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ORIGINAL PAPER

Evaluating CO_2 laser-assisted sclerectomy surgery with mitomycin C combined with or without phacoemulsification in adult Asian glaucoma subjects

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Abstract

Purpose To evaluate and compare outcomes of CO_2 laser-assisted sclerectomy surgery (CLASS) with mitomycin C (MMC) combined with or without phacoemulsification in adult Asian glaucoma patients.

Design Retrospective, comparative study.

Methods Adult Asian glaucoma patients who underwent CLASS alone or combined with phacoemulsification using standardized surgery by two trained glaucoma surgeons between 2014 and 2016 were identified. The main measures of outcome were: intraocular pressure (IOP), use of supplemental medical therapy, best-corrected visual acuity (BCVA), microperforations, macroperforations, and repeated intervention(s) for glaucoma.

Results Forty-one eyes who underwent CLASS alone (13 eyes) or combined with phacoemulsification (28 eyes) were included. Overall, BCVA improved from 0.28 ± 0.19 to 0.14 ± 0.17, and 0.17 ± 0.19, 0.22 ± 0.24, and 0.18 ± 0.23 at 6, 12, 24, and 36 months, respectively. Mean IOP dropped from 17.9 ± 4.7 mmHg to 14.6 ± 5.5, 13.9 ± 3.3, 14.3 ± 3.6, and 14.1 ± 3.7 mmHg, and average number of medications reduced from 2.9 ± 0.9 to 0.2 ± 0.6, 0.5 ± 0.9, 1.0 ± 1.2, and 1.3 ± 1.4 at the same time points. There was no statistically significant difference in BCVA, IOP, and medication reduction between the two groups.

Conclusions CLASS combined with or without phacoemulsification was equally safe and effective, but yielded more modest results in our population.

Keywords Open-angle glaucoma · Angle-closure glaucoma · Laser surgery · CLASS

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Introduction

Glaucoma is the second leading cause of blindness worldwide. It is predicted to affect 79.6 million people by 2020 [1].

Conventional trabeculectomy (TE) is generally considered to be the reference standard for glaucoma

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surgery, but there is an increasing trend in non-penetrating filtration surgery [2]. Non-penetrating deep sclerectomy (NPDS) has been advocated as an equivalent alternative to TE with a higher safety profile [2]. Importantly, it precludes the sudden hypotony that occurs following entry into the anterior chamber (AC) by creating controlled progressive filtration of aqueous into a surgically created intrascleral lake via a thin trabeculo-Descemet window (TDW) [3]. However, traditional NPDS is technically difficult and intraoperative complications include perforation of the TDW during dissection, especially whilst learning [3]. Conversely, if the scleral dissection is not deep enough, effective filtration may not be achieved. Some studies describe NPDS as comparable, [4,5] and others as inferior to TE in terms of intraocular pressure (IOP) reduction [6], especially in the longer term or with a lower target IOP [5,7].

CO_2 laser-assisted sclerectomy surgery (CLASS) uses a CO_2 laser (OT-134 IOPtiMate; IOPtima Ltd, Ramat Gan, Israel) for gradual ablation of scleral tissue layers, leaving a thin TDW. Any percolating fluid readily absorbs the laser energy, thus protecting remaining tissue from further ablation, and avoiding inadvertent perforation with an increased risk of spontaneous iris incarceration or conversion to TE.

Clinical studies of this novel technique are scarce [3,8–11]. At present, the type of ablation, the use of flap sutures, the use of adjuvant antiscarring agents, and the size of flaps are too variable between studies to make comparisons meaningful [3,8–11]. However, as the procedure evolves, there may be a convergence in surgical methods towards an optimized approach.

In this study, we compared the safety and efficacy of CLASS combined with or without phacoemulsification in adult Asian glaucoma patients.

Methods

This was a retrospective, comparative, clinical pilot study.

Fifty-two eyes that underwent CO_2 laser-assisted sclerectomy surgery (CLASS) at a single tertiary institution, Singapore National Eye Centre (SNEC), between 2014 and 2016 were identified from Sunrise Clinical Manager (SCM) or Medical Records Office (MRO).

The inclusion criteria were as follows: adults (18 years or above), diagnosis of primary open angle glaucoma, primary angle closure glaucoma, pseudoexfoliative glaucoma, or normal tension glaucoma in the study eye, no other ocular disorders but cataract, indication for filtration surgery, i.e., IOP not meeting target IOP, defined as IOP level estimated to prevent further glaucomatous optic neuropathy, while on maximal tolerated medications (patients on maximal tolerated medications refer to patients who cannot or will not use medications due to cost issues, memory problems, instillation difficulty, or medication intolerance.).

The exclusion criteria were as follows: diagnosis of glaucoma other than primary open angle glaucoma, primary angle closure glaucoma, pseudoexfoliative glaucoma, or normal tension glaucoma. Concurrent inflammatory or infective ocular disorder, severe systemic disease, or disabling condition were also excluded.

Case records were retrospectively reviewed with the following data extracted: demographics (age at time of surgery, gender, and race); lens status; principal diagnosis (type of glaucoma) and additional diagnoses; previous glaucoma surgery; additional intraoperative procedures at the time of surgery; baseline and postoperative glaucoma medications; baseline (within 2 weeks before surgery) and postoperative (at day 1, week 1, week 4, week 6, month 3, month 6, and yearly thereafter) ophthalmologic examination; intraoperative and postoperative complications; subsequent procedures(s) or operation(s) for glaucoma after surgery.

Ophthalmologic examination included BCVA using Snellen chart, IOP measurement using Goldman applanation tonometry, slit-lamp examination including optic disc evaluation, and visual field assessment using Humphrey (Carl Zeiss Meditec, Jena, Germany) perimetry.

All complications, both intraoperative and postoperative, were recorded and classified according to severity and their relationship to the studied device. Macroperforations were defined as perforations accompanied by iris prolapse. In contrast, microperforations, defined as small holes in the TDW without iris prolapse or loss of AC depth, were not considered to be an adverse event.

The study adhered to the tenets of the Declaration of Helsinki and received approval from the institutional review board.

Surgical procedure

All surgeries were performed under subconjunctival or peribulbar anesthesia with 2% lignocaine without epinephrine.

Following dissection of the perilimbal conjunctiva and tenon capsule, a half-thickness limbal-based 4 × 4 mm superior scleral flap was fashioned with a crescent knife or Beaver blade. The scleral flap was dissected anteriorly until adequate deroofing of the Schlemm's canal (SC). A red laser (HeNe) aiming beam was used to mark the scanning area boundaries. An initial wide scan area was used to repeatedly remove layers of sclera until a percolation zone could be readily identified. The CO2 laser was repeatedly applied with intervals of 2 to 3 s between applications to allow percolation to take place and be detected, until sufficient percolation zone was achieved, then the ablation area was adjusted to deroof the SC across 5 mm of circumference. The scleral flap was then repositioned and sutured with 10–0 nylon sutures or left open in other cases. The conjunctiva was apposed back to its original position with 2 purse string sutures and a mattress suture to ensure a watertight closure.

The MMC used in all patients was either 0.4 mg/ml via sponges for 2 min after scleral flap dissection or 0.2 mg/ml as an intratenon's injection based on surgeon preference. There were adjuvants including Viscoat (Alcon Laboratories, Fort Wort, Texas, USA) in 36 cases, Healaflow (Anteis S.A., Plan Les Ouates, Switzerland) in 6 cases and Duovisc (Alcon Laboratories, Fort Wort, Texas, USA) in 5 cases. Viscoelastic was used as a spacer to prevent the flap from sticking down and to enable the formation of a scleral lake. Clear corneal temporal cataract surgery was performed concurrently if needed.

All patients were treated postoperatively with prednisolone acetate 1% drops and levofloxacin 0.5% drops, 6 times daily for 4 weeks. Patients who had concomitant cataract surgery were treated with the same regimen.

All surgeries were performed by two experienced glaucoma surgeons from SNEC utilizing unified surgical protocols over the same period (2014–2016).

Postoperative safety analysis

Safety outcomes were determined in terms of device malfunctions, device-related macroperforations, or other procedure-related adverse events. To properly assess the safety of the CLASS procedure, any patient who underwent CO2 laser exposure was included in the safety analysis. Patients who subsequently experienced perforation and underwent TE were also included in the safety analysis, but were excluded from the efficacy analysis.

Postoperative efficacy analysis

"Complete success" was defined as $5 \leq \text{IOP} \leq 21$ mmHg and IOP reduction $\geq 20\%$ compared with baseline without medication or repeat filtration surgery. "Qualified success" was defined similarly but with medication. "Failure" was defined as $5 > \text{IOP} > 21$ mmHg or IOP reduction $< 20\%$, loss of light perception, or repeated procedure(s) or operation(s) for glaucoma other than goniopuncture and needling.

Goniopuncture and needling were not considered to be failures or adverse events as both are commonly used as normal postoperative interventions that are required to maintain or augment the operative results of glaucoma surgeries [8]. Goniopuncture was performed if the IOP was creeping up to clinically unacceptable levels for the patient and there was a suggestion of little or no aqueous percolation via the TDW. Needling was performed if the IOP was raised to clinically unacceptable levels for the patient in the presence of a formed bleb, but was scarring up in the subconjunctival region. Hence, needling was considered if the flow through the TDW was thought to be adequate and not the limiting factor, i.e., usually after goniopuncture had been performed.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) Version 24.0 (IBM Corp., Armonk, NY, USA). VA obtained using Snellen was converted to logarithm of the minimum angle of resolution (logMAR) for statistical analysis. Statistical analysis was performed using the paired samples t test

and Wilcoxon signed rank test appropriately to compare preoperative and postoperative data. The Kaplan–Meier survival curves were used to demonstrate the probabilities of success. Statistical significance was defined as a p value of less than 0.05.

Results

Patient data

Fifty-two eyes were initially identified. Five cases were excluded due to diagnosis not meeting inclusion criteria. Two pediatric cases were excluded. One case was excluded due to severe systemic disease of systemic lupus erythematosus complicated by lupus nephritis requiring renal transplant. Three other cases were excluded from the efficacy analysis as the surgical procedure deviated from protocol. This left a total of 41 cases, including 5 bilateral cases.

Apart from two patients who were deceased and lost to follow-up, respectively, after 6 months, all patients were followed up for at least 2 years, and up to 5 years. The mean follow-up was 41.21 ± 2.21 months.

Twenty-nine eyes had primary open-angle glaucoma (70.7%), 8 had primary angle closure glaucoma (19.5%), 3 had normal tension glaucoma (7.3%), and 1 had pseudoexfoliative glaucoma (2.4%). Twenty-eight eyes had concurrent cataract surgery. Demographic data and other baseline characteristics are detailed in Table 1.

Safety analysis

Data on 44 cases were used in the analysis of intraoperative safety outcomes. No device malfunctions occurred. One microperforation and two macroperforations occurred. In these three cases, the decision was made to proceed with trabeculectomy.

Data on the remaining 41 cases were used in the analysis of postoperative safety outcomes. No postoperative procedure-related complications were reported. The AC remained deep and stable in all cases. No wound dehiscence, leaks, or keratopathy were reported. No major complications (such as persistent hypotony or hypotony maculopathy, suprachoroidal hemorrhage or choroidal detachment, malignant glaucoma, hyphema, or endophthalmitis) were reported.

Efficacy analysis

Forty-one cases were included in the final analysis of performance. Standalone ($n = 13$) and combined ($n = 28$) groups were compared. Based on available data at the time point, data were analyzed up to 5 years and compared up to 3 years of follow-up.

Preoperative BCVA of 0.28 ± 0.19 (mean ± SD) improved to 0.14 ± 0.17, and 0.17 ± 0.19, 0.22 ± 0.24, and 0.18 ± 0.23 at 6, 12, 24, and 36 months, respectively (Fig. 1), with statistically significant difference ($p < 0.05$).

The combined group saw greater improvement in BCVA compared to the standalone group (Fig. 1); however, this was not statistically significant. In the combined group, preoperative BCVA of 0.34 ± 0.20 improved to 0.13 ± 0.20, 0.17 ± 0.21, 0.20 ± 0.26, and 0.16 ± 0.26 at 6, 12, 24, and 36 months, respectively, whereas in the standalone group, the eyes had better preoperative BCVA of 0.17 ± 0.11, and minimal change in postoperative BCVA of 0.17 ± 0.08, 0.19 ± 0.12, 0.27 ± 0.17, and 0.24 ± 0.14 at 6, 12, 24, and 36 months, respectively, although the visual acuity in this group was still good at all follow-up time points (BCVA better than 0.3). The difference between the two groups was not surprising given that cataract was extracted in the combined group. Moreover, in the standalone group, 6 out of 13 eyes were phakic preoperatively and out of the 6 phakic eyes, 4 eyes did not undergo cataract surgery throughout the follow-up period while 2 eyes eventually underwent cataract surgery at 2 and 3 years, respectively; hence, any improvement in BCVA would be limited by the natural progression of cataract. All eyes except 1 eye in the standalone group maintained good BCVA of 0.3 or better as of 36 months.

Preoperative use of glaucoma medications decreased from an average of 2.9 ± 0.9 to 0.2 ± 0.6, 0.5 ± 0.9, 1.0 ± 1.2, and 1.3 ± 1.4 at 6, 12, 24, and 36 months, respectively (Fig. 2), with statistically significant difference ($p < 0.05$), yielding a mean medication reduction of 2.6 ± 1.0 (95% CI2.30–2.91), 2.3 ± 1.0 (95% CI 2.00–2.69), 1.8 ± 1.4 (95% CI 1.4–2.27), and 1.5 ± 1.5 (95%

Table 1 Demographic data and baseline characteristics

Number of eyes	41	
Age [mean ± SD (range)] (years)	68.5 ± 9.1 (51.5–84.5)	
Gender	Male	32
	Female	9
Race	Chinese	39
	Malay	2
Lens status	Phakic	34
	Pseudophakic	7
Number of eyes with concurrent cataract surgery	28	
Number of eyes with previous glaucoma surgery	0	
BCVA [mean ± SD (range)] (logMAR)	0.3 ± 0.2 (0.0–0.9)	
IOP [mean ± SD (range)] (mmHg)	17.9 ± 4.7 (11–30)	
Number of glaucoma medications [mean ± SD (range)]	2.9 ± 0.9 (1–4)	
Cup-to-disc ratio [mean ± SD (range)]	0.8 ± 0.1 (0.6–1.0)	
Humphrey visual field	Pattern standard deviation [mean ± SD (range)] (dB)	6.30 ± 3.56 (1.37 − 12.28)
	Mean deviation [mean ± SD (range)] (dB)	− 12.19 ± 9.97 (− 0.88 − − 34.81)
Type of glaucoma	Primary open angle glaucoma	29
	Primary angle closure glaucoma	8
	Normal tension glaucoma	3
	Pseudoexfoliative glaucoma	1

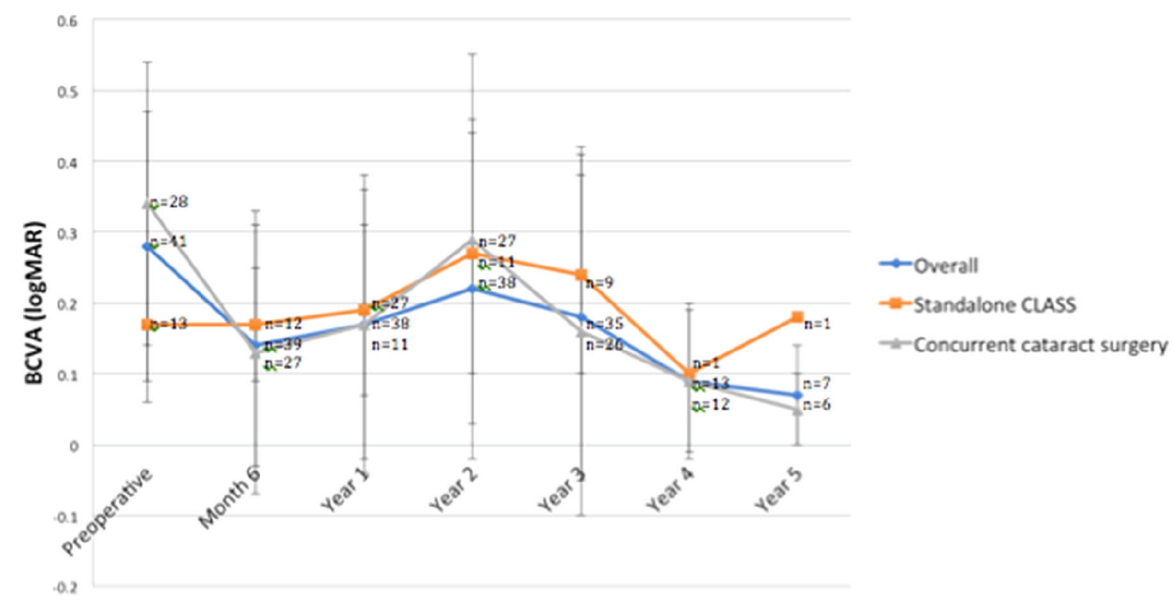

Fig. 1 Best-corrected visual acuity (BCVA) preoperatively and postoperatively

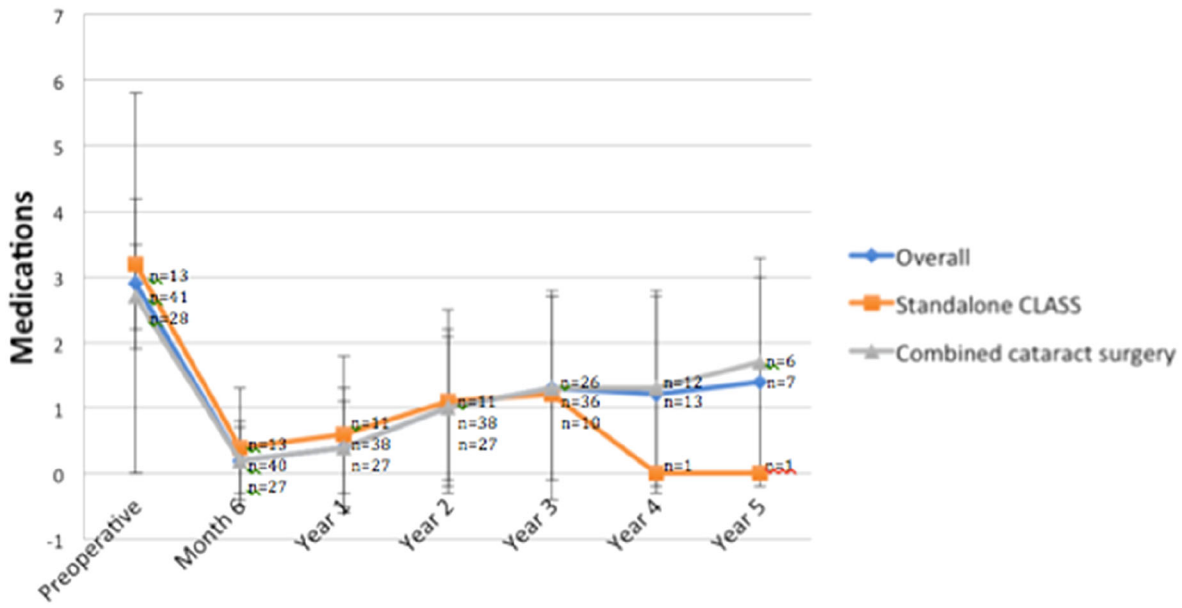

Fig. 2 Medication reduction preoperatively and postoperatively

CI 0.99–2.02) at 6, 12, 24, and 36 months, respectively.

Both groups saw a reduction in medications up to 5 years postoperatively (Fig. 2), and there was no statistically significant difference between the two groups.

Preoperative IOP of 17.9 ± 4.7 mmHg dropped to 14.6 ± 5.5, 13.9 ± 3.3, 14.3 ± 3.6, and 14.1 ± 3.7 mmHg at 6, 12, 24, and 36 months, respectively (Fig. 3), with statistically significant difference ($p < 0.05$), corresponding to a mean percentage reduction of 13.6, 16.5%, 14.7, and 14.1% at 6, 12, 24, and 36 months, respectively.

Both groups saw a reduction in mean IOP (Fig. 3), with no statistically significant difference between the two groups. The standalone group appeared to yield larger percentage reduction (36.7, 40.6, 33.9, and 18.2% at 6, 12, 24, and 36 months, respectively) compared to the combined group (2.6, 6.7, 6.9, and 12.4% at 6, 12, 24, and 36 months, respectively); however, this was not significant except at 1 year ($p < 0.05$), and the combined group had significantly lower preoperative IOP (mean 16.75 mmHg) compared to the standalone group (mean 20.31 mmHg) ($p < 0.05$).

Defining success as $5 \leq$ IOP ≤ 21 mmHg and IOP reduction $\geq 20\%$, complete success rates after 6, 12, 24, and 36 months were 41.5, 41.5%, 34.1, and 24.4%, respectively, whereas qualified success rates were 48.8, 53.7, 61.0, and 51.2%, respectively. The mean survival time was 43.3 ± 10.5 (95% CI 22.8–63.8) weeks. Kaplan–Meier survival curve for probability of CLASS success are presented in.

Thirty-one needling procedures were carried out in 16 eyes between 1 week and 5 years after surgery. Twenty-one YAG laser goniopuncture procedures were carried out in 17 eyes between 1 week and 5 years after surgery. One post-needling hyphema occurred, which was mild and resolved spontaneously shortly after. There were no other procedure-related complications (Fig. 4).

Two eyes had progression of cataract requiring cataract surgery at 2 and 3 years, respectively, during the term of the study.

Discussion

In our study, no hyphema, hypotony, or other postoperative procedure-related complications were reported, which concurred with other published studies of CLASS reporting either no, [8–10] or low rates of hyphema (2.8%) and hypotony (4.6%), [11] and were comparable to those of NPDS [12]. These results confirmed that the safety profile of CLASS is significantly better than TE and at least as good as NPDS. A

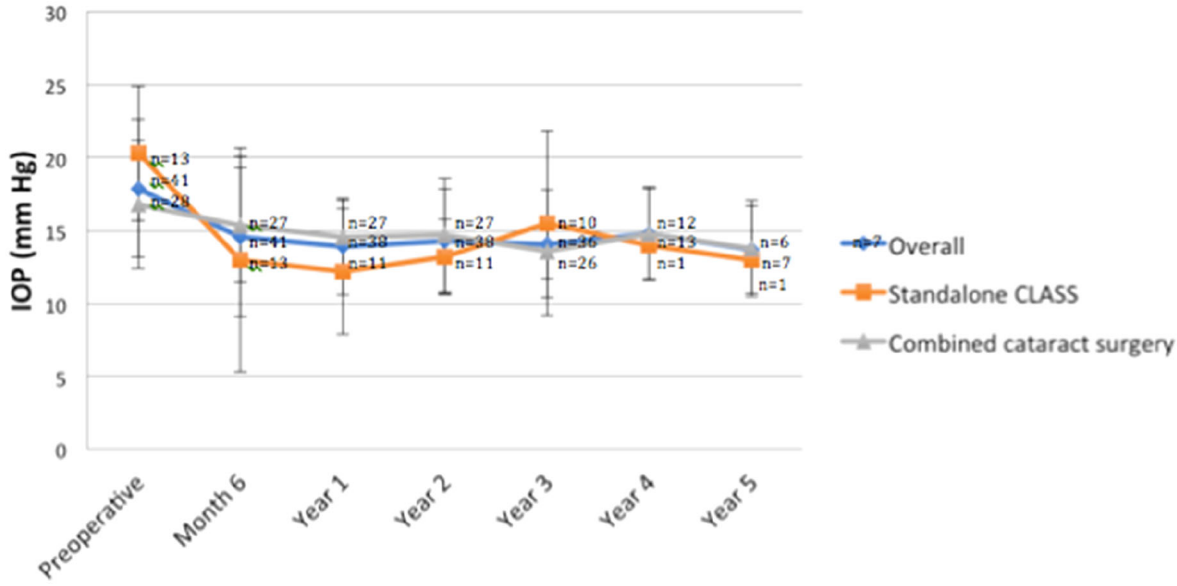

Fig. 3 Intraocular pressure (IOP) reduction preoperatively and postoperatively

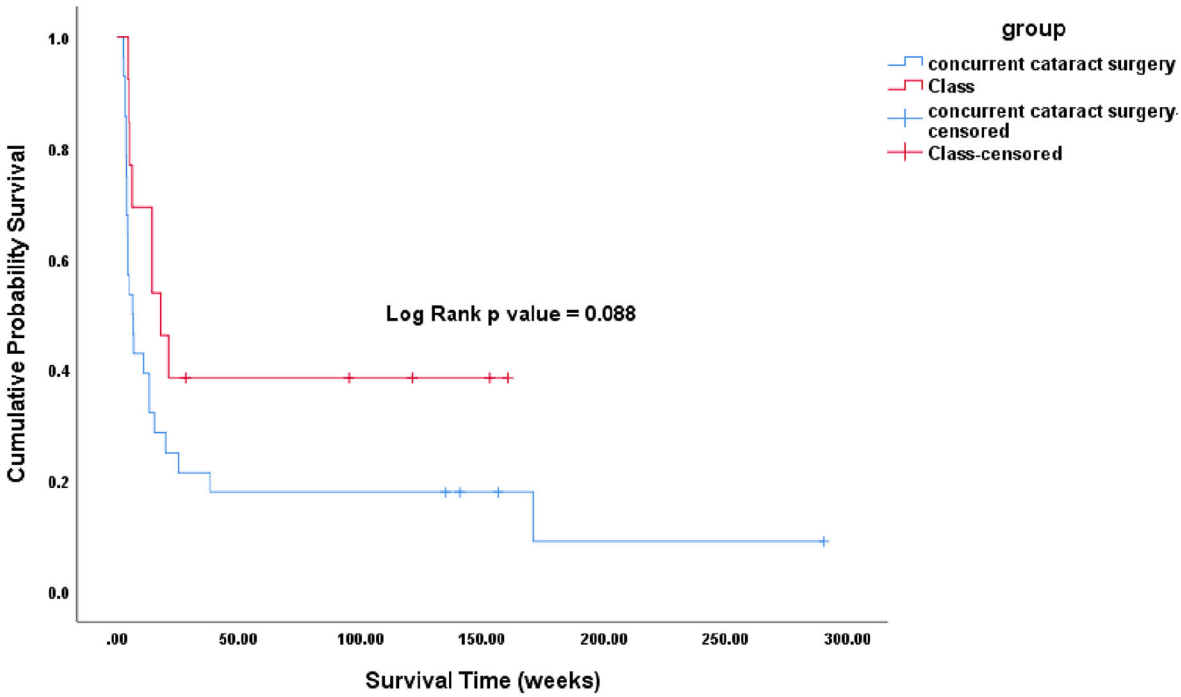

Fig. 4 Survival curves for standalone CLASS and combined cataract surgery

systematic review by Cochrane found that the rates of adverse events were as high as 65% with TE and 17% with NPDS [12]. The rates of hyphema were 13% and 4.6% and those of hypotony were 15.6% and 2.6% in TE and NPDS, respectively. A large study of Asian patients reported hypotony as the commonest complication after TE, followed by bleb leak and hyphema [13]. Hyphema is typically transient and resolves spontaneously. While hyphema is usually not vision threatening, hypotony potentially leads to visual

impairment and may require intervention for resolution.

Intraoperatively, one microperforation and two macroperforations occurred, and all three cases were successfully converted to TE. For the single case of microperforation, although the decision was made to proceed with TE, microperforations are not regarded as harmful, as small holes that are not associated with loss of AC depth or iris prolapse may improve aqueous drainage, and do not necessitate conversion to TE [8]. Some surgeons advocate purposeful micropuncturing of the canal roof to improve fluid flow [8]. However, especially in Asian eyes, there is a risk of iris incarceration.

Even though the risk of inadvertent perforation into the AC is reduced, it is not completely removed. Difficulty finding the landmarks or applying the laser too anterior to the SC can cause macroperforation. For the two cases of macroperforation, we reasoned that the working energy of the laser might have been too high. For this pilot study, we adopted the laser settings as per our Caucasian counterparts and kept the energy level similar for all patients. Another CLASS study, also conducted within an Asian population, concluded that the clinical response of Asian eyes to laser might differ from Caucasian eyes [10].

The macroperforation rate in our study was 5.6% and was consistent with other CLASS studies [3,8–11]. In comparison, the macroperforation rate of NPDS during the initial learning phase may be as high as 30% to 50% [3]. While it is possible to convert to TE for cases of macroperforation, these would be unplanned cases and as such the surgery may not be ideally executed and lead to inferior results or higher complication rates. The conversion rate to TE was 8.6% (comprising one case of microperforation and two cases of macroperforation) in our study. These cases of conversion were necessary as the CO2 laser might have been applied too anteriorly or there was too little aqueous percolating to shield the area adequately. As the CLASS studies including ours reported results of surgeons who performed the procedure while at the beginning of their learning phase, the lower rate of macroperforation suggesting relative simplicity of the CLASS to traditional NPDS is welcome.

The IOP achieved in our study (14.6 ± 5.5, 13.9 ± 3.3, 14.3 ± 3.6, and 14.1 ± 3.7 mmHg at 6, 12, 24, and 36 months, respectively) was comparable to that achieved in published NPDS studies, [2,6,17] with equivalent reduction in glaucoma medications (0.2 ± 0.6, 0.5 ± 0.9, 1.0 ± 1.2, and 1.28 ± 1.4 at 6, 12, 24, and 36 months, respectively) [6,12].

The IOP reduction pattern in our study was similar to other CLASS studies, demonstrating initial postoperative descent that stabilized after 4 weeks postoperatively up to 12 months postoperatively [3,8,9]. However, CLASS appeared to yield more modest results in the Asian eyes of our study (Table 2).

The lower success rates of our study were attributed to several factors. The majority of published studies comprise Caucasian populations, where the amount of scarring is less and the laser may be better calibrated. More exuberant fibrotic responses causing aggressive subconjunctival scarring and surgical failure in Asian eyes compared to Caucasian eyes is well-known [14,15], so the results of our study compared to studies with Caucasian eyes are not surprising. The results of TE are known to vary by population, and there is no reason to expect any difference in CLASS.

There was a greater proportion of PACG cases in our study (19.5%) compared with Yick et al.'s (8.7%) and Yu et al.'s (0%) [10,16], which were susceptible to iris plugging or microperforations and prone to more complications as a result of the anatomic abnormalities that accompany PACG, leading to early failure.

We included the learning curve for the procedure as all results were used and none were excluded as in some studies. Two different laser calibrations were trialed during our term of use with the CLASS laser; hence, the ablation might have been underpowered initially leading to a thicker TDW that is prone to failure. The variable use of spacers based on availability and surgeon preference might have influenced results. We also included the learning curve for postoperative management including goniopuncture.

We recognized the lower baseline IOP (mean 17.9 mmHg) of our patients, who managed to achieve lower IOP (mean 14.1 mmHg) with significantly reduced medications. Such IOP in a patient with milder glaucoma coupled with reduced medication burden might well be an adequate outcome. All 3 eyes with NTG achieved at least qualitative success with IOP reduction $\geq$ 20% as of their last follow-up.

To the best of our knowledge, this is the first study comparing CLASS combined with or without phacoemulsification. The continued advancement of equipment and refinement of technique has paved

Table 2 Comparison of qualified success among CLASS studies

CLASS study	Population	Combined with phacoemulsification	Qualified success (%)
This study	Asian	Yes	46.4, 53.6, and 50.0 (12, 24, and 36 months)
		No	69.2, 76.9, and 53.8 (12, 24, and 36 months)
		Overall	53.7, 61.0, and 51.2 (12, 24, and 36 months)
Geffen et al. (European Ophthalmic Review, 2011)	Caucasian	No	86.6 (12 months)
Geffen et al. (JOG, 2016)	Caucasian	No	84.8 (36 months)
Sakaat et al. (JOG, 2014)	Caucasian	No	90.9 (12 months)
Greifner et al. (JOG, 2016)	Caucasian	No	96 (24 months)
Yick et al. (Medicine, 2016)	Asian	No	81.8 (6 months)
Yu et al. (JOG, 2018)	Asian	Yes	88 (12 months)

the way for combined glaucoma and cataract surgery as a practical strategy for controlling IOP and improving VA. The only study of CLASS with phacoemulsification to date by Yu et al. found that the combined operation effectively reduced IOP and medication use during the early postoperative period [16]. They also demonstrated a greater decrease in IOP (39%) than Yick et al. (19%), which could be attributed to their lower preoperative IOP or the combined surgery. Our study showed that patients who underwent CLASS alone had a greater percentage reduction in IOP than patients who underwent concurrent cataract surgery, although this again could be due to significantly lower preoperative IOP in the combined group.

Phacotrabeculectomy can produce poorer long-term surgical success due to inflammation from phacoemulsification [17,18]. Our comparative results showed comparable efficacy of CLASS with or without phacoemulsification. This can be attributed to the filter being the TDW controlling aqueous flow, as well as providing a means to increase outflow through goniopuncture. The creation of the TDW also induces less inflammation than penetrating procedures. The larger size of the scleral flap may allow higher outflow rate, better pressure regulation, or increased diffusion area. The combined effects of cauterization and ablation of tissues by the laser may cause less scarring overall.

We are aware that interpreting outcomes between different studies can be difficult when outcome definitions vary, preoperative characteristics are not well defined, and outcome assessments are not standardized. Hence our study has several limitations from accepting a certain degree of surgical variation in technique, to its retrospective nature and the inevitable dropout rate. No imaging of the blebs was undertaken to assess the status of the scleral lake and the conjunctival drainage either.

Based on our standardized pilot study with strict inclusion criteria, we found that the results are sufficiently promising to suggest that CLASS combined with or without phacoemulsification are equally simple, safe, and effective at least in the short and intermediate term. Combined surgery is preferred for patients with coexisting cataract.

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Compliance with Ethical Standards

Conflicts of interest The authors have no financial interests or conflicts of interest in this manuscript.

Availability of data and material The authors confirm that the data supporting the findings of this study are available within the article (and/or) its supplementary materials.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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