

2.3 Donor tissue safety

2.3.1 Preventing transmission of communicable diseases by the donor tissue

Historic perspective

From the start of corneal grafting, it has been a concern that there was a risk of disease transmission.

Although it appears to be a rare event, this received continuous attention over the years because of the broad spectrum of diseases that may be involved.^{124,125} The introduction of preventive measures has not always been supported by scientific evidence of transmission as the following historic review will show.

There is one case report of Hata in 1939 suggesting transmission of retinoblastoma after keratoplasty.¹²⁵ Nevertheless donors with malignant choroidal melanoma have been used for years as they were often the only source for corneal grafts. Retrospectively no evidence for tumour transmission has been observed in the patients that were grafted with this tissue.^{81,82,126} In 1992 a case report was published about a papillar adenocarcinoma of the iris, transmitted by corneal transplantation.¹²⁷

Studies for non-ocular solid and hematologic tumours, present in the donor, also did not show tumour transmission after corneal transplantation.^{128,129}

In the 1970s the first cases were reported about donor to host transmission of Creutzfeldt-Jakob disease^{125,130} and rabies.¹³¹ The risk was reduced merely by specific attention for this disease in the medical history of the patient and not by the exclusion on the basis of serological screening.

In the 1980s, the AIDS epidemic brought a change in general awareness of communicable diseases. Acquired Immune Deficiency Syndrome (AIDS) is the last stage in a progression of diseases resulting from an infection with the human immunodeficiency virus (HIV). HIV was never found in the cornea. There are no reports of seroconversion after corneal grafts from HIV infected donors.^{132,133,134} Although it was generally accepted that the risk of HIV transmission was very low, the fear of transmission had a great impact on eye banking, the requirement of serological tests and the development of standards.¹³⁴ As a consequence in the Netherlands local activities in retrieval of donor tissue were discouraged and local eye banking activities were centralized in the Cornea Bank Amsterdam in 1987.

Medical standards were developed in the beginning of the 1980s,¹²⁵ during which absolute and relative contraindications were published; today this is still the state of the art. These standards are regularly reviewed by experts and updated by eye banking organizations.^{121,135,136} All safety measures have been put into perspective bearing in mind that that 20% of major diagnoses was missed at the time of death.¹²⁵ This large percentage of missed diagnoses puts emphasis on autopsy results.

In 1985 the Eye Bank Association of America (EBAA) required its member banks to perform ELISA test screening for HIV in post-mortem blood. Following the serological tests for HIV numerous serological tests were introduced for diseases such as hepatitis B and hepatitis C. In addition to these tests, multi-tissue donors are now also screened for human T-lymphotropic virus (HTLV) as well as for syphilis and are discarded as cornea donors when tested positive. Although a possible corneal transmission of HBV has been documented in two recipients from two different donors,¹³⁷ this was not found for HCV.¹³³ One study showed a weak correlation between seropositivity and the presence of HCV in the cornea¹³⁸ but another could not confirm that finding.¹³⁹ Corneal opacities^{140,141} and chronic interstitial keratitis¹⁴⁰ are reported in HTLV-1 infected patients, but HTLV-I has not been found in the cornea.

At the end of the 1980s, syphilis was thought to be a marker for blood donors at high risk for HIV. Partly for this reason, serological screening used to be required by medical standards of the Eye Bank Association of America (EBAA). Studies in the mid 1990s indicated that there was a poor correlation between reactive syphilis serology and positive HIV testing¹⁴² and the transmission of *Treponema Pallidum* under typical experimental corneal storage conditions was proven to be extremely unlikely.¹⁴³ As a consequence the EBAA revised their regulations by eliminating the requirement for serological testing for syphilis. There have been no reports on syphilis transmission via cornea transplantation. In Europe a large variety of serological tests has been applied till the transposition of European Union Tissue and Cell Directives (EUTCD) into the national laws of European Union Member States (European Union directives 2004/23/EC, 2006/17/EC, 2006/86/EC).^{144,145,146} In these EU directives that do not discriminate between the non-vascularised cornea and vascularised tissue, a validated algorithm to exclude the presence of *Treponema pallidum* and testing for HTLV is prescribed for donors as a minimum requirement. False positive testing results (Lues, HbsAg, HIV, HCV) are commonly found in cadaveric blood samples and serology results are worldwide responsible for > 10% of the discard of donor corneas.^{133,147,148,149} In the Netherlands the discard rate due to serological screening results is slowly increasing to 5% (personal communication, CBA annual reports). Other serological tests (Malaria, Cytomegalovirus, Toxoplasmosis, Epstein-Barr virus and *Trypanosoma cruzi*), although not addressed by EBAA or EEBA medical standards, could be required by organ procurement organizations.

Proven systemic transmissions

Beside the two cases of hepatitis B, the only systemic infectious diseases documented up to 1998 to be transmitted by corneal transplants were 4 cases of rabies¹³³ and one proven case of Creutzfeldt-Jakob disease.¹³⁰ For rabies no serological test is available in post mortem blood and Creutzfeldt-Jacob disease no serological tests are available.

Rabies is an acute encephalitis, uniformly fatal in unvaccinated hosts, caused by the Lyssavirus, genus *Rhabdoviridae*. Since 1979 eight cases of rabies after transplantation have been reported and transmission through the graft is supposed.^{125,131,150,151} Rabies virus can be demonstrated in the cornea¹³¹ but despite this knowledge one relies on the medical history of donors even though this diagnosis as the possible cause of death may easily be missed.¹⁵² For this proven communicable disease a screening test is lacking.

Creutzfeldt-Jakob disease (CJD) is a transmittable human spongiform encephalopathy characterized by the presence of an abnormal intracellular protein. Different forms of the disease exist: sporadic CJD, iatrogenic CJD, familial CJD and variant CJD, the latter following the consumption of infected beef. In addition to the certain transmission of CJD by a corneal transplantation in 1974 the 9 additional cases suggesting transmission of CJD enhanced the concern about prion transmission via corneal transplantation.^{130,152,153,154} It is shown that corneas contain the infective prion although to a lesser extent than other neural structures.^{152,155} In February 1997 in Great Britain, the corneas and the sclera of a patient, who presumably died of lung cancer, were transplanted in 3 recipients.¹⁵⁶ A few months later autopsy of the donor's brain revealed CJD. Although the recipient patients were free of symptoms 7 years after surgery,¹⁵⁶ this incident has stressed the importance of reliable tests. Up till now no validated test for CJD infectivity is available. For the time being one has to rely on good screening of the medical history of the donor. In addition to appropriate donor selection criteria, in most eye banks disposable instruments are used in order to reduce the risk of CJD cross contamination.¹⁵⁷

Local transmissions

Cytomegalovirus keratitis in the penetrating keratoplasty of a HIV-patient has been described and histopathologically confirmed once.¹⁵⁸ A donor to host transmission could not be proven. Evidence for the transmission of Herpes simplex virus type 1 (HSV-1) by penetrating keratoplasty with subsequent reactivation of donor derived HSV-1 in the transplanted cornea has been described by several authors since the beginning of this century.^{59,160,161,162} Herpes simplex replication is a cause of endothelial necrosis in organ cultured corneas.^{160,163,164} HSV-1 is acknowledged as a cause of primary failure.^{164,165,166,167,168} On the one hand screening of donor cornea culture fluid for HSV DNA is not considered feasible.^{160,169} On the other hand herpes simplex replication is a cause of endothelial necrosis in organ cultured corneas, this donor tissue will be discarded at the time of endothelial evaluation shortly before surgery.^{163,166,170}

Concluding remarks

Worldwide over a 100.000 corneal transplants are performed annually.¹⁷¹ Some tests for transmittable diseases have been added and others have been discontinued. Every new test has its price, not only regarding finance but also regarding the ensuing false positive test results and consequently the waste of donor tissue which leads to prolonged visual impairment of patients.¹⁷² Decisions to implement new tests should be based on sound scientific evidence.¹⁷² The EBAA and the European Eye Bank Association (EEBA) act in the best interest of patients and corneal surgeons. Their well-documented regulations and broad acceptance of the regulations in the field of corneal transplantation should inform the politicians. The best interest of the patients is served when EBAA, EEBA, surgeons and policy makers cooperate in balancing benefits and risks.

2.3.2 Prevention of donor related ocular infections

The overall rate of postoperative endophthalmitis following penetrating keratoplasty (PKP) has been found to be around 0.4%,¹⁷³ in a literature review of 90549 penetrating keratoplasties. This is higher than the observed 0.1% following cataract surgery.¹⁷⁴ This demonstrates an increased risk of postoperative endophthalmitis in association with PKP. However the rate has been decreasing over the last decade and this is ascribed to improved procedures in eye banking.¹⁷³

For the surgeon it is therefore of paramount importance to have insight in the microbiological condition of received donor tissue by knowing the risk of contamination, the decontamination steps and measures and the microbiological tests performed in the eye bank. Compared to the eye in a living person, where the tears together with the blinking reflex keep the eye surface sterile, donor corneas have a high incidence of microbial surface contamination.^{175,176} External bacteria on a donor cornea are mainly skin bacteria (especially staphylococcus) and internal bacteria are mainly intestinal bacteria and may be due to perimortem bacteraemia.¹⁷⁷ Procurement and processing techniques in the eye bank have been developed and adapted with the aim to remove these contaminants as much as possible and to keep the contamination of delivered corneas as low as possible.

Decontamination procedures

Starting with donor selection the risk of contaminating microbes is reduced in each subsequent decontamination step, similar to the procedure in dilution series.

Donor tissue selection

Varying with the availability and efficacy of decontamination procedures, donor selection may take place based on risk factors. Facial trauma may possibly predispose for the presence of microbes on the surface of the globe. Also patients that have been on a respirator may be at an increased risk for ocular surface contamination.¹⁷⁸ In a recent study, post-keratoplasty endophthalmitis was found to be associated with recent hospitalization and fatal cancer among donors.¹⁷⁹ A donor to host microbial transmission was suggested for those cases. Septic donors are also considered to be a risk factor.¹⁸⁰

During retrieval

Vigorous rinsing of the eye with sterile saline may be another step. Irrigation has long been believed to significantly reduce surface contamination¹⁷⁶ but an increase of contaminated eyes has also been described, explained by 1) washing out the microbes trapped within crypts in the conjunctival epithelium and 2) organisms washed into the eye from the lids and periocular skin.¹⁸¹ This emphasizes the importance external eye cleaning before decontamination procedures.¹⁸² The role of antibiotics added to the rinsing solution might

be debated as the microbes are hardly metabolically active at the rather low temperature and time is too short for the antibiotics to be effective. It is suggested that washing at retrieval is as effective as washing of the eyes in the eye bank.¹⁸³ However, by omitting one step from the cascade the total result of decontamination becomes less effective. With corneoscleral disc excision in situ the decontamination procedure is reduced with one step: the decontamination of the eye on arrival in the bank. There are reports describing that more contamination is found in these in situ retrieved corneas when compared to the corneas excised in the eye bank.^{184,185} Others do not corroborate this.^{186,187}

On arrival in the eye bank

Vigorous irrigation of the eye with saline solutions to remove bacteria in addition to mucus, dead cells and other particles reduce the risk for bacterial contamination. This is also valid for the following measures: immersion of the whole eye in an appropriate antibiotic solution or povidone iodine solution which has shown to be a better alternative,¹⁸¹ and the subsequent rinsing to remove toxic ingredients in the decontamination solutions.^{188,189}

During-storage

If the tissue is stored without antibiotics it turns out that more than 30% of the corneas contain remaining microbes despite all previous decontamination steps.¹⁸⁹ The following measures have been developed to minimize the risk during storage.

a. Hypothermic storage

During hypothermic storage proliferation of these bacteria and to a lesser extent proliferation of fungi is reduced. In addition, storage time is limited. Consequently, increase of contamination is prevented. Direct reduction is not expected. Antibiotics added to the storage medium have little effect during the storage at 2-8°C.^{190,191} In the strategy to combat bacteria additional antibiotics have been added or substituted and newer types have been proposed.¹⁰² Antimycotics are not added to the hypothermic storage media. A prophylactic effect of the antibiotics in corneal storage media is shown after warming up the cornea for 1 hour before surgery to room temperature, which is usually done.^{192,193} Microbiological tests are rarely performed during hypothermic storage. The storage time is too short to perform these tests before grafting.

b. Organ culture

In organ culture antibiotics as well as antimycotics are always present. At this temperature range, they are most effective because contaminating microbes are metabolically active. In addition, resistant microbes are more evident because they continue to grow. Nevertheless, when corneas after organ culture are subjected to a mechanical extraction technique using a laboratory blender, microbes are still found.¹⁹⁴ This indicates that they may penetrate the tissue and thus escape detection. A quarantine period and microbiological screening by taking samples of the storage solution is therefore mandatory. In this way organ culture exploits its vulnerability for microbes to discard contaminated corneas before surgery. Culturing of the transport solution is advocated to detect microbes that may have penetrated the cornea since in this medium, more epithelial layers shed and internal fluids are extruded.¹⁹⁵ Direct microbiological screening of the corneoscleral rim does not always belong to the standard procedures after grafting. The observed difference in distribution of bacteria and contamination between the central and peripheral parts of the cornea after organ culture causes to question the value of routine postoperative corneoscleral rim cultures after keratoplasty.¹⁹⁴ Storage of the transport medium with the corneoscleral rim and keeping it available in case of suspicion of keratitis/endophthalmitis may be an alternative.

Effectiveness of the decontamination process

The effectiveness of the decontamination process depends on the retrieval method (corneoscleral disc excision in situ or enucleation), and the storage methods (hypothermic or organ culture). This might explain the considerable risk variations as reported in the literature: 12.4 - 47.9% positive donor corneoscleral rims.^{180,185,186,196} The reported isolates include *Staphylococcus aureus*, *Staphylococcus epidermidis*, hemolytic and non-hemolytic *Streptococci*, *Pseudomonas* and *Propionibacterium*. Donor corneas have a significantly higher incidence of *Streptococcus* and gram negative bacteria in cultures.¹⁹⁶

The frequency of positive rim cultures after PKP is found to be higher in hypothermic storage (8.6-9.8%)^{197,198} than in organ culture (0.26 - 1.4%).^{197,199} In the past a case report about the transfer of *Torulopsis glabrata* strengthens the doubt about the safety of OC in the USA while experiments in Europe prove that this criticism is not justified.²⁰⁰

Donor related keratitis and endophthalmitis

Fungal keratitis and endophthalmitis

These are uncommon but devastating complications following keratoplasty. *Candida* species, especially *Candida albicans* are responsible for the majority of reported cases of these fungal infections.^{197,201} Donor to host transmission is described.^{201,202} An association with positive donor rims has been observed and appears to be higher (75%) than for bacterial contamination (33%).¹⁷⁹

Bacterial keratitis and endophthalmitis

Post-keratoplasty infections are supposed to be caused by the few surviving bacteria that can multiply in the human body after transplantation. Fortunately, the occurrence of post penetrating keratoplasty endophthalmitis is far less than the observed frequency of positive cultures.^{179,203,204,205} The incidence of reported endophthalmitis after hypothermic storage is 0.1-2%.²⁰⁶ Cases of bacterial endophthalmitis, where the same organism was cultured from the donor and recipient are very few.^{203,207} The incidence of endophthalmitis reported after a properly performed organ culture procedure, including a quarantine period and microbiological testing to discard contaminated corneas before surgery, is even less (0–0.1%)¹²¹ Whether organ culture really has a lower risk for post operative endophthalmitis still needs to be confirmed by a prospective study. The discrepancies in the rates show that more reliable data have to be collected. The mandatory notification to national authorities and the EU of endophthalmitis as serious adverse reaction is a way to do this.