ORIGINAL ARTICLE

Rapid fixation of brains: a viable alternative?

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Aim: Recent events surrounding postmortem practices have necessitated several changes in the way necropsies are conducted both within the medicolegal and hospital settings. These changes have resulted in a striking reduction in the frequency of brain retention for neuropathological examination. Aberdeen Royal Infirmary, responding quickly to rising concerns among bereaved relatives regarding organ retention, instituted a change in practice regarding the examination of brains at necropsy as early as 2001. The new protocol involved subjecting the brain to rapid fixation using combined immersion and perfusion techniques. This allows return of the organ to the body without unreasonable delay to the funeral. In this article, we present the results of a retrospective audit comparing the efficacy of the new method against the conventional fixation protocol.

Methods: An equal number of postmortem brains were selected randomly from the year 1998 (following the usual fixation protocol), and from the year 2001 (following the new method). A three tier scoring system was devised to assess section and staining quality, and a single observer scored all the cases.

Results: No statistical difference was noted in the quality of sections prepared by rapid fixation those by conventional fixation. Furthermore, the rapid method was more frequently adequate for diagnosis (considering scores of 2 and 3 together) than the conventional method.

Conclusion: Rapid fixation offers a viable alternative for detailed examination of brains at necropsy, without necessarily prolonging the retention of the organ.

The practice of pathology in the UK has been shaken in the recent past by several incidents related to organ retention. These incidents resulted in a number of investigations, all of which made recommendations regarding consent and organ retention.^{1–3} They also exposed pathologists to severe criticism regarding traditionally accepted necropsy practice, leading to a reluctance to retain brains for detailed neuropathological examination, and consequently, a decline in the number of cases where the brain has been kept. In addition, it imbued in pathologists an urgent need to institute changes in post-mortem practice, which would enable them to accommodate the sentiments of bereaved relatives, while maintaining a high standard of examination. In addition to the initiative already taken by pathologists, the Human Tissue Act of 1961 has been replaced by new legislation, which regulates these issues, among others.⁴

Examination of the brain fresh at postmortem examination, as is increasingly the practice, can lead to a decline in standards. For example, it is known that the accuracy of clinical diagnosis of neurodegenerative disorders is relatively low, being in the region of 70–80% for most dementias.⁵ Accurate diagnosis requires examination of well prepared, representative sections of the brain, ideally by a neuropathologist.

In an attempt to find a solution to this problem, we initiated in 2001 a change to our practice in examination of the brain following medicolegal necropsies. Apart from those cases where full fixation of the brain was mandatory, owing to likely or inevitable criminal proceedings, all brains requiring neuropathological examination were rapidly fixed using 20% neutral buffered formalin (NBF). The quality of sections and staining in these rapidly fixed brains was then compared with that in brains that had undergone conventional fixation, to see if there was a significant difference in the quality of the sections.

METHODS

Change in practice

Rapid fixation was achieved by instilling 20% NBF through the blood vessels at the base of the brain and into the lateral

ventricles through the floor of the third ventricle. The brain was then suspended in a bucket containing 20% NBF and fixed for a short period of time, ranging from 1–4 days. This is in contrast to the practice before 2001, when the brain would be suspended in 10% NBF for 3–4 weeks without injection of NBF through the blood vessels/ventricles. The brain was then cut by a neuropathologist, and relevant blocks selected for histology. The sectioned brain was returned to the body prior to funeral, thereby avoiding the current issues associated with retention.

Case selection

Equal numbers ($n = 36$) of necropsy cases were randomly selected from the year 1998, when the brains had been retained for conventional fixation, and from 2001, when the brain had undergone rapid fixation without retention.

The number of 36 was selected as it represented one quarter of the total number of brains retained in 1998, and this was thought to be representative of all cases.

Scoring system

A scoring system was devised to assess the sectioning and staining quality separately, as follows: 1 = poor quality of sectioning or staining, such that an accurate diagnosis was not possible; 2 = reasonable quality of sectioning and/or staining, which was deemed adequate for diagnosis; and 3 = excellent quality, at par with the best of conventionally fixed brains, which was taken as the gold standard. A single observer, blinded to the fixation status, then scored all the cases.

RESULTS

Section quality

The scores for section quality in the two selected years were compared first. Of the cases from 1998, 4 were scored 1, 11 received a score of 2, and the remaining 21 were all scored 3.

Abbreviations: NBF, neutral buffered formalin; RICT, raised intracranial tension

Table 1 Frequency: section and stain quality

Score	Section quality, n (%)		Stain quality, n (%)	
	1998	2001	1998	2001
1	4 (11.1)	2 (5.6)	2 (5.6)	0 (0)
2	11 (30.6)	18 (50.0)	6 (16.7)	15 (41.7)
3	21 (58.3)	16 (44.4)	28 (77.8)	21 (58.3)

Scoring of the cases from 2001 produced 2 cases with a score of 1, 18 with a score of 2, and 16 scored 3 (table 1).

At a glance, there appears to be a slight difference in the distribution of scores between the two years, with a greater number of the rapidly fixed brains scoring 2, and a greater number of conventionally fixed brains scoring 3. However, following cross tabulation, and using the Pearson χ^2 test, no statistical difference between the scores for section quality between the two years was found, the χ^2 value being 2.077 ($p = 0.722$; >0.05 , thus not significant) (table 2).

Staining quality

The scores for staining quality were similarly compared. Of the cases from 1998, 2 scored 1, 6 scored 2, and 28 cases scored 3. Of the rapidly fixed brains from 2001, none scored 1, 15 scored 2, and 21 cases scored 3. These figures again show slight differences in distribution, with more of the conventionally fixed brains scoring 3 compared with rapidly fixed brains (table 1).

Once again, on crosstabulation, using the Pearson χ^2 test, there was no statistical difference between the scores for the two years, the χ^2 value being 1.861 ($p = 0.394$; >0.05 , thus not significant) (table 2).

DISCUSSION

Our results show that there does not appear to be any statistical difference in the section and stain quality between conventionally fixed and rapidly fixed brains. Interestingly, the rapidly fixed brains had a higher percentage of adequates for diagnosis (grades 2 and 3) both for section quality (94.4% v 88.9% for conventionally fixed brains) and stain quality (100% v 94.5% for conventionally fixed brains).

Factors that might possibly have influenced fixation include the time interval between death and postmortem examination, and raised intracranial tension (RICT) prior to death, which is associated with the condition known as "pump brain". In the series from 1998, the majority of necropsies were performed within 3 days of time of death (mean time interval 2.16 days; range 0–5). Similarly, in the series from 2001, most necropsies were performed within 3 days (mean time interval 2.86 days; range 1–11). Interestingly, the series from 2001 showed a slightly longer interval from death to postmortem examination compared with the series from 1998. In all cases, the bodies were

Table 2 Cross tabulation: section and stain quality

	Section quality				Stain quality			
	2001			Total	2001			Total
	1	2	3		2	3	Total	
1998								
1		1	3	4		2	2	
2	1	6	4	11	2	4	6	
3	1	11	9	21	13	15	28	
Total	2	18	16	36	15	21	36

Figure 1 Axial section through the basal ganglia following rapid fixation in 4 days. **Figure 2** Coronal section through the basal ganglia following rapid fixation in 3 days.

refrigerated from the time of receipt in the mortuary. With regard to the issue of "pump brain", there were five instances in the cases from 1998 in which a neurological cause of death associated with RICT was identified. All five cases were scored 3 for both section and stain quality. In the series from 2001, four cases were observed, and the scores for section and stain quality respectively were 3 and 3, 2 and 2, 2 and 3, and 2 and 2. It is clear that even in those instances where there was a period of RICT prior to death, none of the brains subjected to rapid fixation were considered inadequate for reaching a diagnosis (none scored <2 for either section or stain quality).

Therefore, as all the brains were cut by the same pathologist, subsequently handled by the same technologist, and stained using the same protocol, it follows that the only variable that could have resulted in differences in the section and staining quality was the fixation of the brains.

The concept of rapid fixation of the brain is not an entirely novel one. In a review article on the sampling of brain in neurodegenerative disorders, Love suggests that in instances where prolonged fixation is not feasible, the fresh brain should be cut into slices, which are then fixed flat for about 3 days prior to sampling.⁵ Another study used perfusion of

Figure 3 Section from rapidly fixed brain scored 1 (section and stain quality).

Figure 4 Section from rapidly fixed brain scored 3 (section and stain quality).

10% formalin as a method of rapid fixation.⁶ Their results were similar to those in this study, with comparable and occasionally superior sections using their rapid fixation protocol. Barrett *et al*, in their study, approached the problem using heat accelerated fixation (using 10% NBF) of the brain in postmortem examinations of paediatric tissue. They, too, found the results to be more than adequate not only for examination of routinely stained slides, but also for immunocytochemical staining.⁷

In our experience, the single most important factor determining the quality of pathological examination is the involvement of a neuropathologist in the gross examination and block selection. The role of the neuropathologist is also invaluable in the interpretation of the features seen on histology. This has been elegantly proven by Katelaris *et al* in their paper comparing the rate of detection of abnormalities in brains examined by forensic pathologists and neuropathologists.⁸ Although other studies have looked into the use of rapid fixation, this is, to our knowledge, the first to combine the use of both immersion and perfusion techniques. When the change in protocol was first instituted in 2001, fixation was achieved using 20% formalin. However, since then we

have resorted to the more conventional 10% NBF, while maintaining the other components of the fixation process. A future audit will be directed to establishing the efficacy of 10% NBF as opposed to 20% NBF, which was used in this retrospective analysis.

We have shown that this method provides good quality sections enabling a detailed neuropathological examination of the post-mortem brain without the need for retention (figs 1–4). In our experience, use of this method has encouraged thorough examination of the brain in appropriate medicolegal and hospital necropsies, without adding to the anxiety of relatives; and has also resulted in increased training opportunities. This is desirable not only for the purposes of accurate diagnosis, but also for the continued education of pathologists.

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TAKE HOME MESSAGES

- Recent changes in UK law have impacted on the way post-mortem examinations are conducted. There has been a large decrease in the frequency of brain retention for neuropathological examination.
- Responding to changes in practice, a new protocol involving rapid fixation in formalin was developed, and compared with conventional fixation.
- Using a three tier scoring system, no statistical difference was noted in the quality of sections prepared by the two methods.
- The rapid fixation was more likely to give results adequate for diagnosis than conventional fixation, and is a viable alternative.

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