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Donor-Dependent Factors Influencing Contamination Rates of Conjunctival Swabs of Human Donor Eyes

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Background: This study investigated the effect of donor-dependent factors on contamination rates of conjunctival swabs of human donor eyes.

Material/Methods: From July 2015 to September 2017 a total of 1008 conjunctival swabs from 504 consecutive human donor eyes were analyzed. Cross-tabulation, chi-squared tests, and Fisher's exact tests were used to evaluate the effect of donor-dependent factors on contamination rates of conjunctival swabs.

Results: The mean conjunctival swabs contamination rate was 28.4%. Donors with the diagnosis of carcinoma or metastases were associated with an increased conjunctival swab contamination rate [odds ratio (OR)=1.8, 95% confidence interval (CI)=1.2–2.6, p=0.007; OR=1.7, 95% CI=1.1–2.6, p=0.016; respectively].
However, the age, sex, diagnosis of diabetes mellitus, and donors who received chemotherapy did not significantly increase the conjunctival swab contamination risk.

Conclusions: Donors with the diagnosis of a carcinoma or metastases seemed to be predisposed to increased conjunctival swab contamination risk.

MeSH Keywords: Cornea • Corneal Transplantation • Decontamination • Eye Banks • Microbiology • Tissue Banks

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Background

Corneal transplantation is still the most frequent type of transplantation performed worldwide to treat corneal blindness and restore vision in affected patients; however, complications can occur after corneal transplantation, among which endophthalmitis is the most serious.

Transplantation of contaminated corneal tissue may lead to postoperative endophthalmitis and this can have devastating consequences for vision, sometimes leading to loss of the eye [1–6]. Therefore, extensive decontamination procedures have been advocated to minimize the risk of donor cornea contamination. Up until January 2018, the German guidelines on the collection and processing of donor corneas have required 2 microbiological examinations of the donor's cornea using conjunctival swabs after disinfection and before the corneal excision or enucleation [7,8]. Despite donor eye decontamination with povidone-iodine, persistent microorganismal colonization has been demonstrated [9–11]. Without decontamination, different authors describe a conjunctival swab contamination rate of around 90% in cadaver donors [12–16]. Table 1 shows different studies with the contamination rate of conjunctival swabs with and without decontamination of the donor globes.

In 2018, our study group showed that a prolonged time interval between death and conjunctival swab collection, a hospitalization time of 2–7 days prior to death, and corneal collection outside the university hospital seemed to be the main factors responsible for an increased conjunctival swab contamination risk [17]. This exciting investigation inspired us to examine the effect of particular donor-dependent factors on the contamination rates of conjunctival swabs of human donor eyes. To the best of our knowledge, this is the first study that has looked at these special donor-dependent factors as they affect the contamination rates of conjunctival swabs of human donor eyes.

Material and Methods

Eye donors

In the period from July 2015 to September 2017, 504 corneas from 252 consecutive donors were stored at the Tübingen Eye Bank in Tübingen, Germany. In 2016, our study group reported that our eye bank would have lost approximately 14% of the transplantable corneal tissues by using an upper age limit of 80 years [18]. Due to the scarcity of corneal transplantable tissues in Germany, we came to the conclusion that older donors cannot be commonly disqualified from corneal donation. For this reason, no maximum age limit for eye donation exists in our eye bank.

An accurate medical history of each donor was collected and donor serology was evaluated from a donor blood sample taken up to 24 h postmortem. The postmortem blood samples had been collected from the subclavian vessels, femoral vessels, or by direct intracardiac puncture. The procedure was practiced in the order mentioned above until blood was found.

Samples from cornea donors were transported and stored at +4°C. All samples had been tested immediately at the Institute of Medical Virology (University Hospital of Tübingen). Commercially available tests for screening donor serum had been used according to the manufacturers' instructions. Blood samples had been drawn for mandatory tests of infectious diseases, including antibodies to hepatitis B core antigen (anti-HBc), hepatitis C virus (anti-HCV), HIV antigen and antibodies (anti-HIV 1/2, p24 antigen), and also for hepatitis B surface antigen (HBs-antigen). Donors' corneas were discarded based on reactive serological testing for at least 1 marker [19]. Any potential donors with high-risk sexual behaviors or intravenous drug use and consequently at high risk for infectious pathogens (e.g., HIV, HBV, or HCV) were not eligible to donate. High-risk sexual behaviors include being a male having sex with other males, having multiple sexual partners, being male or female sex workers, or being the sex partner of any of these persons or of a person known to have HIV for any reason. Specific antibodies against HIV can usually be detected for the first time at 2–10 weeks after infection. Therefore, these potential donors were not eligible to donate.

This research was approved by the Institutional Review Board of the University of Tübingen, and it adhered to the tenets of the Declaration of Helsinki.

Decontamination protocol and collection

The periocular area (cheeks, eyelids, eyelashes, and brows) and the ocular surface (cornea, conjunctiva, fornices, and adnexal structures, including the anterior lamellae of the eyelids) were cleaned using a 0.75% povidone-iodine solution [1 ml of 7.5% Braunol (B. Braun Melsungen AG, Melsungen, Germany) diluted with 10 ml of sterile 0.9% NaCl (B. Braun Melsungen, AG)] for at least 3 min.

The head of the donor was covered with a sterile surgical ophthalmic drape. The medical doctor performing the enucleation wore a sterile gown and gloves, a surgical cap, and a disposable surgical mask. After insertion of the sterile eye speculum, 2 conjunctival swabs (from the upper and lower conjunctival fornices) were taken from each eye (BBL® CultureSwab Plus; Becton Dickinson, Franklin Lakes, NJ). Then, the medical doctor could start with the enucleation. After a limbal-based conjunctival incision, a 360-degree peritomy was performed. Following the blunt dissection of the muscles from the conjunctiva and

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Table 1. Contamination rate of conjunctival swabs with and without decontamination of the donor globes. This table shows different studies with the contamination rate of conjunctival swabs with and without decontamination of the donor globes.

Study	Donor eyes [n]	Decontamination with	Contaminated conjunctival swabs [%]
Boberg-Ans et al. 1962 [12]	63	No decontamination	87.3
Gopinathan et al. 1998 [13]	60	No decontamination	90.0
	60	No decontamination	93.3
	60	No decontamination	91.6
	60	Donor globe for 3 min in 5% PVP-I, then irrigated with 10ml saline	56.0
	60	Donor globe for 3 min in ciprofloxacin 0.3%, then irrigated with 10 ml saline	78.6
	60	Donor globe for 3 min in gentamicin 0.3%, then irrigated with 10ml saline	76.4
Wilhelm et al. 2001 [14]	76	No decontamination	82.9
Panda et al. 2006 [15]	200	No decontamination	77.5
	200	20 ml saline wash for 10 min	60.5
	40	Donor globe for 3 minutes in 1% PVP-I	27.9
	40	Donor globe for 10 min in 0.2% ciprofloxacin	40.6
	40	Donor globe for 10 min in 0.3% gentamicin	60.7
	40	Donor globe for 10 min in Neosporin	47.7
	40	Donor globe first for 10 min in 2.5% cefazolin followed by 10 min in 0.3% amikacin	44.6
Tandon et al. 2008 [16]	376	No decontamination	88.8
	166	Gentamicin 0.4% for 3 min	50.0
	1457	Combination of gentamicin 0.4% for 3 min and PVP-I 1% for 3 min	61.8
	668	Combination of amikacin 0.4% for 3 min and PVP-I 5% for 3 min	31.3
Matsumoto et al. 2011 [22]	98	No decontamination	61.2
	98	Gentamicin	36.7
Li et al. 2014 [11]	2009: 316	No decontamination	38.3
	2010: 341	No decontamination	53.7
	2011: 381	No decontamination	55.6
Fuest et al. 2016 [23]	1054	Periocular skin with 10% PVP-I solution for 3 minutes and conjunctival sac with 5% PVP-I solution for 3 minutes, then rinsed with saline solution	22.8% when incubated in 22.5° C and in 32.5% when incubated in 32.5°C

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Table 1 continued. Contamination rate of conjunctival swabs with and without decontamination of the donor globes. This table shows different studies with the contamination rate of conjunctival swabs with and without decontamination of the donor globes.

Study	Donor eyes [n]	Decontamination with	Contaminated conjunctival swabs [%]
Laubichler et al. 2017 [26]	184	No decontamination	100.0
	92	Octanisept for 30 sec before enucleation	68.0
	92	Octanisept for 30 sec before enucleation and after enucleation globes were completely covered in a 0.75% PVP-I solution for 3 minutes then rinsed with 500ml NaCl 0.9%	1.1
Röck et al. 2018 [17]	504	Periocular area and ocular surface with 0.75% PVP-I solution for at least 3 minutes	28.4

% – percentage; n – number, PVP-I – povidone-iodine.

Tenon's capsule, the muscles were transected using a muscle hook and scissors. The optic nerve was cut with curved blunt scissors to allow a complete enucleation. The 2 globes were stored separately in sterile urine cups (Sarstedt, Numbrecht, Germany). We placed each eye in gauze at the bottom of the sterile cup, which was filled with 10 ml of 0.9% physiological NaCl (B. Braun Melsungen AG) and 5 ml of gentamicin eye drops (Merck Pharma GmbH, Darmstadt, Germany). Sterile instruments were used throughout the whole process.

The eyes were transported to our eye bank on a cool pack in a cool box (temperature between 1°C and 10°C). Here, the donor globes were stored in a refrigerator at 4°C until the preparation of the corneoscleral discs in a class II biological safety cabinet.

The processing of each whole globe was performed in a class II biological safety cabinet by 1 experienced technician. Before preparing the corneoscleral discs, all excess conjunctiva was removed from the donor globes, and they were immersed separately in a 0.375% povidone-iodine solution [2.5 ml of 7.5% Braunol diluted with 50 ml of sterile 0.9% NaCl (B. Braun Melsungen AG)] for 5 min, followed by rinsing with 50 ml of sterile 0.9% NaCl (B. Braun Melsungen AG). Then, a 15-mm trephine was used to cut the sclera around the cornea, and the cut was completed using scissors. The corneoscleral disc was carefully separated from the iris and the uveal tissue using scissors and tweezers. After that, the corneoscleral disc was placed on a corneal holder (Bausch & Lomb, Heidelberg, Germany) and put into a tissue culture flask (Corning Incorporated) filled with Culture Medium I (Biochrom AG) with 2.5% FBS (Merck Millipore). Culture Medium I is supplemented with 60 µg/ml of penicillin G sodium, 130 µg/ml of streptomycin sulfate, and 2.5 µg/ml of amphotericin B. The corneoscleral discs were stored in an incubator at 37°C, 5% CO_2, and 95% humidity. Sterile instruments were used throughout the whole process, including the enucleation, corneoscleral disc excision, and endothelial examination.

All corneas were procured by members of the Tübingen Eye Bank, which means that the swabs and samples taken from medical doctors.

Microbiological testing

Conjunctival swabs

The conjunctival swabs were transported and stored at room temperature. All the samples were tested immediately at the Institute of Medical Microbiology and Hygiene (University Hospital of Tübingen). Supplemented Columbia sheep blood agar plates (Oxoid GmbH, Wesel, Germany), Endo agar plates, and supplemented brain heart infusion (BHI) agar plates (Institute of Medical Microbiology and Hygiene) were incubated at 37°C to test for bacterial contamination. A liver broth was also used. Additionally, yeast-gentamicin plates (Institute of Medical Microbiology and Hygiene) were used to detect fungal contamination, and they were incubated at 30°C. The plates were incubated for 10 days. They were read after 24 and 48 h and after 10 days.

If there was cultural growth, the microorganisms were detected by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Microflex; Bruker Corporation, Billerica, MA). Any proof of bacterial or fungal microorganisms was documented as contamination.

Evaluation

In this study, we collected data on the donor-dependent factors, including donor age, sex, the diagnosis of diabetes mellitus,

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the diagnosis of a carcinoma or metastases, and donors who have received chemotherapy, on the contamination rates of the conjunctival swabs of human donor eyes. We assessed these donor-dependent factors because we hypothesized that patients with a carcinoma have a higher germ load in the conjunctival swab than in those without carcinoma. Therefore, we additionally assessed whether the patients had metastases or had received chemotherapy. We hypothesized the same for diabetes mellitus.

Statistical analysis

The statistical analysis of the data was managed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY). The categorical data were analyzed using cross-tabulations and Pearson's chi-squared tests. Fisher's exact tests were used as tests of association. The quantitative data are reported as mean with standard deviation. The odds ratios (ORs) are presented with 95% confidence intervals (CIs), and $p<0.05$ was considered to be statistically significant.

Results

In the period from July 2015 to September 2017, a total of 1008 conjunctival swabs from 504 consecutive donor eyes from the Eye Bank Tübingen, University of Tübingen, Tübingen, Germany were microbiological investigated at the Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany.

The male-to-female ratio of the donors was 62% to 38%. The overall contamination rate of the conjunctival swabs was 28.4%. The mean donor age was 68 ±15 years old (range 17–96 years).

Donors with the diagnosis of a carcinoma were significantly associated with an increased conjunctival swab contamination rate [odds ratio (OR)=1.8, 95% confidence interval (CI)=1.2–2.6, p=0.007]. Donors with the diagnosis of metastases were significantly associated with an increased conjunctival swab contamination rate (OR=1.7, 95% CI=1.–2.6, p=0.016) (Table 2).

The mean time between death and the conjunctival swab collection was 12.4±15.0 h. In 2018, our study group showed that a prolonged time interval between death and the conjunctival swab collection was significantly associated with an increased conjunctival swab contamination risk [17].

The mean time between death and the conjunctival swab collection in donors with a carcinoma was 12.4±8.3 h and in donors without a carcinoma it was 11.3±8.4 hours. Those with carcinoma had no statistically significant longer duration.

The mean hospitalization time of donors with a carcinoma was 14.1±16.9 days and in donors without a carcinoma it was 11.5±13.9 h.

These time factors in association with or without the diagnosis of a carcinoma are not confounders and had no statistically significant influence on the conjunctival swab contamination rate.

Additionally, the following donor-dependent factors had no statistically significant influence on the conjunctival swab contamination rates: sex (p=0.4), donor age (p=0.5), the diagnosis of diabetes mellitus (p=0.08), and donors who have received chemotherapy (p=0.5).

Donors with the diagnosis of a carcinoma and who have received chemotherapy had a contamination rate of the conjunctival swabs of 35.0%, and the rate in donors with the diagnosis of a carcinoma and who have not received chemotherapy was 40.5%. The difference was not significantly associated with an increased conjunctival swab contamination rate (p=0.5).

The leucocyte counts and other immune status values of the donors were not recorded in the study. In our next study we will consider this important topic.

Contaminating organisms

We found that 95.1% of the conjunctival swab contamination organisms were bacteria. The most commonly found bacteria belonged to the normal skin bacterial flora (70.7%), including coagulase-negative staphylococci, *Corynebacterium* spp., and *Streptococcus* spp., but excluding *Streptococcus pneumoniae*. The most common bacteria not belonging to the normal flora were *Staphylococcus aureus* (8.2%), *Enterococcus* spp. (3.8%), and *Escherichia coli* (3.3%). We found that 4.9% of the conjunctival swab contamination organisms were fungi (exclusively *Candida* spp). The overall contamination rates for the organ culture media were 1.0%.

Discussion

Our investigation illustrates the influence of donors with a diagnosis of a carcinoma or metastases on the conjunctival swab contamination risk in 1008 conjunctival swabs from 504 consecutive donor eyes at the Tübingen Eye Bank.

The positive conjunctival swab rate of 28.4% found in our investigation is within the range found in previous studies (Table 1). The wide range may be due to the varying decontamination protocols and different conjunctival swab origins. While Reddy and Paul found a 7.0% (7 out of 100 conjunctival specimens) positive

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Table 2. Factors influencing the contamination risk of donor eye conjunctival swabs. The categorical data were analyzed using cross-tabulations and Pearson's chi-squared tests. Fisher's exact tests were used as tests of association, as appropriate. The table illustrates the significant influences of donors with the diagnosis of a carcinoma (p=0.007) or metastases (p=0.016) on the conjunctival swab contamination rate of 1008 conjunctival swabs from 504 consecutive donor eyes from the Tübingen Eye Bank. No other donor-dependent factor such as sex (p=0.4), donor age (p=0.5), the diagnosis of diabetes mellitus (p=0.08), and donors who have received chemotherapy (p=0.5) affected the risk of conjunctival swab contamination.

Factor	Donors in%	Contaminated conjunctival swabs [%]	OR	95% CI	P
Donor age [years]					
<75	60.7	30.6	1.0	–	–
≥75	39.3	28.0	0.8	0.6 1.3	0.5
Diabetes mellitus					
No	72.5	27.4	1.0	–	–
Yes	27.5	35.6	1.5	1.0 2.2	0.08
Infections					
No	40.5	31.1	1.0	–	–
Yes	59.5	27.5	0.8	0.6 1.3	0.4
Cancer					
No	65.9	25.7	1.0	–	–
Yes	34.1	37.7	1.8	1.2 2.6	0.007
Metastases					
No	75.4	26.8	1.0	–	–
Yes	24.6	38.3	1.7	1.1 2.6	0.016
Chemotherapy					
No	82.1	28.5	1.0	–	–
Yes	17.9	35.4	1.4	0.8 2.3	0.5

% – percentage; OR – odds ratio; CI – confidence interval.

rate in patients admitted for cataract surgery (eyes in a living state, control group) after inserting 1 drop of proparacaine into the conjunctival sack [20]. Mindrup et al., Matsumoto et al., and Fuest et al. obtained rates in line with our results – 28.9%, 36.7%, and 22.8%, respectively – after disinfection with povidone-iodine in corneal donor cadavers [21–23]. In living persons, the germ growth is kept in check because of the antibacterial effect of the tear film. Germs can easily grow over the donor cornea in cadaver eyes because this tear film is absent. Without decontamination before swab collection, Capriotti et al. obtained a rate of 86.2% contaminations in healthy eyes from a rural population [24].

Decontamination protocols and decontamination procedures vary in many ways. In our study, in the periocular region (both the fornices and ocular surfaces) were cleaned using a 0.75% povidone-iodine solution for at least 3 min. After insertion of a sterile eye speculum, 2 conjunctival swabs were taken from each eye. Without decontamination, different authors described a conjunctival swab contamination rate of around 90% in cadaveric donors [12–16]. Table 1 shows the contamination rates of conjunctival swabs with and without decontamination of the donor globes in different studies.

After sterile enucleation, the donor globes were also immersed separately in a 0.375% povidone-iodine solution for 5 min (see our decontamination protocol). However, in total, we decontaminated the donor eyes for at least 8 min in low concentrations of povidone-iodine and transported the eyes separately in cooled sterile urine cups filled with 5 ml of gentamicin eye drops between the 2 decontaminations. Our extensive decontamination protocol, as described above, could explain why our organ culture media contamination rate of 1.0% was lower than

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the rates of 4.5% to 10.8% found by Li et al., who cleaned the periocular region with a 7.5% povidone-iodine solution spray and the ocular surface with a 1.25% povidone-iodine solution for 3 and 5 min, respectively [11,17].

Donor eye decontamination before graft processing plays a significant role in preventing tissue contamination. Many years ago, povidone-iodine was regarded as one of the best antiseptic solutions as a skin preparation for the reduction of microorganisms. In 1982, the bactericidal activity of various concentrations of povidone-iodine was tested by Berkelman et al., showing that low concentrations (i.e., 0.1% to 1%) were more rapidly bactericidal than a full-strength treatment (10% solution) [25].

Nationally and internationally, there are no uniform guidelines for decontamination protocols and procedures. The procedures vary in many ways. In accordance with our study, Laubichler et al. recommended use of a 0.75% povidone-iodine solution for at least 3 min to decontaminate donor eyes [26].

Despite the slightly similar steps, no complete performance of the entire detailed decontamination protocol described in our investigation could be found in any of the other studies considered.

In our investigation, donors with the diagnosis of a carcinoma were significantly associated with an increased conjunctival swab contamination rate (p=0.007). Moreover, donors with the diagnosis of metastases were significantly associated with an increased conjunctival swab contamination rate (p=0.016).

Reasons for the significantly higher rate of positive conjunctival swabs of cancer patients can be seen in the reduced general condition and reduced abilities of the immune system of these patients. As a result, cancer patients have a higher germ load than in healthy people, which is associated with poorer survival rates [27].

In addition to the germ load, the physiological germ spectrum is also subject to cancer-related changes [28].

However, some points should be considered. Investigations in the future will require a larger sample size of conjunctival swabs and donor eyes, which would improve the results and the validity of findings. Due to different donor eye decontamination protocols, different povidone-iodine concentrations, and different frequency of repetitions there is a limited comparability of these data with other reports on the results of the microbiological testing of conjunctival swabs. Furthermore, other studies used different methods of microbiological testing, which makes the comparison of the study results even more difficult.

Future studies should investigate antibiotic and antimycotic treatments before donor death and their effect on contamination rates of conjunctival swabs.

Conclusions

Our investigation shows that donors with the diagnosis of a carcinoma or metastases seemed to be predisposed to an increased conjunctival swab contamination risk. A particularly extensive decontamination protocol is recommended in donors with the diagnosis of a carcinoma or metastases.

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