial symptoms:
Onset site: Medulla oblongata □ upper limbs □ chest □ lower limbs □ cognitive impairment □
Cognitive impairment: 0 = none, 1 = yes □
Family history: 0 = none, 1 = yes (relationship to patient)
History of trauma: 0 = none, 1 = yes □, if yes, specify location
Past medical and personal history
Final clinical diagnosis:
ALS □ Progressive Bulbar Palsy □ PMA □ PLS □, Motor Neuron Disease with Frontotemporal Dementia (MND-FTD) □ Flail Arm Syndrome (FAS) □ Flail Leg Syndrome (FLS) □ Other □
Ancillary examinations
Electromyography (EMG) results: extensive neurological damage 1 = Yes □, 0 = No □
Cervical, lumbar, and cranial MRI (excluding, or combined with cervical spondylosis, lumbosacral spinal stenosis, cerebral infarction, cerebral atrophy, etc.):
Genetic testing results:

Table 1. Antemortem clinical information from ALS donors.

information may be collected at this time point, including the medical history and medical records of the donor if available (see SOP of human brain bank for detail). The tissue harvesting procedure should be started within 24 h after the death of the donor. Efforts should be taken to keep the postmortem delay as short as possible, ideally within 12 h and a maximum of 24 h.

Before conducting the autopsy, the body is observed and recorded (see Table 2 for details), especially the lower limb, trunk, and upper limb muscles. After the surface examination, the body is placed prone with the back facing up and a wooden pillow is placed under the chest to facilitate the operation. A brief description of the dissection procedure of the spinal cord and related nerve roots was provided in Figure 1, including the following 5 steps:

Step 1. A longitudinal incision is made along the middle of the back, from the external occipital protuberance level along the spinous process to the level of the lower edge of the first lumbar (opening to the sacrum if the cauda equina of the spinal cord is required, Figure 1A).

Step 2. The ligaments and paravertebral muscles attached to the spinous process and the vertebral plates on both sides are peeled away to expose the posterior bony structures such as the spinous process, the vertebral plates, and the transverse process (Figure 1B).

Step 3. The posterior wall of the spinal canal is incised longitudinally at the junction of the vertebral plate and the lateral mass (cervical spine), or the vertebral plate and the articular processes, on both sides, respectively, using tools such as a spinal saw, bone chisel, and bone clamps (Figure 1C).

Step 4. The laminae are lifted to reveal the dura mater. The focus of this step is to do a laminectomy to expose the spinal canal. After the junction is cut, the spinous process can be lifted upward with cloth towel clamps. During lifting, if bones have not been completely incised, they can be incised again (Figure 1D).

Step 5. The spinal nerve is cut with scissors outside the dura and the spinal cord is removed along with the dura (Figure 1E).

4. PROCESS AND PRESERVATION OF TISSUE SAMPLES FROM ALS DONORS

The spinal dura mater is cut to open along the midline of the anterior surface of the spinal cord and spread apart on both sides to expose the spinal cord, which is fixed to the posterior dura mater. A longitudinal incision is made on the left side of the spinal cord as a marker. Each part of the spinal cord should be examined for possible pathological abnormalities, and photographic records are taken.

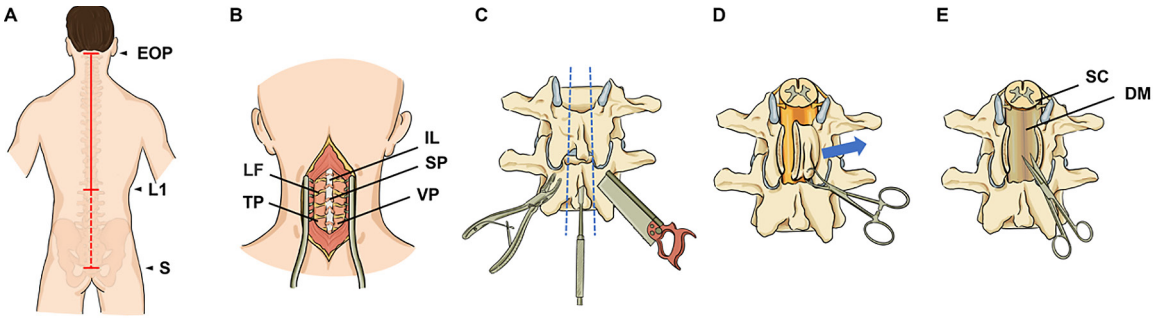

Figure 1. Diagram of the dissection procedure of the spinal cord and related nerve roots. A. longitudinal incision (red dotted line) is made along the middle of the back, from the external occipital protuberance (EOP) level along the spinous process to the level of the lower edge of the first lumbar (L1); B. The spinal process, vertebral plates, and transverse process are exposed after the removal of ligaments and paravertebral muscles attached to the spine. IL: interspinous ligament; LF: ligamentum flavum. C. The posterior wall of the spinal canal is incised longitudinally at the junction of the vertebral plate and the lateral mass (cervical spine), or the vertebral plate and the articular processes using bone clamps, bong chisel, and saw. The dotted lines represent the junction between the vertebral plate and the articular processes. D. The laminae are lifted (arrow) to reveal the dura mater using a cloth towel clamp. E. The spinal nerves are cut with a micro-scissor outside the dura matter so that the spinal cord can be removed along with the dura.

The spinal cord is sectioned horizontally according to the nerve roots and photographed with the head side up, to distinguish the various sections of the spinal cord. One slice (approximately 0.5 cm) of each of the following levels: C2, C7, T2, T8, L2, L4, S1, and S2, which will be used for pathological diagnosis (Figure 2). The sections are fixed and stored in an embedding box, while the remaining part is frozen for preservation. Recommended freezing method is freezing the tissue in liquid nitrogen in a small metal container (with a flat bottom), then transfer to a sealed bag to be stored at −80 °C for preservation.

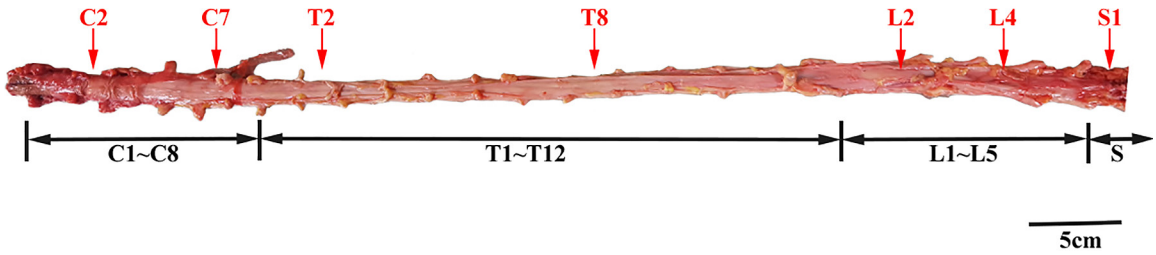

Figure 2. Sampling of the spinal cord tissues from ALS donors. Note the spinal cord regions and specific sections to be sampled and processed for neuropathological evaluation.

The collection of the brainstem and brain tissue should follow the SOP of the human brain bank. In particular, the pons should be sliced to include the facial colliculus and trigeminal nerve root level.

5. NEUROPATHOLOGICAL EVALUATION OF TISSUE SAMPLES FROM ALS DONORS

Macroscopically, abnormality of the brain is usually unremarkable in patients with ALS. In some cases, there may be obvious atrophy of the precentral gyrus, which is best detected by removing the pia mater after fixation. The key histological changes are the loss of motor neurons with associated astrocytosis in the anterior (ventral) horn of the spinal cord, motor nuclei of the brainstem (cranial nerves XII, motor VII, motor V), and the motor cortex. Various inclusions can be seen in surviving motor neurons on hematoxylin and eosin staining (Figure 3A).

Macroscopic appearance: There is anterior root atrophy with thinning and a gray-brown color, and the spinal cord may appear atrophied, particularly at the cervical and lumbar enlargements. On the transverse section, the atrophy of the anterior horns can be observed. Most brains appear to be normal,

but some may show atrophy of the precentral gyrus. When concomitant with dementia, atrophy of the frontal and temporal lobes may occur.

Microscopic appearance: There are degeneration and loss of lower motor neurons in the anterior horn of the spinal cord and motor nuclei of the brainstem (such as the hypoglossal nucleus, facial nucleus, and motor nucleus of the trigeminal nerve), as well as upper motor neurons (Betz cells) in the motor cortex of the brain. The loss of neurons is accompanied by astrocytosis. There is diffuse degeneration of the corticospinal tract and corticobulbar tract, including the internal capsule, cerebral peduncle, and anterior and lateral columns of the spinal cord (Figure 3B). Muscles exhibit neurogenic atrophy (grouped atrophy). Surviving motor neurons in the spinal cord and brainstem often contain intracytoplasmic Bunina bodies and ubiquitin/p62-positive inclusion bodies, which are fibrous, linear, or spherical and mostly are TDP-43-positive (Figure 4). Familial ALS with FUS gene mutations that begin in adolescence can also have FUS-positive inclusion bodies.

Recommended immunohistochemistry and special staining: primary antibodies against NeuN, GFAP, NF, CD68, ubiquitin, p62, TDP43; LFB myelin staining (also known as Kluver staining, see Figure 3B).

Region	HE	LFB (Kluver)	TDP43	Ubiquitin/ p62	NeuN	GFAP	NF	CD68
Spinal cord C2	√							
C7	√	√	√	√	√	√	√	√
T2	√							
T8	√	√	√	√	√	√	√	√
L2	√							
L4	√	√	√	√	√	√	√	√
S1	√							
S2	√							
Medulla oblongata (middle olivary nucleus level including hypoglossal nucleus)	√							
Pons (facial colliculus level including facial nerve nucleus)	√							
Pons (trigeminal nerve root level including the motor nucleus of the trigeminal nerve)	√							
Precentral gyrus	√	√	√	√	√	√	√	√
Basal ganglia including the internal capsule	√							

Table 2. The list of neuropathological evaluation of ALS samples

6. PROSPECT OF ALS BRAIN BANK

The human brain bank is an indispensable platform for research on various neurological diseases, especially for diseases such as ALS that threaten patients' lives and quality of life but have unclear pathogenesis and still lack effective prevention and treatment measures. However, due to the low incidence of this disease, it has been neglected for a long time, and the construction of corresponding biological sample libraries has been relatively lagging until recently (Kjaeldgaard *et al.*, 2020). To our knowledge so far there is only a very limited number of publicly accessible brain banks specifically dedicated to patients with ALS both domestically and internationally (https://www.cdc.gov/als/NationalALSBiorepositorySamples.html). Fortunately, with the gradual attention of the whole society to this disease, more and more patients, doctors, and researchers have recognized the crucial importance of building an ALS brain bank. The experiences of well-known patients such as Stephen William Hawking (January 8, 1942–March 14, 2018), as well as the "ice bucket challenge" that has been popular worldwide since 2014, have attracted widespread social attention and raised funds for

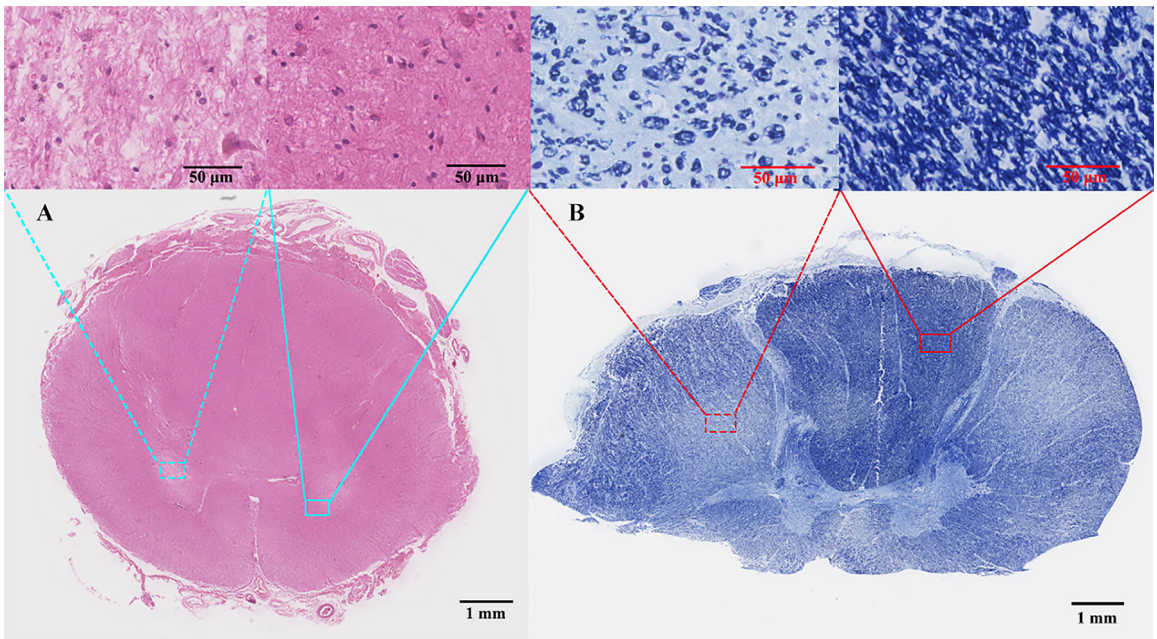

Figure 3. Hematoxylin and eosin (HE) and Kluver staining of the spinal cord sections from an ALS donor. A. Typical image of HE staining indicating the loss of motor neurons in the spinal cord anterior horn at the L1 segment. B. A typical image of Kluver staining demonstrating the demyelination of the bilateral corticospinal tract at the T2 segment of the spinal cord. Inset on the top are enlarged images of the framed part in the panels.

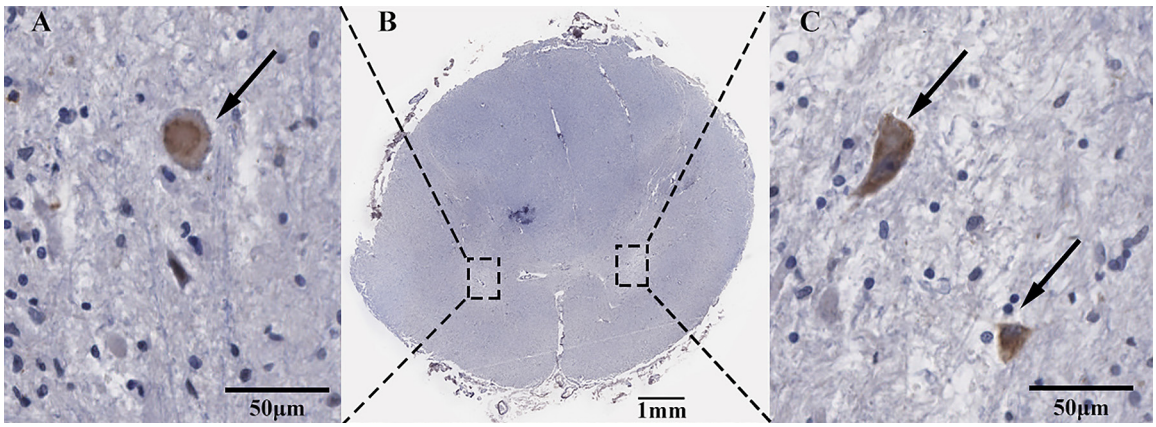

Figure 4. pTDP-43 immunohistochemical staining of the spinal cord from an ALS donor. The anterior horn at the L1 segment of the spinal cord shows inclusions with immunoreactive for pTDP-43 in the motor neurons (brown plaques as indicated by arrows in A and C). Panels A and C are enlarged images of the framed part in panel B. The cell nuclei were counterstained as blue color in the background by hematoxylin.

patients with ALS. In China, starting from 2022, through the efforts of an patient with ALS named Cai Lei and his mutual aid organization "Home for Heal and Help" for many years, with the support of multiple institutions such as the China Organ Transplantation Development Foundation, the Red Cross Society of China, the China Human Brain Bank Consortium, the Peking Union Medical College of the Chinese Academy of Medical Sciences, and Zhejiang University, the "The ALS Brain Bank Initiative in China" was launched and an ALS-specific brain bank was established in the national brain bank. The bank has begun to collect and preserve organs such as the brain and spinal cord of ALS voluntary donors after their death and provide samples to relevant research institutions for scientific research. The SOP of the ALS brain bank described in this article was jointly formulated by

the expert teams from participating universities, clinical, and scientific institutions in this program. It is believed that with the gradual establishment and improvement of the ALS brain bank, the continuous deepening of relevant basic and clinical research, and the continuous increase in attention and investment in this disease, humans can eventually overcome this incurable disease and benefit patients and society.

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Conflict of Interest Disclosures

The authors declare no conflict of interest.

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