ALLEN INSTITUTE for BRAIN SCIENCE

Aging, Dementia and TBI Study

TECHNICAL WHITEPAPER: TISSUE COLLECTION

OVERVIEW

This Technical White Paper describes the screening, case selection, and neuropathology protocols for the project. These include consent protocols and IRB consent, inclusion/exclusion criteria, case selection algorithms, tissue acquisition and rapid autopsy protocols, and distribution of tissues across the different elements of the project. The cohort is from the ACT (Adult Changes in Thought) study described in detail in an accompanying white paper (Documentation). ACT subjects with a traumatic brain injury (TBI) with loss of consciousness (LOC) were selected, and each TBI case was matched with a control case using an algorithm to identify a single best matched control. A rapid autopsy dissection was performed on all cases with post-mortem interval (PMI) < 8 hr to ensure highest quality tissue. This rapid protocol isolates ventricular cerebrospinal fluid (CSF) and approximately 60 flash frozen tissue samples from 15 regions of a hemisection of brain. Non-rapid autopsy brains (PMI > 8 hr) had a portion of cerebellum removed and frozen, after which the entire brain was fixed in 10% neutral buffered formalin. Intact fixed cerebrum, cerebellum, and brainstem from rapid and non-rapid cases were embedded and serially sectioned into 4 mm coronal sections, from which samples were isolated for analysis.

CASE SELECTION

Consent Protocols and IRB Consent

Authorization to conduct research was approved under University of Washington Human Subjects IRB application #24250 “University of Washington Neuropath Core, Brain Aging and Neurodegeneration Brain Bank”.

Participants enrolled in the ACT clinical study who elected to participate in the brain autopsy program completed a consent packet that included:

- Consent for Autopsy
- UW Neuropath Core Brain Aging and Neurodegeneration Brain Bank Autopsy and Tissue Donation to Brain Bank Consent Form
- HIPAA Authorization (HIPAA: Health Insurance Portability and Accountability Act)
- Autopsy Contact Information Sheet

At the time of death, the consent for autopsy was updated with legal next of kin.

Inclusion/Exclusion Criteria

The cohort includes participants from the ACT study who died with an autopsy consent that was updated with legal next of kin and who had a rapid autopsy with dissection and banking of frozen brain tissue. All subjects with a history of TBI with LOC documented in their ACT study records were included. Controls with no known TBI with LOC were selected from the same cohort matched to rapid autopsy status, sex, age, date of death, and post-mortem interval (see Case Selection Algorithms section below for further details).

Case Selection Algorithms

All ACT subjects with a TBI with LOC and rapid autopsy with available banked frozen tissue were identified. Starting with the youngest participants, each TBI case was matched with a control case with rapid autopsy and available frozen tissue and no known TBI with LOC exposure of the same sex. An algorithm was applied (below) that identified a single best matched control for every case. Once a control was identified for a case, that control was not available for additional TBI cases. The algorithm (below) was performed for the entire cohort resulting in a single best match for every case (see **Table 1**). It was then repeated to identify a second best control for each of the TBI cases.

Algorithm is as follows with comparison to the index TBI case:

- Exact age – yes
    - If exactly 1, then take
    - If more than 1, then closest year of death
        - If exactly 1, then take
        - If more than 1, then closest post-mortem interval
            - If exactly 1, then take
            - If more than 1, then closest date of death
- Exact age – no, then age +/- 1 year and repeat algorithm
- Age +/- 1 year no, then age +/- 2 years and repeat algorithm until a match is identified.

This selection was performed to generate an initial cohort with two matched controls for every TBI exposed case. Every six months the process was repeated to select two matched controls for new autopsy cases with TBI that were added to the cohort during the process of the study.

Table 1. TBI and control cohort characteristics.

	TBI Cohort	Control Cohort
Number of cases	55	55
Age	89.0 +/- 6.3	89.0 +/- 6.2
Sex	32 male / 23 female	32 male / 23 female
Year of death	2008.4 +/- 3.8	2008.7 +/- 3.4
Post mortem interval (hours)	4.6 +/- 1.5	4.7 +/- 2.0

TISSUE COLLECTION AND PROCESSING

Tissue Acquisition and Rapid Autopsy Protocol

A rapid autopsy dissection was performed on all cases with post-mortem interval (PMI) < 8 hr to ensure highest quality tissue for research. Cerebral cortical tissue pH was recorded as a measure of tissue integrity[1]. The rapid protocol isolates ventricular cerebrospinal fluid (CSF) and approximately 60 flash frozen tissue samples from 15 regions of a hemisection of brain (see **Table 2**) that are stored at -80˚C. All tissue remaining after rapid dissection was immersion-fixed in formalin for two weeks. Non-rapid autopsy brains (PMI > 8 hr) had a portion of cerebellum removed and frozen for possible future genetic studies, then the entire brain was fixed in 10% neutral buffered formalin. Intact fixed cerebrum, cerebellum, and brainstem from rapid and non-rapid cases were embedded in agarose gel and precisely (mechanically) serially sectioned using a spinning blade meat slicer into 4 mm coronal sections which were then sampled (see **Table 3**) and processed in a manner that preserves utility for unbiased stereological studies. Routine processing was performed for Formalin-Fixed Paraffin-Embedded (FFPE) sections.

Table 2. Fresh frozen brain dissections/storage protocol (rapid autopsies only).

Frozen Tissue Block Number	Brain Region
A1-A5	Midfrontal gyrus (5 sections)
B1-B5	Inferior parietal lobule (5 sections)
C1-C5	Posterior superior temporal gyrus (5 sections)
D1-D5	Occipital pole (5 sections)
E1-E5	Hippocampus (rostral to caudal, 5 sections)
F1-F2	Amygdala (2 sections)
G1-G3	Putamen (1 pre-commissural, 1 at anterior commissure, 1 post commissural)
H1-H2	Globus pallidus (1 pre-commissural, 1 at anterior commissure, 1 post commissural)
I1-I3	Caudate (1 pre-commissural, 1 at anterior commissure, 1 post commissural)
J1-J4	Midbrain (3 sections containing substantia nigra)
K1-K5	Pons (5 sections including locus coeruleus)
L1-L5	Medulla (4 sections)
M1-M5	Cerebellar cortex (5 sections)
N1-N5	Prefrontal white matter
O1	Optic nerve
CSF 1-10	Lateral ventricle cerebrospinal fluid (removed prior to brain dissection)

Table 3. Routine fixed brain sampling and stains (all cases).

FFPE Tissue Block Number	Anatomic Region	Routine Diagnostic Stains
A1	Right mid frontal gyrus	H&E/LFB
A2	Left mid frontal gyrus	H&E/LFB, Bielschowsky, Aβ (6E10), Tau (Tau2), α-synuclein (LB509)
A3	White matter	H&E/LFB
A4	Right inferior parietal lobule	H&E/LFB
A5	Left inferior parietal lobule	H&E/LFB, Bielschowsky
A6	Right superior/middle temporal gyri	H&E/LFB
A7	Left superior/middle temporal gyri	H&E/LFB, Bielschowsky
A8	Right occipital (calcarine) cortex	H&E/LFB, α-synuclein (LB509)
A9	Left occipital (calcarine) cortex	H&E/LFB, Tau2, Congo Red
A10	Anterior cingulate gyrus	H&E/LFB
A11	Hippocampus at level of uncus	H&E/LFB, Tau2

A12	Hippocampus at lateral geniculate	H&E/LFB
A13	Amygdala	H&E/LFB, α-synuclein (LB509)
A14	Right striatum at anterior commissure with Nucleus Basalis of Meynert (NBM)	H&E/LFB, Aβ (6E10)
A15	Left striatum at anterior commissure with NBM	H&E/LFB
A16	Right thalamus with subthalamic nucleus (STN)	H&E/LFB
A17	Left thalamus with STN	H&E/LFB
A18	Midbrain with substantia nigra	H&E/LFB, Aβ (6E10)
A19	Pons including locus ceruleus	H&E/LFB
A20	Medulla including dorsal motor nucleus of the vagus	H&E/LFB
A21	Cerebellar cortex and dentate nucleus	H&E/LFB, Aβ (6E10)
A22	Spinal cord/pituitary (as available)	H&E/LFB

Abbreviations: H&E, hematoxylin and eosin; LFB, luxol fast blue.

Tissue Dissection and Distribution for Molecular Studies

Once cases were selected and banked tissues identified, frozen tissues were processed for each component of the study. Specifically, banked frozen tissue from each region for each TBI and control case was used for immunohistochemistry (IHC)/*in situ* hybridization (ISH)/RNA sequencing (RNAseq), targeted proteomic analysis, and quantification of free radical injury (isoprostanes) in immediately adjacent tissue sections. In most cases, two adjacent flash frozen tissue blocks from parietal lobe, temporal lobe, and hippocampus were available, with one sent to the Allen Institute for Brain Science for IHC/ISH/RNAseq and the other saved for studies at the University of Washington (UW). In cases where only one tissue block was available for parietal lobe and temporal lobe, the tissue section was divided on dry ice in a plane perpendicular to the surface of the gyrus to provide depth of sulcus cortex and underlying white matter to both the study sites. For the hippocampus, when only one frozen sample was available the entire sample was submitted to the Allen Institute for IHC/ISH/RNAseq rather than attempting to divide the tissue.

For specimens to be analyzed at UW, the frozen tissue block was divided perpendicular to the cortex at mid gyrus to provide equivalent samples for gas chromatography-mass spectrometry (GCMS) (isoprostanes) and for immunoassays. The entire tissue sample designated for GCMS was used for the GCMS assay. For immunoassays, a 1 cm punch was used to selectively isolate gray and white matter. Specifically, punch biopsies of frozen parietal cortex and white matter, and temporal cortex, were obtained and submitted for protein extraction (see below). Residual frozen tissues were saved but not analyzed. In cases where only one frozen tissue block was available and the tissue was already divided and half submitted to the Allen Institute, punches were taken for immunoassays and the remainder submitted for GCMS. In all cases, punches of cerebral cortex were targeted to depth of sulcus regions and white matter punches were targeted to the deepest available layers in each tissue block, which in most instances underlie the depth of sulcus samples. Punch biopsies resulted in approximately 1 cm^3 of tissue from each region. For hippocampus, due to lack of available tissue, the entire hippocampus tissue block was submitted for immunoassays. Tissue in all cases was handled, dissected, and distributed frozen on dry ice.

For IHC of FFPE tissues, blocks were taken from parietal cortex and temporal cortex from the same side of the brain that was sampled during the rapid autopsy for frozen tissues. FFPE hippocampus for IHC was taken from the opposite side as the entire hippocampus hemisphere was used for frozen tissue processing. These blocks were submitted for sectioning, histochemical, and immunohistochemical staining, and slides were submitted to the Allen Institute for Brain Science for scanning and image analysis. Although tissue from these blocks was

not collected exactly adjacent to the frozen tissue sampling, the tissues were from the same anatomical regions and are thought to be representative of general pathologic processes that likely involved the frozen samples.

REFERENCES

1. Kingsbury, A.E. et al. Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res Mol Brain Res.* **28**, 311-8. (1995).