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Extended post mortem delay times should not be viewed as a deterrent to the scientific investigation of human brain tissue: A study from the Brains for Dementia Research Network Neuropathology Study Group, UK

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Although animal and cellular models can recapitulate certain aspects of the pathology and pathogenesis of human neurodegenerative diseases such as Alzheimer's disease, frontotemporal lobar degeneration and Parkinson's disease, none of these models is perfect. The study of human post mortem tissues is still essential for investigation of neurodegenerative disorders. It is a common perception that the long post-mortem delays (PMDs) that often limit the acquisition of brains after death are detrimental to brain quality, and therefore prejudicial to the reliability of scientific data based on such materials. However, this viewpoint is largely anecdotal and has not been supported by scientific evidence. We have sought to address this issue by investigating how putative measures of tissue quality such as brain pH or values of RNA integrity (RIN) relate to PMD.

The study encompassed 556 brains, consecutively acquired through the Brains for Dementia Research Network between 2013 and 2016. Five Centres participated, Bristol (52 brains), Manchester (93 brains), Newcastle (76 brains), Oxford (213 brains) and Kings College, London (122 brains). These comprised 300 males (mean age 76.6 ± 12.8 , range 6-104 years) and 256 females (mean age 80.3 ± 14.2 , range 8-102 years). PMD varied from 6 to 279 hours (mean 59.4 ± 32.9 hr). Brains were divided along the mid-line. One hemi-brain was dissected fresh into coronal slices for pH measurements and frozen tissue sampling, while the other hemi-brain was placed whole into 10% buffered formalin for diagnostic investigations. Brain pH was measured on the freshly sliced cerebral hemispheres at each of the 5 Centres at 4 standard locations – frontal lobe white matter anterior to the genu of the corpus callosum, occipito-parietal white matter posterior to the splenium, occipital pole white matter and cerebellar white matter – using the same pH meter in each Centre (Hanna Instruments Skin pH Portable Meter - HI99181). RIN was measured in frontal cortex (London, 89 cases) or cerebellum (Oxford, 118 cases). Briefly, RNA was extracted manually from frozen cerebellum using Allprep DNA/RNA minikit (Qiagen); RNA concentration was measured with Nanodrop 2000c; RIN was measured using Agilent 2100 Bioanalyzer. PMD was calculated as the interval between time of death and time when the dissected brain was placed in the freezer/into formalin. The relationships between, pH and PMD were analysed on all 556 cases - collectively and when stratified according to agonal status in terms of sudden versus protracted death, and the presence or absence of sepsis at time of death, where this could be reliably determined from clinical notes or death certification. Sudden death involved conditions such as cardiac arrest and pulmonary embolism, whereas protracted death mostly involved carcinomatosis or neurodegenerative disease where dying had occurred over a period of days. Sepsis was assumed if respiratory infection (pneumonia), biliary infection or urinary tract infection was listed as cause of death. RIN measures were correlated with pH and PMD separately for each of the 2 participating Centres.

There were no significant differences in mean pH values between all 4 brain areas measured either when assessed on the complete cohort ($F_{3,1292} = 0.672$, $p=0.569$) or when stratified according to collecting Centre (Oxford, $F_{3,108} = 0.273$, $p=0.844$; Bristol, $F_{3,204} = 0.222$, $p=0.881$; Newcastle, $F_{3,288} = 1.19$, $p=0.313$; London, $F_{3,484} = 0.688$, $p=0.560$; Manchester,

$F_{3,1292} = 1.15, p=0.332$) (see also [3]). Likewise, there were no significant differences in mean pH values between sudden or protracted death, and sepsis and non-sepsis, deaths for all brain areas (Table). These data imply that there is no real value in making 4 separate measures of pH, even under any death scenario, and that any one region can be representative of the brain as a whole. Similarly, there were no significant differences in pH values between sudden and slow death cases, or between sepsis and non-sepsis death cases, for each brain region (Table). There were no significant correlations between pH in any area and PMD for all cases, or when stratified for agonal status (Table). As would be expected, each pH measure correlated highly significantly ($p<0.001$ in every instance - data not presented) with each other in the same brain; this correlation applied across the whole cohort but also when it was stratified for Centre or agonal status. RIN value significantly declined with falling pH ($r=0.349, p=0.001$ Oxford; $r=0.349, p=0.004$ London) but did not correlate with PMD ($p=0.248$, Oxford, $p=0.294$, London).

Present analysis supports the following conclusions:

1. There is no significant correlation between brain pH and PMD.
2. Brain pH does not significantly vary across regions, and any area can therefore be employed as representative of the brain as a whole.
3. Agonal status, evidenced by sudden versus protracted death or presence/absence of sepsis appears to have no bearing on brain pH at death.
4. RIN values decline with falling pH, but do not correlate with PMD.

The present study refutes the widely held presumption that extended PMD *per se* is detrimental to brain tissue quality, and indicates that this is likely to be determined by brain pH. We have seen that it is not uncommon for a brain which has been received within 12 hours of death to have just as low a pH as one obtained 5 days after death, and conversely one received 5 days after death to have just as 'normal' a pH as one obtained within 12 hours of death. Furthermore, pH can be used as a surrogate measure of RIN, a measure of global RNA integrity, which is adversely affected by low brain pH but does not correlate with PMD.

There have been previous studies investigating relationships between brain pH, PMD and markers of tissue quality, the most comprehensive coming from the BrainNet Europe Consortium [1,2]. Like us, Monoranu et al [2] found no correlation between pH and PMD, but noted that a prolonged agonal state with global ischaemia was associated with a lowering of pH. Durrenberger et al [1] analysed the effects of ante-mortem and post-mortem variables on human mRNA and found brain pH to be a good indicator of overall RNA quality. Although no correlation between cerebrospinal fluid pH, and overall RNA quality, with PMD was noted, some individual RNAs did decrease in quality with ante-mortem events and PMD. Hence, while global RNA and DNA quality may not be adversely affected by PMD, the preservation of protein and also individual mRNA transcripts may well be affected. Collectively, present evidence suggests it is likely to be better to use shorter PMD material, combined with a favourable pH, when studying these more labile molecules.

What factors might influence, or determine, brain pH are not clear. Although our collection of clinical data was subject to all the limitations of a retrospective study, the absence of even a trend towards a difference in pH between cases dying suddenly and those where death was protracted, or between those with and without terminal sepsis, suggests strongly that the

mode of dying does not play a major role in determining post-mortem brain pH (but see [2]). The explanation for variations in pH most likely lies with the extent the brain can continue to employ glycolysis during/around/after the time of death and thereby generate different amounts of lactic acid until this form of metabolic activity ceases. Nonetheless, this would appear not to be influenced substantially by what is happening in the rest of the body in terms of those events which actually bring about death.

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	Frontal	Parietal	Occipital	Cerebellum	PMD
All Centres	6.26±0.39	6.31±0.38	6.27±0.39	6.23±0.39	59.4±32.9
protracted death	6.28±0.38	6.31±0.36	6.27±0.37	6.26±0.36	61.3±34.7
sudden death	6.22±0.36	6.31±0.47	6.30±0.46	6.12±0.39	51.6±24.4
sepsis	6.29±0.35	6.31±0.35	6.27±0.32	6.24±0.34	54.5±27.3
non-sepsis	6.28±0.38	6.32±0.37	6.28±0.38	6.26±0.37	62.4±35.5
All Centres	0.811	0.665	0.735	0.169	
protracted death	0.467	0.330	0.308	0.780	
sudden death	0.527	0.364	0.425	0.311	
sepsis	0.585	0.799	0.494	0.224	
non-sepsis	0.585	0.498	0.329	0.691	

Table: Mean pH values in each of the 4 brain regions, and post mortem delay (PMD), for all Centres combined and when stratified according to agonal status. Correlations between pH and PMD are shown, again for all Centres combined and when stratified according to agonal status.