Surgical Success Evaluation in Limbal Stem Cell Deficiency Due to Chemical Injury – How to Be Accurate?

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Research Article

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Abstract

Purpose: To evaluate limbal stem cell transplantation (LSCT) success in limbal stem cell deficiency (LSCD) due to chemical injury in a tertiary eye care center in Turkey by using 'Limbal Stem Cell Working Group' LSCD grading system.

Methods: Medical records of 80 eyes of 80 patients who underwent LSCT for LSCD secondary to chemical injury were included. The patients were divided into 3 groups according to performed surgery as limbal autograft, limbal allograft, cultivated limbal epithelial cell transplantation (CLET). Surgical success was defined as improvement in the post-operative 1st year LSCD stage.

Results: The mean age of the patients was 37.9±15.7(4-71) with a Male/Female ratio of 2.4. Forty-five(56.3%) patients were injured with alkaline and 16(20%) were injured with acid substance. The mean follow-up time was 60.3±30.6(6-118.6) months. Limbal autograft, allograft and CLET were performed in 58(72.5%), 12(15%) and 10(12.5%) eyes, respectively. The interval between injury and surgery in limbal autograft, limbal allograft and CLET were 43.3±94.1(0.5-592); 14.5±10.6(2.4-32.5) and 122.8±158.9(21.1-504) months, respectively (p=0.02). The overall surgical success rate was 65%. Surgical success rates in each groups were 65.5%, 41.7% and 90%, respectively (p=0.03).

Conclusion: It is vital to accurately determine the stage of the LSCD in order to evaluate the surgical success rates. Surgery type and a longer time interval between the chemical injury and surgery seems to be the most important factors associated with a higher surgical success rate. Despite the limited subject number for subgroups, the results were remarkable to emphasize the significance of the novel LSCD grading system.

Introduction

Chemical injury of the ocular surface is a true ocular emergency that requires urgent and intensive management.[1] Dua classification is used for grading the severity of ocular chemical injury and also may predict the visual prognosis.[2, 3]

Severe ocular chemical injury may cause limbal stem cell deficiency (LSCD), which occurs due to dysfunction of limbal stem cells and impairment of the limbal barrier. LSCD secondary to chemical injury is usually unilateral, and may either be total or partial.[4–6] The accurate evaluation of LSCD has a great importance in choosing the proper management of the disease. Although medical treatment may be useful in mild LSCD, replacement of the limbal stem cells via graft or cell transplantation is mandatory in severe LSCD.[7–11] There was not a consensus about the grading of LSCD until 2019. By then, to evaluate the severity of LSCD, a new grading system was defined by "Limbal Stem Cell Working Group". [9]

After limbal stem cell transplantation (LSCT), the overall surgical success rates vary between 50–80% changing according to the tissue source. Beside the tissue source selected for LSCT, there are several

other factors such as, surgery type, LSCD grade, the duration between the chemical injury and surgical intervention, that may affect the success rates as well.[10–12]

Also for accurate evaluation of the surgical success, objective criteria use is particularly vital.[13, 14] The aim of this study is to accurately evaluate the LSCT success in LSCD due to chemical injury in a tertiary eye care center in Turkey by using 'Limbal Stem Cell Working Group' LSCD grading system.

Materials And Methods

This retrospective study was conducted with the approval of the Ethics Committee of Ege University and in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Patients who were admitted to Ege University Department of Ophthalmology between the years of 2007 and 2019 with LSCD secondary to ocular chemical injury and underwent LSCT were included. Medical records of patients including age, gender, nature of the chemical agent, accompanying ocular injuries (eyelid malformation, symblepharon, etc.), the time interval between injury and LSCT, and transplantation methods were reviewed. Pre- and post-operative (6th month, 1st year, 3rd year follow ups) best-corrected visual acuity (BCVA) measurements (Snellen chart) as well as intraocular pressure (IOP) measurements were documented. Anterior segment photographs of the patients were analyzed by two different researchers, and chemical injury grades and LSCD stages were recorded. Patients with Grade 3 and higher chemical injury grade according to Dua classification[2] were included. Pre- and post-operative (6th month, 1st year, and 3rd year) LSCD stages were categorized according to the staging system established by the 'Limbal Stem Cell Working Group'.[9] According to this classification, Stage 1 was defined as normal corneal epithelium within the central 5 mm zone of the cornea. Additionally, according to the degree of limbal involvement this stage was divided into three subgroups as A, B and C. In Stage 2, the central 5 mm zone of the cornea and also limbus are both affected. In Stage 3, the entire corneal surface is affected. In the presented study, medical records of patients with Stage 1B and/or higher LSCD stages at pre-operative examination were included.

The performed LSCT treatments were limbal autograft, living relative limbal allograft and cultivated limbal epithelial transplantation (CLET). CLET was performed as an explant culture, which was expanded on human amniotic membrane with autologous serum.

'Successful surgical outcome' was defined as improvement in the post-operative 1st year LSCD stage, regardless of visual acuity improvement. The patients were divided into 3 groups according to the used LSCT type as Group 1: limbal autograft, Group 2: limbal allograft, and Group 3: CLET.

LSCD secondary to other causes rather than ocular chemical injury, follow-up less than 6 months, previous ocular surgery or other trauma history, and absence of anterior segment photographs or regular medical records were the exclusion criteria.

Descriptive statistics were given as mean, standard deviation, median, minimum, maximum, frequency and percentage. The Shapiro-Wilk test was used to test the normality assumption of quantitative variables. In univariate analyzes, one-way ANOVA was used for normally distributed variables, and Kruskal-Wallis test (Dunn test for paired comparisons) for non-normally distributed variables. Categorical variables were compared using the Pearson Chi-square test and Fisher's exact test. To examine relationship between quantitative variables, Spearman correlation analysis was performed. Univariate logistic regression analysis was performed to determine the factors affecting the surgical success rate. Parameters with $p < 0.10$ in univariate analyzes were included in the multiple logistic regression analysis. IBM SPSS Statistics 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used in the statistical analysis. $P < 0.05$ was considered significant.

Results

The mean age of the patients was 37.9 ± 15.7 (4–71) with a male to female ratio of 2.4. Forty-five (56.3%) patients were injured with an alkaline and 16 (20%) patients were injured with an acid substance. Nineteen (23.7%) patients were exposed to unknown or mixed substances. The mean follow-up after the surgical intervention was 60.3 ± 30.6 (6-118.6) months. The chemical injury severity of 48 of 80 patients (60%) was Grade 5 or higher at presentation. The majority of them were Grade 6 (n = 27, 33.8%), followed by Grade 5 (n = 21, 26.2%), Grade 4 (n = 18, 22.5%) and Grade 3 (n = 14, 17.5%). The LSCD stage of 58 patients (72.5%) were Stage 2B or above at pre-operative examination. The majority of them were Stage 3 (n = 30, 37.5%), followed by Stage 2B (n = 28, 35%), Stage 2A (n = 10, 12.5%), and Stage 1B (n = 12, 15.0%).

Pre-operative, post-operative 6th month and post-operative 1st year LSCD stages showed positive correlation with chemical injury grade and the results were statically significant ($p < 0.05$) (r: 0.73; 0.61; 0.44, respectively).

Overall, 52 of 80 patients (65%) showed surgical success according to the novel LSCD grading system described by ‘Limbal Stem Cell Working Group’. The mean improvement in LSCD stage in patients with successful surgical outcome was 1.9 ± 0.9 (1–4). The LSCD stages were stable in 22 patients (27.5%) and decreased in 6 patients (7.5%). In these 6 patients with worsening LSCD scores, the mean decrease in the LSCD stage was 1.3 ± 0.8 (1–3). There was not any significant relationship between gender, age, nature of chemical agent, presence of symblepharon and chemical injury grade with surgical success ($p > 0.05$ for all categories). Preoperative LSCD stage did not have statistically significant effect on surgical success rates ($p = 0.1$). In a multivariate logistic regression analysis controlling for age, nature of chemical agent, presence of symblepharon and chemical injury grade; time interval between injury and transplantation predicted surgical success. Longer time interval between injury and transplantation, was associated with higher surgical success rates ($p = 0.05$).

The patients were divided into three groups according to the type of surgery performed. Limbal autograft was performed in 58 (72.5%) eyes, limbal allograft was performed in 12 (15%) eyes, and CLET was

performed in 10 (12.5%) eyes (Fig. 1). There was no significant difference between these three groups in terms of mean age, gender, nature of chemical agent, chemical injury grades and pre-operative LSCD stages ($p > 0.05$ for all categories). The time interval between injury and surgery in limbal autograft, limbal allograft and CLET were 43.3 ± 94.1 (0.5–592); 14.5 ± 10.6 (2.4–32.5); 122.8 ± 158.9 (21.1–504) months, respectively; being significantly higher in CLET group compared to other 2 groups ($p = 0.02$). (Table 1)

Table 1
Variables for different LSCT methods.

Variables	Group 1 (Limbal Autograft)	Group 2 (Limbal Allograft)	Group 3 (CLET)	p value
Number of eyes (n (%))	58 (72.5%)	12 (15%)	10 (12.5%)	
Age (years)				
Mean ± SD	37.19 ± 16.66	38.58 ± 16.02	41.30 ± 8.61	0.74
Range	4–71	6–63	30–56	
Gender (n (%))				
Male	38 (65.5%)	10 (83.3%)	9 (90.0%)	0.17
Female	30 (34.5%)	2 (16.7%)	1 (10.0%)	
Chemical agent (n (%))				
Alkaline	33 (56.9%)	5 (41.7%)	7 (70.0%)	0.39
Acid	12 (20.7%)	4 (33.3%)	0 (0.0%)	
Other/mixed	13 (22.4%)	3 (25.0%)	3 (30.0%)	
Dua chemical injury grade				
Mean ± SD	4.59 ± 1.25	5.33 ± 0.77	4.80 ± 0.91	0.16
Range	2–6	4–6	4–6	
Preoperative LSCD stages (n (%))				
Stage 1B	11 (19.0%)	1 (8.3%)	0 (0.0%)	0.06
Stage 2A	10 (17.2%)	0 (0.0%)	0 (0.0%)	
Stage 2B	17 (29.3%)	4 (33.3%)	7 (70.0%)	
Stage 3	20 (34.5%)	7 (58.4%)	3 (30.0%)	
Time interval between injury and surgery (months)				
Mean ± SD	43.3 ± 94.1	14.5 ± 10.6	122.8 ± 158.9	0.02*
Range	0.5–592	2.4–32.5	21.1–504	

* P<0.05 was considered significant.

Abbreviations: LSCT, limbal stem cell transplantation; CLET, cultivated limbal epithelial cell transplantation; LSCD, limbal stem cell deficiency; SD, standart deviation.

Variables	Group 1 (Limbal Autograft)	Group 2 (Limbal Allograft)	Group 3 (CLET)	p value
Surgical success at the post-operative 1st year (n (%))	38 (65.5%)	5 (41.7%)	9 (90%)	0.03*
* P<0.05 was considered significant.				
Abbreviations: LSCT, limbal stem cell transplantation; CLET, cultivated limbal epithelial cell transplantation; LSCD, limbal stem cell deficiency; SD, standart deviation.				

At the post-operative 1st year, 38 (65.5%) of 58 patients in limbal autograft group had surgical success. In limbal allograft group, surgery success rate was 41.7%, and in CLET, surgery success rate was 90%. The mean improvement in LSCD stage in limbal autograft, limbal allograft and CLET group were 1.7 ± 0.8 (1–3), 2.1 ± 1.1 (1–4) and 2.3 ± 1.2 (1–4), respectively. Surgical success at the post-operative 1st year was significantly higher in CLET group (p = 0.03). Although, limbal autograft group had a better surgical success rate than limbal allograft group, the difference was not statistically significant (p > 0.05). (Table 1)

Additional penetrating keratoplasty (PK) following LSCT had to be performed in 11 (13.7%) of 80 patients. 7 patients were in the limbal autograft and 4 patients were in limbal allograft group. The mean time between LSCT and PK was 22.4 ± 17.7 (4.8–67.7) months. After PK, at the postoperative 6th month, the mean BCVA improved in 10 of 11 patients (90.9%) from 20/5000 ± 20/2222 (range, 20/20000-20/666) to 20/137 ± 20/143 (range, 20/20000-20/50). In 1 patient (9.1%), BCVA and LSCD stage remained the same. No increase in LSCD stage and no decrease in BCVA were observed after PK in any patients. The increase in BCVA after PK was found to be significantly higher in those with a higher initial LSCD stage (p = 0.04).

Discussion

Chemical injury of the ocular surface is a significant public health problem with a serious economic effect due to prolonged hospitalization, long-term medical treatment, multiple surgery requirement, work-day loss, and even blindness.[15] In severe ocular chemical injury, the main cause of blindness is LSCD. The current and definitive treatment of LSCD is the replacement of healthy limbal stem cells.[7, 16, 17]

Young male individuals are at a higher risk of ocular chemical injuries as well as other traumas, especially due to work accidents.[18, 19] In accordance with the literature, in the present study the majority of the patients (n = 57,71.2%) were male and the mean age of the patients was 37.9 ± 15.7.[20]

In chemical ocular surface injury, exposure to alkaline agents is reported to be more frequent than acid agents.[6, 21] In the present study, 56.3% of the overall injuries were caused by alkaline substances in consistent with the literature.

The severity of the injury generally depends on exposure time and type of chemical agent.[1] Potent corrosive agents (mostly alkaline substances) may penetrate deeper in the eye. Therefore, it develops prolonged inflammation not only on the ocular surface, but also in deeper structures causing more severe LSCD and other complications such as glaucoma and cataract formation.[22, 23] In the present study, the eyes with higher chemical injury grades tend to have higher LSCD stages.

For evaluating the surgical success, different success criteria for LSCT were defined among literature. While some authorities described the anatomical improvement in ocular surface as surgical success; in other studies, functional improvement and the patient-reported symptoms are used to evaluate surgical success.[13] In a recent meta-analysis including 40 clinical studies (2202 eyes), the overall success rate of all LSCT was found to be 67.4%, and improvement rate of ocular surface was found to be 74.5%.[14] Furthermore, the lack of standardized criteria for evaluation of LSCD made it difficult to compare the outcomes of different treatment modalities. Although the increase in visual acuity was considered as a success criterion in most previous studies, LSCT actually aims to treat LSCD findings, not to improve the vision. Stromal opacities are the main cause of poor vision in patients with severe ocular chemical injury, so the absence of visual improvement does not actually indicate any treatment failure.[14, 24, 25] Recently in 2019, 'Limbal Stem Cell Working Group' established an objective scale to evaluate LSCD.[9] This staging system provided insight not only in evaluating surgical success, but also in the diagnosis and definition of LSCD, and determination of the appropriate treatment option.[9]

There are several surgical intervention options for LSCD. Limbal autograft is the most commonly performed surgical option in unilateral cases.[25–27] In consistence with the literature, limbal autograft was the most common surgical intervention in the present study.

Surgery type is one of the most important factors that affects success rate. Many different techniques have been described according to the source of the cells and the carrier tissue.[7] While limbal autograft is generally preferred in unilateral LSCD; in bilateral cases, limbal allograft is needed.[28] The main disadvantages of limbal autograft are unrepeatability and LSCD risk at the contralateral healthy eye.[10] In limbal allograft, there are main problems such as risk of rejection and requirement of systemic immunosuppressive therapy use.[29] CLET, on the other hand, is a relatively new and promising method with a success rate of approximately 80%.[4] While repeatability is the most important advantage of CLET, the main disadvantage is the procedures' expensive nature.[30] In a meta-analysis including 40 clinical studies (2202 eyes), improvement of the ocular surface was 85.7% (33–100%) for limbal autograft, 57.8% (0–89) for limbal allograft and 84.7% (44–91) for CLET.[14] Herein, surgical success rates at the post-operative 1st year were; 65.5% for limbal autograft, 41.7% for limbal allograft and 90% for CLET. The improvement in postoperative LSCD stage was statistically higher in the CLET. Although there was no difference between three groups in respect to nature of the chemical agent, chemical injury grades and preoperative LSCD stages, the time interval between injury and limbal transplantation was significantly longer in CLET group. These results suggest that the successful surgical results of CLET may be associated with the longer time interval between chemical injury and LSCT surgery due to the inflammation regression by time, which provides the limbal graft survival better.

It is known that different factors may also affect LSCT outcomes, beside the surgery type.[31] Cheng et al.[32] reported that preoperative symblepharon grade and presence of inflammation both play a significant role in CLET outcomes. In their series with 80 eyes with symblepharon secondary to chemical or thermal injury; success rate of CLET was higher in eyes with grade 1/2 symblepharon than eyes with grade 3 and 4 symblepharon.[32] El-hofi et al.[33] reported better final BCVA results after limbal allograft in Dua-Grade 4 chemical injury than in Dua-Grade 5 chemical injury. In their study with 20 chemical injury patients who underwent limbal allograft, all patients that needed regrafting were Dua-Grade 5. They also reported that all eyes with delayed re-epithelization after limbal transplantation were alkali injury.[33] However, in the present study, there was no significant relationship between gender, age, nature of chemical agent (alkaline/acid/other), presence of symblepharon and chemical injury grade with post-operative 1st year surgical success rates.

It is known that the inflammation is the main cause of graft failure in LSCT.[34] And in the subacute phase of chemical injury, a low degree of inflammation may persist despite the use of anti-inflammatory therapy.[23] Therefore, most of the authorities have indicated that performing LSCT in the chronic period of chemical injury is much better for graft survival.[23, 33] However, publications showing the direct effect of LSCT timing on LSCT outcomes are limited, and the ideal timing of LSCT after chemical injury has not been determined.[26] Rao et al.[35] indicated that surgery at the acute phase of injury (< 4 months) is associated with delayed corneal re-epithelialization and poorer visual outcomes. Sejpal et al.[23] reported a high failure rate in eyes who underwent CLET within 4 months after chemical injury. On the contrary, Ozdemir et al.[36] stated that early LSCT may prevent corneal neovascularization in chemical injury with large epithelial defects and small limbal ischemia. Herein, with 80 eyes, the longer interval between injury and transplantation was associated with the higher surgical success rates.

Also, the pre-operative LSCD stage may also be associated with the prognosis after LSCT. Due to the lack of LSCD grading consensus, there was not any detailed study that evaluates the pre- and post-operative LSCD grading association. In the present study, although lower LSCD stages at the initial examination was tent to have better surgical outcomes at 1st year, the statistical difference was not significant. To the best of our knowledge, this study will be one of the earlier studies, which accurately demonstrates the relationship between pre-operative LSCD stages and LSCT success by using the staging system established by ‘Limbal Stem Cell Working Group’.

LSCT is an effective treatment for ocular chemical injury where only the corneal epithelium and limbus are affected.[37] It provides ocular integrity and improves visual acuity. However, if the corneal stroma is affected, PK is required for visual improvement.[24] PK simultaneously with LSCT is solely preferred in the presence of corneal perforation, since it has a high graft failure rate.[38, 39] It is known that the presence of inflammation and vascularization in the recipient bed increases the risk of rejection of the corneal graft.[40] Therefore, it is recommended to perform PK once ocular surface stability is achieved with LSCT.[38] Although there is no consensus about ideal timing of PK after LSCT, it is recommended to be performed at least 12 months later.[25] In addition, there is limited information in the literature regarding the potential effect of PK on limbal graft survival. Figuerdo et al.[37] recommended performing

PK at least 12 months following CLET. They reported that performing PK at least 12 months after CLET did not negatively affect CLET survival and provided significant improvement in visual acuity.[37] In the present study, 11 of 80 patients underwent PK after LSCT, and the mean time between LSCT and PK was found to be 22.4 months. None of the patients had worsening of LSCD stage and BCVA after PK. Although the number of patients who underwent PK was limited, it was enough to show that PK did not affect limbal graft survival. In 10 out of 11 patients, a significant increase in BCVA was achieved following PK. The increase in BCVA after PK was found to be higher in those with an advanced initial LSCD stage. In LSCD staging, involvement of the central 5 mm zone of the cornea was stated as the most important criteria. The LSCD stage is expected to be high in patients with central corneal involvement. So, in patients with effected central cornea, better visual acuity will be achieved after PK.

Although the main limitations of the study are its retrospective nature and the limited number of patients in the CLET and limbal allograft groups, the results of the current study are remarkable to highlight the impact of the novel LSCD grading system.

In conclusion, LSCT is the surgical treatment of severe ocular chemical injuries that cause LSCD. It is vital to determine the stage of the LSCD accurately in order to evaluate the surgical success rate. The most important factors affecting the outcomes of LSCT are, the method of transplantation and the time between surgery and injury. For this reason, in order to achieve higher success rates, rushing surgeries following chemical injury should be avoided.

Declarations

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The authors have no relevant financial or nonfinancial interests to disclose.

Ethics approval:

The study was approved by Institutional Ethics Committee of Ege University and followed the tenets of Helsinki Declaration

Consent to participate:

Informed consent was obtained from all individual participants included in the study.

Consent for publication:

All authors read the final manuscript and give consent for the article to be published.

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Authors' Contributions:

Ilayda Korkmaz was responsible for conducting research, screening potentially eligible studies, extracting and analyzing data, drafting manuscript. Melis Palamar was responsible for designing and writing the study protocol, coordinating the study and helping to draft the manuscript. Sait Egrilmez was responsible for screening potentially eligible studies, analyzing data and explication of study results. Mehmet Gurdal was responsible for analyzing and conceiving of study results. Ayse Yagci was responsible for updating tables, figures and reference list. Ozlem Barut Selver was responsible for designing the study protocol, coordinating the study, interpreting results and providing feedback on the final report. All authors read and approved the final manuscript.

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Figures

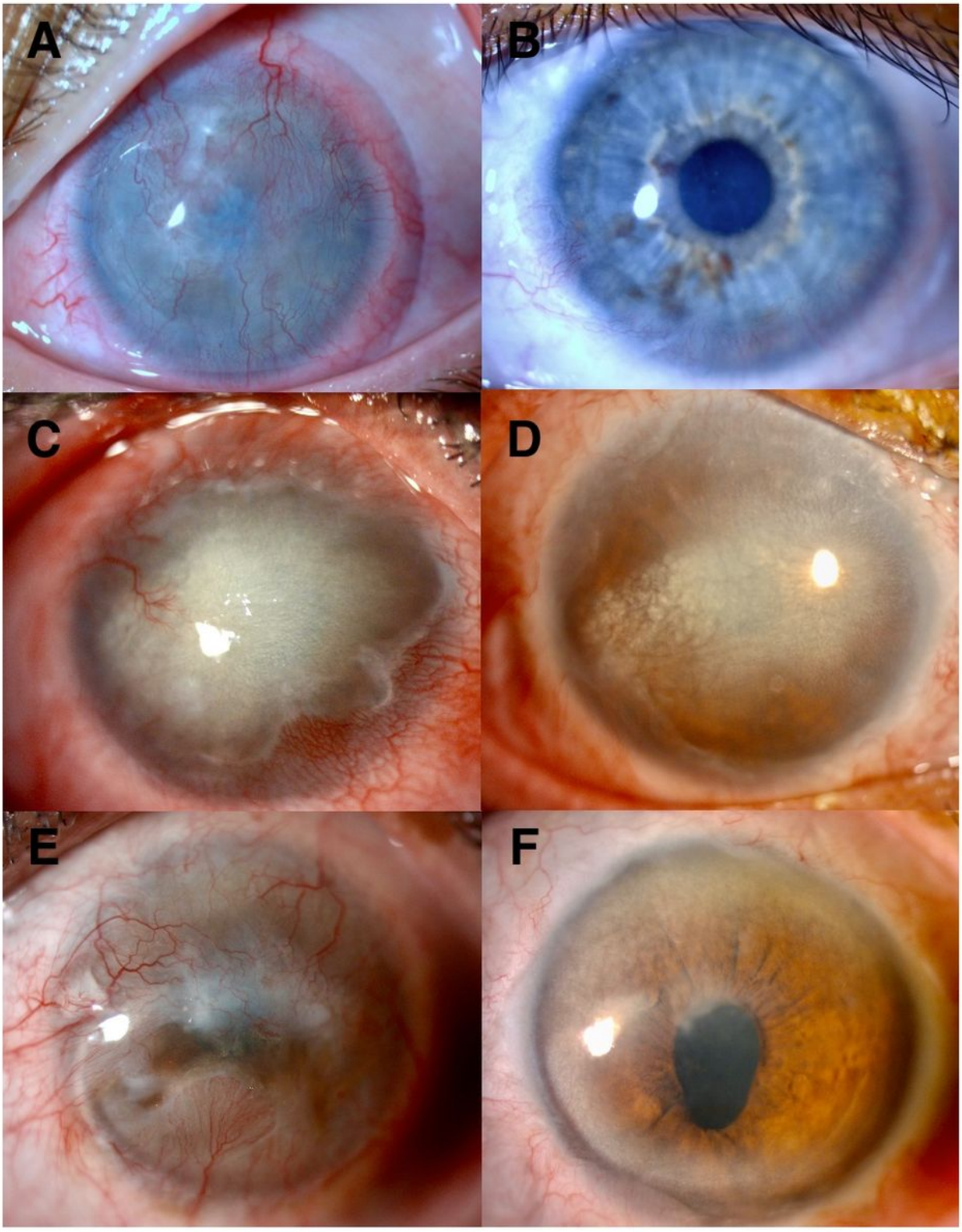

Figure 1

Pre-operative and post-operative 1st year anterior segment image samples of the patients of limbal autograft (A-B), limbal allograft (C-D) and CLET (E-F). (CLET: cultivated limbal epithelial cell transplantation)