JPAC 16-12

Joint UKBTS Professional Advisory Committee [1]
Summary Sheet

1. Paper for the JPAC meeting on:	10th March 2016
2. Date submitted:	4th March 2016
3. Title (including version no.):	Post mortem retrieval time limits and time from retrieval to processing for ocular tissue
4. Author(s):	Dr Akila Chandrasekar, Chair SAC on Tissues and Cellular Therapy Products
5. Brief summary:	The attached paper was discussed at the SAC-TCTP meeting on 10th September 2016 and it's recommendations accepted. It is recommended that section 21.12 of the Red Book be amended as proposed below.
6. Action required by JPAC: (What do you want JPAC to do in response to this paper?) e.g. • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within the work plan • provide a steer on policy	To endorse the recommendation.
7. Any other relevant information:	

[1] **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices **Professional Advisory Committee**

SAC-TCTP Paper 15-46

Standards for post-mortem retrieval and processing times for ocular tissue

Background

Corneas are procured from deceased donors either by enucleation of the whole eye and subsequent excision of the corneoscleral disc in an eye bank or by in situ excision of the corneoscleral disc directly from the donor's eye, the rest of the eye remaining in the orbit. Enucleation is currently favoured in the UK and accounts for virtually all cornea donation. The advantages are that sclera is also obtained for reconstructive and glaucoma surgery, and non-corneal tissue is obtained for research into the causes and treatment of eye disease such as retinal degeneration and cataract. The presumed advantage of in situ excision is the reduced death to preservation time for the cornea since the corneoscleral disc is placed immediately into a storage medium. The disadvantages, however, are that sclera for reconstructive surgery and tissues for research are not obtained, the procedure risks damage to the corneal endothelium, and it is carried out in an open environment such as a mortuary. In situ excision is used extensively in North American and by French eye banks. In other countries, the preference is at the discretion of the eye bank medical director.

It is clear that tissue quality and cellular viability will decline after death. There are, however, no standards setting out acceptable time limits from death of a donor to the processing of corneas. These can vary from just a few hours with in situ excision to 48 hours or more for enucleation and corneoscleral disc excision in an eye bank. The purpose of this paper is to set out the evidence from the literature in order to arrive at recommendations for the procurement of corneas in the UK for maximum death to enucleation and enucleation to processing times.

The EU Tissues and Cells Directive (2004/23/EC) and its accompanying Commission Directives (2006/17/EC, 2006/86/EC and 2012/39/EU) (together "the EUTCD") and the Human Tissue (Quality and Safety for Human Application) Regulations 2007 ("the Regulations"), which transposed the EUTCD into UK law, are silent on the matter of death to processing times for corneas and other tissues: the same is true for the JPAC *Guidelines for the Blood Transfusion Services in the United Kingdom* ("the Red Book"). The Royal College of Ophthalmologists ("the RCOphth") *Standards for the retrieval of human ocular tissue used in transplantation, research and training* merely state that enucleation should be carried out as soon as possible after a donor's death, but post mortem times up to 24 hours are acceptable. No recommendation is given for time from enucleation to processing. Eye banking organizations (European Eye Bank Association, Eye Bank Association of America, Eye Bank Association of Australia and New Zealand) leave post-mortem times to the discretion of the eye bank medical director. The post-mortem times reported in the European Eye Bank Association 2015 Directory (data for 2013 from 66 eye banks in 21 countries) are 18.5 hours for death to enucleation and 23 hours for death to processing. The comparable figures for the UK in 2014/15 are, respectively, 16.8 (SD 5.8) hours and 33.6 (SD 8.2) hours. The longer death to preservation time in the UK includes the time taken for transport from donor centres to the Bristol and Manchester eye banks.

The only procurement-related time limits specified by the EUTCD and the Regulations refer to the taking of the blood sample for the mandatory testing of markers of transmissible disease: the sample must not be taken more than 24 hours after death or, if taken ante mortem, the sample must have been obtained not more than 7 days before the death of the donor. However, it has been accepted in the UK that death to enucleation (or in situ excision) should not exceed 24 hours partly because the donor's blood sample is often taken at the same time as the eyes are retrieved and the blood sample must be taken within 24 hours of death.

Definitions

For the purpose of this paper, the following definitions are used:

Time of death – the date and time of death recorded by the individual certifying or verifying death or, for organ donors, the time of cessation of ventilation/circulatory arrest/'cross-clamp' time.

Time of enucleation – the date and time of enucleation of the eyes from a deceased donor.

Time of processing – the date and time of excision of the corneoscleral disc from an eye, whether in situ or from an enucleated eye, and its placement into hypothermic storage medium or organ culture medium.

Death to enucleation time – the time in hours between a donor's death and enucleation of the eyes.

Enucleation to processing – the time in hours between enucleation of an eye and excision of the corneoscleral disc and placement into storage medium.

Death to processing time – the time in hours between the death of a donor and excision of the corneoscleral disc and placement into storage medium. For procurement by enucleation, this will

equal the sum of the death to enucleation and the enucleation to processing times. For in situ excision, there is no death to enucleation interval and death to processing time is the time in hours between death of a donor and in situ excision of the corneoscleral disc and placement into storage medium.

Evidence for the setting of maximum post-mortem times

Published evidence from the UK, Denmark, France, Italy, Germany, New Zealand and Australia was reviewed. Data from the USA were not included because the surgeon demand for very short death to preservation times, which is not evidence-based, means that analyses such as the prospective Cornea Donor Study (death to preservation time ≤12 hours: Cornea 2005;24:389-396) are unable to inform the setting of maximum death to processing times relevant to the UK. The papers are divided between those that report analyses of the influence of post-mortem times on the suitability of corneas for transplantation based on endothelial criteria (principally endothelial cell density, ECD) and those that report the influence of post-mortem times on clinical outcomes (graft survival and postoperative changes in ECD).

Evidence from the suitability of corneas for transplantation based on ECD

The primary source of evidence comes from an analysis by Armitage et al. (2014). All corneas in this study (n=7107) were retrieved by enucleation and stored by organ culture between 1999 and 2005. Mean times from death to enucleation, from enucleation to processing and from death to processing were, respectively, 14.9 (SD 7.2) and 16.5 (SD 7.5) hours and 31.4 (SD 9.6) hours. None of these post-mortem time intervals had any effect on the risk of corneas being unsuitable for transplantation owing to ECD <2200 cells/mm² (p=0.9, p=0.3 and p=0.2, respectively). However, increasing time from enucleation to processing did have an effect on endothelial quality (defined as an ECD ≥2500 cells/mm²): the percentage of corneas with an ECD <2500 cells/mm² increased from 44.4% for times ≤12 hours to 47.6% for times between 19 and 24 hours (OR 1.2, 95%CI 1.0-1.4, p=0.01) and further increased to 50.9% for times >24 hours (OR 1.4 95%CI 1.1-1.8, p=0.006). Therefore, while the percentage of corneas suitable for transplantation was unaffected by post-mortem times, there was a small (3%) increase in percentage of corneas with <2500 cells/mm² for enucleation to processing times between 19 and 24 hours. It would appear reasonable, therefore, to accept death to enucleation times up to 24 hours and enucleation to processing times also up to 24 hours, albeit acknowledging the 3% increase in corneas with <2500 cells/mm² for enucleation to processing times between 19 and 24 hours. The further increase in percentage of corneas with <2500 cells/mm² for enucleation to processing times >24 hours, suggests that a maximum of 24 hours is a reasonable and safe limit to adopt.

Qualified support for little or no influence of post-mortem times (within given limits) on the suitability of corneas for transplantation is provided by other studies, including an earlier study by Armitage and Easty (1997). All corneas in this study (n=9250) were also retrieved by enucleation and stored by organ culture. The likelihood of corneas being suitable for transplantation (i.e., ECD ≥2200 cells/mm²) was affected by increasing post-mortem times; but the odds ratios (OR) derived from the logistic regression analyses of 0.988 (95% CI 0.982-0.995) for death to enucleation time and 0.994 (95%CI 0.992-.0997) for enucleation to processing time suggested that the effects were slight.

A study from France by Gavrilov et al. (2010) only included corneas (n=2596) retrieved by in situ excision. On retrieval, the corneas were immediately placed in storage medium at ambient temperature and transported to the eye bank for later transfer to organ culture at 31℃. Death to retrieval times up to 24 hours and retrieval to organ culture times up to 48 hours or more were accepted. There was an increase in percentage of corneas discarded with increasing post-mortem times and the study concluded that the percentage of discarded corneas could be reduced by restricting the times for death to retrieval and retrieval to organ culture to <6 hours and <24 hours, respectively. However, the increased percentage of discarded of corneas with longer post-mortem times was not due to an increase in percentage of corneas not meeting the minimum ECD but rather a result of higher rates of contamination in organ culture and positive donor serology. The death to retrieval time categories in this analysis were <6, 6-12 and >12 hours and times up to 24 hours accepted (i.e., the >12 hour category was equivalent to 13-24 hours). The percentage of corneas discarded for endothelial reasons varied from 21.7% for times <6 hours to 21.4% for times >12 hours. This therefore supports the acceptability of death to retrieval times up to 24 hours so far as the impact on ECD is concerned. Retrieval to organ culture times >48 hours had little effect on the percentage of corneas discarded as a result of poor quality endothelium; however, during this time, the cornea was already in a storage medium, albeit at ambient temperature. These data do not therefore support the

acceptability of enucleation to processing times up to 48 hours when, as in the UK, the whole eye is kept in a moist chamber on ice before processing and transfer to organ culture storage.

Evidence from corneal transplant outcome studies

In a series of 3014 corneal transplants, Armitage et al. (2014) found that provided corneas met or exceeded the minimum ECD of 2200 cells/mm², none of the post-mortem times had a negative influence on 5-year graft survival. This provides support from clinical outcome data that death to enucleation up to 24 hours and enucleation to processing also up to 24 hours are acceptable.

Williams et al. (2008) in an analysis of data from the Australian Corneal Graft Registry, reported that death to enucleation times, grouped as 0-3, >3-6, >6-9, >9-12 and >12 hours, had no influence on graft survival. Since median death to enucleation time was 6 hours with a range of 1 to 35 hours, this analysis also suggests that an upper limit of 24 hours should be acceptable.

An analysis from New Zealand by Patel et al. (2005) reported a mean death to preservation time of 15.2 (SD 6.2) hours. The analysis showed that the there was no difference in mean death to preservation times for corneas that were suitable for transplantation based on endothelial criteria and those that were unsuitable.

Andersen and Ehlers (1988) from Denmark reported no difference in 5-year graft survival for corneas with death to preservation times ≤24 hours (range 9-23 hours) compared with >24 hours (range 25-75 hours). The criteria for endothelial suitability are not described and the influence of increasing post-mortem time on suitability for transplantation was not investigated.

Parekh et al. (2013) from Italy found no influence of death to preservation times up to 26 hours on the percentage loss of endothelial cells during organ culture storage, which averaged 3-4%. However, they did find that death to preservation time >10 hours was associated with an increased rate of postoperative endothelial cell loss in a small series (n=64) of corneal transplants for keratoconus. It should be noted that graft survival for this indication is high (95% at 3 years and 84% at 5 years – data from the Australian Corneal Graft Registry 2015 Report) and the clinical significance of this finding is therefore uncertain; moreover, the evidence for such an effect is equivocal. In another small series (n=53), Böhringer et al. (2002) from Germany also found an influence of post-mortem time on postoperative endothelial cell loss in transplants for keratoconus. Their mean death to preservation time was 13.3 (SD 14.0) hours: the large SD indicates a wide range of values and post-mortem times up to 72 hours were included. These longer post-mortem times may have had a disproportionate influence on the postoperative cell loss predicted by their regression equation, which again makes interpretation difficult. On the other hand, in a series of 1248 patients Langenbucher et al. (2003), also from Germany, found no influence of post-mortem times (death to preservation) up to 30 hours on postoperative endothelial cell loss in transplants for keratoconus or other indications.

Recommendation

Given the paucity of reports in the literature that can directly inform a decision about acceptable upper limits for post-mortem times, it is suggested that studies that used UK data should form the primary evidence base for such recommendations. Approximately 3500 corneal transplants are reported each year to the NHSBT UK Transplant Registry. Eye donor data, including post-mortem times and endothelial assessment (ECD), are recorded along with corneal transplant recipient information and clinical outcome data reported at 1, 2 and 5 years. The most recent report (Armitage et al., 2014) suggests little influence of post mortem times on the suitability of corneas for transplantation (ECD ≥2200 cells/mm²) or on endothelial quality (ECD ≥2500 cells/mm²) except a small (3%) increase in corneas with ECD <2500 cells/mm² where enucleation to processing times are between 19-24 hours. This study also shows that where corneas meet or exceed the minimum criteria for penetrating keratoplasty, post-mortem times within limits have no negative influence on 5-year graft survival. The main limitation of this analysis is that it includes only penetrating and not endothelial keratoplasty (PK and EK, respectively). Currently, the criteria for suitability of a cornea for PK and EK are the same. As more EK data are accrued to the UK Transplant Registry, the influence of donor factors on EK outcome can be investigated and, if required, the selection criteria reviewed.

Other studies provide a degree of support both from eye bank endothelial quality assessment and clinical outcome data. The findings do not always agree (e.g., the influence of post-mortem times on postoperative endothelial cell loss) and most of the studies cannot provide clear-cut guidance on acceptable post-mortem times because of the way the data were structured and/or the way the analyses were carried out.

In conclusion, it is recommended that the maximum time from death to enucleation should be confirmed as 24 hours and that the enucleation to processing time also should not exceed 24

hours. These times should be considered as separate standards; i.e., a death to enucleation time less than 24 hours does not mean that the enucleation to processing time can be increased beyond 24 hours.

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6 August 2015

Current Entry

21.12: Ocular tissue retrieval and storage

21.12.1: Eye retrieval

An 'NHSBT Tissue Retrieval Site Risk Assessment' form must be completed by the eye retriever to ensure the suitability of the retrieval site. This must be done for every eye retrieval as circumstances may change even within the same premises.

Eye retrieval must be carried out by a person who is trained and competent in enucleation. Either this individual must be employed by an HTA-licensed eye bank or there must be a third party agreement in place between the eye bank and the individual's employing authority.

Enucleation should be carried out as soon as possible, but no longer than 24 hours after death. The eye retriever must be satisfied that lawful consent/authorisation has been obtained and that at the time of retrieval there is no known medical reason to suggest that the eyes should not be retrieved. Sterile, single-use instruments must be used and disposed of safely after the retrieval. The NHS Blood and Transplant (NHSBT) Human Tissue Transport Box contains all the required documentation, including an enucleation protocol, and a set of sterile, single-use instruments. All required documentation must be fully completed by the eye retriever, including the NHSBT Ocular Tissue Donor Information form and body map.

The NHSBT enucleation protocol must be followed.[8] After enucleation a stump of optic nerve at least 5 mm long must remain attached to the eye, which is then secured in a plastic eye stand. The eye stand and eye (cornea uppermost) are placed on top of a moist cotton wool ball or gauze swab and placed in a sterile pot (moist chamber). The eye must not be immersed in any liquid in the moist chamber. The moist chambers are then packed in an NHSBT Human Tissue Transport box together with a plastic bag containing melting ice. At least 1 kg of ice is needed to keep the contents of the transport box below 5℃ for up to 24 hours during transportation to the eye bank. The donor's eye sockets should be packed with cotton wool and lids closed over plastic eye caps to restore the original profile of the lids. The final cosmetic appearance is of critical importance as family or friends may wish to view the body. Any bleeding or bruising resulting from the enucleation must be noted on the body map.

21.12.2: Ocular tissue storage

Corneas may be stored for up to 2 weeks at 4℃ in an ap propriate hypothermic storage solution. Alternatively, the great majority of corneas in the UK are stored for up to 4 weeks in organ culture at 34℃. The corneal endothelium is exa mined by light microscopy a few days before use to ensure its suitability for transplantation in patients with corneal endothelial disease/deficiency. Organ-cultured corneas are delivered to hospitals in medium containing 5% dextran to reverse the stromal oedema that occurs during storage. Corneas with an inadequate endothelium may still be suitable for anterior lamellar grafts. These corneas may also be transferred to 70% ethanol and stored at room temperature for up to 12 months for use in glaucoma surgery. Sclera, which is also stored in 70% ethanol for up to 12 months, is used for glaucoma or other reconstructive surgery. Ocular surface stem cells may be isolated from the limbus and expanded in ex vivoculture for treating limbal stem cell deficiency.

Proposed revised entry

21.12: Ocular tissue retrieval and storage

21.12.1: Eye retrieval

An 'NHSBT Tissue Retrieval Site Risk Assessment' form must be completed by the eye retriever to ensure the suitability of the retrieval site. This must be done for every eye retrieval as circumstances may change even within the same premises.

Eye retrieval must be carried out by a person who is trained and competent in enucleation. Either this individual must be employed by an HTA-licensed eye bank or there must be a third party agreement in place between the eye bank and the individual's employing authority.

Enucleation should be carried out as soon as possible, but no longer than 24 hours after death. The eye retriever must be satisfied that lawful consent/authorisation has been obtained and that at the time of retrieval there is no known medical reason to suggest that the eyes should not be retrieved. Sterile, single-use instruments must be used and disposed of safely after the retrieval. The NHS Blood and Transplant (NHSBT) Human Tissue Transport Box contains all the required documentation, including an enucleation protocol, and a set of sterile, single-use instruments. All required documentation must be fully completed by the eye retriever, including the NHSBT Ocular Tissue Donor Information form and body map.

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21.12.2: Ocular tissue processing and storage

Corneas should be excised and placed in an appropriate storage solution as soon as possible, but no longer than 24 hours after enucleation. Corneas may be stored for up to 2 weeks at 4℃ in an appropriate hypothermic storage solut ion. Alternatively, the great majority of corneas in the UK are stored for up to 4 weeks in organ culture at 34℃. The corneal endothelium is examined by light microscopy a few days before use to ensure its suitability for transplantation in patients with corneal endothelial disease/deficiency. Organ-cultured corneas are delivered to hospitals in medium containing 5% dextran to reverse the stromal oedema that occurs during storage. Corneas with an inadequate endothelium may still be suitable for anterior lamellar grafts. These corneas may also be transferred to 70% ethanol and stored at room temperature for up to 12 months for use in glaucoma surgery. Sclera, which is also stored in 70% ethanol for up to 12 months, is used for glaucoma or other reconstructive surgery. Ocular surface stem cells may be isolated from the limbus and expanded in ex vivoculture for treating limbal stem cell deficiency.
