The impact of amphotericin B-fortified preservation media on donor rim cultures and posttransplant infection

Vincent Hussey, Catherine R Sheils, Nasim Salimiaghdam, Kirsten Young, Marjan Farid

Purpose: To determine the impact of amphotericin B supplementation to donor cornea preservation solutions on the rates of positive donor rim fungal cultures and postkeratoplasty fungal infections. Methods: This was a retrospective analysis of cases undergoing corneal transplantations at a single tertiary referral center from 2016 to 2021. Patients undergoing corneal transplantations with and without amphotericin B supplementation to the storage media were reviewed for donor rim culture results and postoperative infection. The primary outcome measures were positive donor rim fungal culture results and postkeratoplasty fungal infection. Results: A total of 1238 corneal transplants were analyzed. Of these, 849 were stored in preservation solution without amphotericin B, while 389 had amphotericin B included. There was a lower incidence of positive donor rim fungal cultures in cases with amphotericin B supplementation (1.8%) compared to the cases without amphotericin B (2.9%), although this difference was not statistically significant (P = 0.24). Of the 389 cases with amphotericin B supplementation, one (0.25%) went on to develop clinically significant infection, while three of 849 (0.35%) cases without amphotericin B developed infection. The sample size was too small to determine the effect of amphotericin B on the incidence of postkeratoplasty fungal infection. Conclusion: The addition of amphotericin B to donor cornea preservation solution resulted in a downward trend of positive donor rim fungal cultures and postkeratoplasty fungal infections, although these differences did not reach statistical significance. Further studies with larger sample sizes are necessary to appropriately determine the impact of amphotericin B supplementation in the storage solution on positive donor rims and postkeratoplasty fungal infections.

Key words: Amphotericin B, donor rim cultures, fortified preservation media, transplant infection

Fungal infection is a rare but potentially serious postoperative complication of corneal transplantation. Reports from the Eye Banking Association of America (EBAA) show a trend of increasing fungal infections following keratoplasty from 2005 to 2014. Although donor rim cultures are not required by the EBAA and the appropriateness of their universal use may be debated, the literature suggests there is a relatively high risk of postoperative fungal infection in cases with positive donor rim cultures. Amphotericin B, an antifungal agent, is offered by some eye banks as an additive to donor cornea storage media. Recent in vitro studies have shown promising results for the efficacy and safety of amphotericin B in donor cornea storage solutions. However, to the authors’ knowledge, no studies have investigated the impact of amphotericin B supplementation on the rates of postkeratoplasty fungal infections in humans.

This work retrospectively examines positive donor rim culture results and fungal infection rates in keratoplasties done with and without amphotericin B in donor cornea storage media at a single academic institution from 2016 to 2021. Herein, we aim to determine the effect of the addition of amphotericin B to corneal preservation media on the rates of positive donor rim cultures and incidence of fungal infection following corneal transplantation.

Methods

Selection criteria
Corneal transplantations performed between April 2016 and May 2021 at a single academic tertiary referral center in Southern California were reviewed. Transplants were performed by a total of 21 primary surgeons including attending surgeons and cornea fellows. All transplantation types including penetrating keratoplasty, endothelial keratoplasty, anterior lamellar keratoplasty, keratolimbal allograft, and patch grafts were included in the study. Beginning July 2018, when transplant media containing amphotericin B became available, all transplant cases were reviewed to determine whether the donor media contained amphotericin B, which was determined by suture preference. At our institution, it is practice to send all donor rims for culture. Cases without a documented positive or negative donor rim culture result were excluded from the analysis.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKLHPMedknow_reprints@wolterskluwer.com

Cite this article as: Hussey V, Sheils CR, Salimiaghdam N, Young K, Farid M. The impact of amphotericin B-fortified preservation media on donor rim cultures and posttransplant infection. Indian J Ophthalmol 2022;70:3294-7.
Variables
All charts were reviewed for positive or negative donor rim culture results, and in case of positive results, the isolated bacterial or fungal organism was noted. The patient charts were reviewed up to 3 months postoperatively to determine the incidence of both infectious and noninfectious complications.

Statistical analysis
Statistical analyses were performed to compare the incidence of positive donor rim cultures and posttransplant infection between the group with transplant media containing amphotericin B and the group without amphotericin B using Chi-squared tests, with \( P < 0.05 \) considered statistically significant. The University of California Institutional Review Board approval was obtained for this retrospective review.

Results
There were 1238 corneal transplantations included in the analysis, the majority of which were penetrating keratoplasty (PKP), followed by Descemet’s stripping automated endothelial keratoplasty (DSAEK) and Descemet’s membrane endothelial keratoplasty (DMEK), with a smaller number of keratolimbal allografts (KLAL), deep anterior lamellar keratoplasty (DALK), keratoprostheses, and corneal patch grafts [Table 1]. The majority of transplants were stored in Optisol without amphotericin B (849 of 1238), with a smaller number stored in Optisol with amphotericin B (389 of 1238). The type of transplants performed in the amphotericin B group was similar to the types of transplants in the non-amphotericin B group, with the exception of a significantly higher proportion of KLAL grafts performed in the amphotericin B group (\( P = 0.01 \)) [Table 1].

In total, there were 32 positive donor rim fungal cultures. *Candida* species accounted for the majority of fungal organisms identified on culture (72%) [Table 2]. There were 29 positive bacterial rim cultures.

Overall, the incidence of a positive donor rim culture in our study for both bacterial and fungal organisms was 4.6%. There was a higher incidence of a positive donor rim culture in the group stored in Optisol without amphotericin B (5.3%) compared to the group stored in Optisol with amphotericin B (3.1%), though this difference did not reach statistical significance (\( P = 0.08 \)) [Table 3]. Similarly, there was a lower incidence of positive fungal culture (1.8% vs. 2.9%) and bacterial culture (1.3% vs. 2.8%) in the group stored in Optisol with amphotericin B compared to the group stored without amphotericin B, though these differences also did not reach statistical significance in our sample (\( P = 0.24 \) and 0.10, respectively) [Table 3]. There was a higher incidence of donor-positive rim cultures in PKP grafts compared to other types of transplanted tissues [Table 4]. Among PKP grafts alone, there was a higher incidence of a donor-positive rim culture in the group without amphotericin B (7.2%) compared to the group with amphotericin B (4.7%); however, this difference did not reach statistical significance (\( P = 0.22 \)). Table 5 further subcategorizes the positive bacterial and fungal culture results based on the type of transplanted tissue and the study group.

Among cases of positive fungal culture results in the amphotericin B group (seven in total), one patient developed infectious keratitis. For this patient, immediate repeat PKP was performed and pathology demonstrated fungal structures on stains of donor cornea. Among the cases of positive fungal culture results in the group without amphotericin B (25 in total), there were three cases with suspected infectious complications. The first patient developed a chronic postoperative endophthalmitis beginning at postoperative month 1, with intravitreal culture

---

**Table 1: Type of transplants included in the study**

| Type of transplant | Optisol with amphotericin B | Optisol without amphotericin B | Total cases | \( P \) |
|--------------------|-----------------------------|-------------------------------|-------------|--------|
| PKP               | 213                         | 489                           | 702         | 0.35   |
| DSAEK/EK          | 90                          | 179                           | 269         | 0.42   |
| DMEK              | 57                          | 144                           | 201         | 0.31   |
| KLAL              | 17                          | 16                            | 33          | 0.31   |
| Kpro              | 7                           | 7                             | 14          | 0.13   |
| DALK              | 4                           | 11                            | 15          | 0.69   |
| Corneal patch graft | 1                          | 3                             | 4           | 0.78   |
| Total             | 389                         | 849                           | 1238        |        |

**Table 2: Fungal organisms identified on donor rim cultures**

| Fungal organism | All cases | Optisol with amphotericin B | Optisol without amphotericin B |
|-----------------|-----------|-----------------------------|-------------------------------|
| *Candida albicans* | 9         | 1                           | 8                             |
| *Candida glabrata* | 8         | 3                           | 5                             |
| *Candida parapsilosis* | 4         | 1                           | 3                             |
| *Candida lusitaniae* | 2         | 0                           | 2                             |
| Mold *Scedosporium* species, not *prolificans* | 2         | 0                           | 2                             |
| *Aspergillus* species | 1         | 1                           | 0                             |
| Dematiaceous mold nonsporulating | 1         | 0                           | 1                             |
| *Fusarium* | 1         | 0                           | 1                             |
| *Penicillium* | 1         | 0                           | 1                             |
| *Phialemonium* inflatum | 1         | 1                           | 0                             |
| *Rhodotorula* | 1         | 0                           | 1                             |
| *Acremonium* | 1         | 0                           | 1                             |

**Table 3: Incidence of positive bacterial and fungal cultures and of fungal infection**

|                           | Optisol with amphotericin B | Optisol without amphotericin B | \( P \) |
|---------------------------|----------------------------|-------------------------------|--------|
| Total transplants         | 389                       | 849                           |        |
| Positive cultures         | 12 (3.1%)                 | 45 (5.3%)                     | 0.08   |
| Positive bacterial culture | 5 (1.3%)                 | 24 (2.8%)                     | 0.10   |
| Positive fungal cultures  | 7 (1.8%)                  | 25 (2.9%)                     | 0.24   |
| Fungal infection          | 1 (Confirmed)             | 3 (Suspected)                 |        |
Table 4: The incidence of donor rim-positive cultures by the type of transplant

| Type of transplant | Optisol with amphotericin B | Optisol without amphotericin B | Total cases | P      |
|--------------------|-----------------------------|-------------------------------|-------------|--------|
| PKP                | 10 (4.7%)                   | 35 (7.2%)                     | 45 (6.4%)   | 0.22   |
| DSAEK/EK           | 2 (2.2%)                    | 4 (2.2%)                      | 6 (2.2%)    | 0.99   |
| DMEK               | 0 (0.0%)                    | 5 (3.4%)                      | 5 (2.5%)    | n/a    |
| KLAL               | 0 (0.0%)                    | 1 (6.2%)                      | 1 (3.0%)    | n/a    |
| Kpro               | 0 (0.0%)                    | 0 (0.0%)                      | 0 (0.0%)    | n/a    |
| DALK               | 0 (0.0%)                    | 0 (0.0%)                      | 0 (0.0%)    | n/a    |
| Corneal patch graft| 0 (0.0%)                    | 0 (0.0%)                      | 0 (0.0%)    | n/a    |
| Total              | 12 (3.1%)                   | 45 (5.3%)                     | 57 (4.6%)   |        |

DALK=deep anterior lamellar keratoplasty, DMEK=Descemet’s membrane endothelial keratoplasty, DSAEK=Descemet’s stripping automated endothelial keratoplasty, KLAL=keratolimbal allografts, PKP=penetrating keratoplasty

Table 5: The type of surgeries performed among the donor rim-positive bacterial and fungal cultures

| Type of transplant | Optisol with amphotericin B | Optisol without amphotericin B | Total cases |
|--------------------|-----------------------------|-------------------------------|-------------|
| PKP                | 10                          | 35                            | 45          |
| Positive bacterial culture | 5                          | 22                            | 22          |
| Positive fungal culture | 5                          | 17                            | 17          |
| DSAEK/EK           | 2                           | 4                             | 6           |
| Positive bacterial culture | 0                          | 0                             | 0           |
| Positive fungal culture | 2                          | 4                             | 4           |
| DMEK               | 0                           | 5                             | 5           |
| Positive bacterial culture | 0                          | 2                             | 2           |
| Positive fungal culture | 0                          | 3                             | 3           |
| KLAL               | 0                           | 1                             | 1           |
| Positive bacterial culture | 0                          | 0                             | 0           |
| Positive fungal culture | 0                          | 0                             | 0           |
| Kpro               | 0                           | 0                             | 0           |
| DALK               | 0                           | 0                             | 0           |
| Corneal patch graft | 0                           | 0                             | 0           |
| Total              | 12                          | 45                            | 57          |

DALK=deep anterior lamellar keratoplasty, DMEK=Descemet’s membrane endothelial keratoplasty, DSAEK=Descemet’s stripping automated endothelial keratoplasty, KLAL=keratolimbal allografts, PKP=penetrating keratoplasty

Discussion

Fungal keratitis and endophthalmitis are serious complications following corneal transplant surgery with an upward trend in the rates of fungal infections in the literature.\cite{1,2} Amphotericin B-supplemented donor cornea preservation media has been proposed as a benefit of decreasing positive fungal donor rim cultures and postkeratoplasty fungal infections. The current study suggests that amphotericin B-fortified donor cornea storage media may decrease the rate of positive donor rim fungal and bacterial cultures, although this trend did not reach statistical significance. The incidence of fungal infection after keratoplasty was extremely low in both groups, and a larger cohort of cases would need to be studied to yield potential benefit.

Previous studies investigating positive donor rim fungal culture rates in storage media without amphotericin B have found the positivity rate to be relatively low, from 1.13% to 5.95%.\cite{6,11-13} Our study is in agreement with these findings, with a donor rim fungal culture positivity rate of 2.9% in cases without amphotericin B supplementation in Optisol. In this study, in cases with amphotericin B added to Optisol, the positive donor rim fungal culture rate decreased to 1.7%; however, this change was not statistically significant (P = 0.24). Multi-center studies with larger sample sizes will be necessary.

In the present study, the incidence of fungal infection postkeratoplasty in cases without amphotericin B supplementation was extremely low, at 0.35% (3/853), similar to previous studies.\cite{6,11-13} The incidence was 0.25% (1/393) in cases with amphotericin B supplementation in Optisol. In this study, with amphotericin B added to Optisol, the positive donor rim fungal culture rate decreased to 1.7%; however, this change was not statistically significant (P = 0.24). Multi-center studies with larger sample sizes will be necessary.

The patient underwent repeat KLAL in addition to PKP. Clinical results being negative for fungal organisms and bacterial organisms and cytology being negative for lymphoproliferative disorder; the cause of the endophthalmitis remained unknown. The second patient developed infiltrates at the graft–host junction at postoperative day 10, which improved on treatment with oral voriconazole; cultures of the graft–host junction infiltrates were not performed. The third patient, a KLAL recipient, developed a newly defined infiltrate at postoperative month 2 on the KLAL segment, in addition to a corneal opacity. The patient underwent repeat KLAL in addition to PKP. Clinical and intraoperative cultures and pathology were negative for fungal structures or fungal growth. In all three cases, we are unable to confirm that infection was related to a positive donor culture, but cannot exclude the possibility; we have, therefore, categorized these cases as suspected donor-related infections. Overall, the incidence of infectious complications in our sample was low; therefore, statistical tests (Chi-squared and Fischer’s exact test) were not performed.

...
The main limitation of this study is that the study is a single-center retrospective review with an inadequate sample size to determine a statistically significant difference in the incidence of positive culture results or fungal complications. Second, the speciation and frequency of posttransplant fungal infections vary regionally, and as our data was collected from a single center in Southern California, the infectious organisms and rates presented here may differ from those in other locales. Additionally, this retrospective analysis did not allow for the optimal control of variables, with all cases of amphotericin B-fortified culture media occurring since 2018, whereas the control group occurred from 2016 to 2021. Finally, there are many donor factors related to donor positive rim culture that were not analyzed in our study, including cause of death, length of hospital stay, and death to preservation time. Despite these limitations, this study is the first to analyze the impact of adding amphotericin B on positive culture results and infection rates.

The use of universal donor rim fungal cultures is controversial.\textsuperscript{3,14} Studies have shown that of the positive donor rim cultures, only about 6% will go on to develop clinically significant infections.\textsuperscript{6,14} In our study, one of seven patients (14%) with a positive donor rim culture went on to develop clinical infection in the group fortified with amphotericin B, compared to three of 25 (12%) in the group without amphotericin B. The number of infections was too small to determine if amphotericin B-fortified storage media impacts the proportion of “true-positive” culture results, or in other words, the number of patients who will go on to develop clinically significant infections after receiving a positive culture result. Further multisite studies may reach sufficient power to determine if amphotericin B impacts the proportion of patients with a positive culture result who will develop a clinical infection later on.

**Conclusion**

Herein, we present one of the first studies investigating the efficacy of amphotericin B supplementation to donor cornea preservation media on donor rim fungal cultures and postkeratoplasty fungal infections. The data show a decreased rate of positive fungal donor rims and posttransplant fungal infections in cases with amphotericin B supplementation, although these differences did not reach statistical significance. Future studies investigating the use of amphotericin B supplementation to donor cornea preservation media with larger sample sizes are necessary to determine its efficacy.

**Acknowledgement**

The authors acknowledge departmental support from an Research to Prevent Blindness (RPB) unrestricted grant.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Aldave AJ, DeMatteo J, Glasser DB, Yu EY, Iliakis B, Nordlund ML, \textit{et al}. Report of the eye bank association of America medical advisory board subcommittee on fungal infection after corneal transplantation. Cornea 2013;32:149-54.

2. Edelstein SL, DeMatteo J, Stoeger CG, Macsai MS, Wang CH. Report of the eye bank association of america medical review subcommittee on adverse reactions reported from 2007 to 2014. Cornea 2016;35:917-26.

3. Ritterband D. The role of corneal donor rim fungal cultures: Pros side. Cornea 2021;40:1085-6.

4. Terry MA. The role of donor rim fungal cultures. Cornea 2021;40:1087-8.

5. Wilhelmus KR, Hassan SS. The prognostic role of donor corneoscleral rim cultures in corneal transplantation. Ophthalmology 2007;114:440-5.

6. Vislisel JM, Goins KM, Wagoner MD, Schmidt GA, Aldrich BT, Skeie JM, \textit{et al}. Incidence and outcomes of positive donor corneoscleral rim fungal cultures after keratoplasty. Ophthalmology 2017;124:36-42.

7. Kiatos E, Armstrong JJ, Hutnik CM, Tsioros SM, Malvankar-Mehta MS, Hodg W G. The value of corneoscleral rim cultures in keratoplasty: A systematic review and cost-effectiveness analysis. Clin Outcomes Res CEOR 2017;9:459-74.

8. Layer N, Cevallos V, Maxwell AJ, Hoover C, Keenan JD, Jeng BH. Efficacy and safety of antifungal additives in Optisol-GS corneal storage medium. JAMA Ophthalmol 2014;132:832-7.

9. Duncan K, Parker J, Hoover C, Jeng BH. The effect of light exposure on the efficacy and safety of Amphotericin B in corneal storage media. JAMA Ophthalmol 2016;134:432-6.

10. Tran KD, Aldrich BT, D’Amato Tóthová J, Skeie JM, Kondratick CM, Giurgola L, \textit{et al}. Efficacy and safety of various Amphotericin B concentrations on candida albicans in cold storage conditions. Cornea 2020;39:110-7.

11. Tsai E, Luong PM, Fogel J, Fogel ES, Zegans ME. Microbiial analysis of donor corneoscleral rims and storage media. Ocul Immunol Inflamm 2019;27:817-20.

12. Alshabeeb RS, Aldayel AA, Martinez-Osorio H, Ahad MA. Incidence and outcome of transplantation of fungal culture-positive donor corneoscleral tissue in optical keratoplasty. Int Ophthalmol 2021;41:867-73.

13. Keyhani K, Seedor JA, Shah MK, Terraciano AJ, Ritterband DC. The incidence of fungal keratitis and endophthalmitis following penetrating keratoplasty. Cornea 2005;24:288-91.

14. Mian SI, Aldave AJ, Tu EY, Ayres BD, Jeng BH, Macsai MS, \textit{et al}. Incidence and outcomes of positive donor rim cultures and infections in the cornea preservation time study. Cornea 2018;37:1102-9.