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Indocyanine green-augmented diode laser treatment of port-wine stains: clinical and histological evidence for a new treatment option from a randomized controlled trial

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Summary

Background Complete clearance of port-wine stains (PWS) is difficult to achieve, mainly because of the resistance of small blood vessels to laser irradiation. Indocyanine green (ICG)-augmented diode laser treatment (ICG+DL) may overcome this problem.

Objectives To evaluate the feasibility of ICG+DL therapy of PWS and to compare the safety and efficacy of ICG+DL with the standard treatment, flashlamp-pumped pulsed dye laser (FPDL).

Methods In a prospective randomized controlled clinical study, 31 patients with PWS were treated with FPDL (λ_{em} = 585 nm, 6 J cm^{-2}, 0·45 ms pulse duration) and ICG+DL (λ_{em} = 810 nm, 20–50 J cm^{-2}, 10–25 ms pulse duration, ICG-concentration: 2 mg kg^{-1} body weight) in a split-face modus in one single treatment setting that included histological examination (haematoxylin and eosin, CD34). Two blinded investigators and the patients assessed clearance rate, cosmetic appearance and side-effects up to 3 months after treatment.

Results ICG+DL therapy induced photocoagulation of medium and large blood vessels (> 20 μm diameter) but not of small blood vessels. According to the investigators' assessment, clearance rates and cosmetic appearance were better after ICG+DL therapy than after FPDL treatment (P = 0·114, P = 0·291, respectively), although not up to a statistically significant level, whereas patients considered these parameters superior (P = 0·003, P = 0·006, respectively). On a 10-point scale indicating pain during treatment, patients rated ICG+DL to be more painful (5·81 ± 2·12) than FPDL treatment (1·61 ± 1·84).

Conclusion ICG+DL represents a new and promising treatment modality for PWS, but laser parameters and ICG concentration need to be further optimized.

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Port-wine stains (PWS) are congenital, progressive vascular malformations of the dermis that are often disfiguring, particularly when located in the head and neck area where most lesions occur. The incidence is estimated at 3 per 1000 live births.[1] PWS are characterized by ectatic capillary and venule-sized vessels within the papillary and reticular dermis. These ectatic blood vessels show varying diameters ranging from 10–150 μm in plain PWS and up to 500 μm in hypertrophic lesions.[2–4]

Because of its proven efficacy and low incidence of side-effects, the flashlamp-pumped pulsed dye laser (FPDL) with a wavelength of 585 nm and a pulse duration of 0·45 ms has become the gold standard for the treatment of PWS.[5] Complete clearing of PWS is obtained only rarely.[6,7] Furthermore, about 20% of PWS are resistant to FPDL treatment.[8] We showed in a recent study that smaller blood vessels (< 29 μm) of PWS are often resistant to FPDL treatment,[9] which could be one reason for limited clearance or even complete resistance of some PWS to laser treatment. Another reason could be the unsteady red blood cell concentration within smaller blood vessels resulting in an inhomogeneous concentration of the chromophore haemoglobin.[10,11] To improve the selective thermal damage to smaller blood vessels, our group used indocyanine green (ICG) as an exogenous

target chromophore in an animal model.[12] The water-soluble tricarbocyanine dye ICG, which has been used in medical diagnosing since 1956,[13] has been approved for numerous function tests.[14,15] In general, the intravenous administration of ICG has a low potential for side-effects. However, ICG contains sodium iodide and should be used with caution in patients with a history of allergy against iodides. Allergic reactions occurring after intravenous injection of ICG are described in the literature.[16] Rarely, patients may develop haematoma, nerve damage, phlebitis or vasovagal episodes as a result of venipuncture.

One advantage of ICG is the fact that the chromophore shows maximum absorption at about 810 nm, thus a diode laser (805 nm) with a deeper light penetration into tissue than FPDL irradiation (585 nm) can be used for excitation. In an animal study, we showed that even small blood vessels can be coagulated by ICG-augmented diode laser (ICG+DL) irradiation;[12] thus, this treatment was more effective than FPDL therapy in the same animal model.[9]

Based on the broad body of preliminary work, we compared the impact of ICG+DL therapy with the standard treatment, i.e. FPDL therapy, in 31 patients with PWS in a randomized controlled clinical trial. To the best of our knowledge, this study is the first report on ICG+DL of PWS in the literature.

Patients and methods

Patients

Thirty-two caucasian patients of skin type I–IV according to Fitzpatrick with PWS (age > 6 years, 16 female, 16 male) were randomized, and 31 patients received treatment by an experienced dermatologist (Fig. 1). All patients were given written and verbal information on the nature of this pilot study. Signed informed consent was obtained before treatment from patients or their parents if patients were under the age of 18 years. The study was approved by the local ethical committee and by the federal authority (Federal Institute for Drugs and Medical Devices; EudraCT No 2009-009956-20) and carried out in accordance with the Declaration of Helsinki. The study was conducted between April 2010 and April 2011 at the Department of Dermatology, University Hospital Regensburg, Germany.

Laser devices, randomization, treatment parameters and indocyanine green application

Lesions were photo documented before treatment. Areas of 20 cm^2 that were representative with regard to the colour and surface structure of the PWS were chosen for laser treatment.

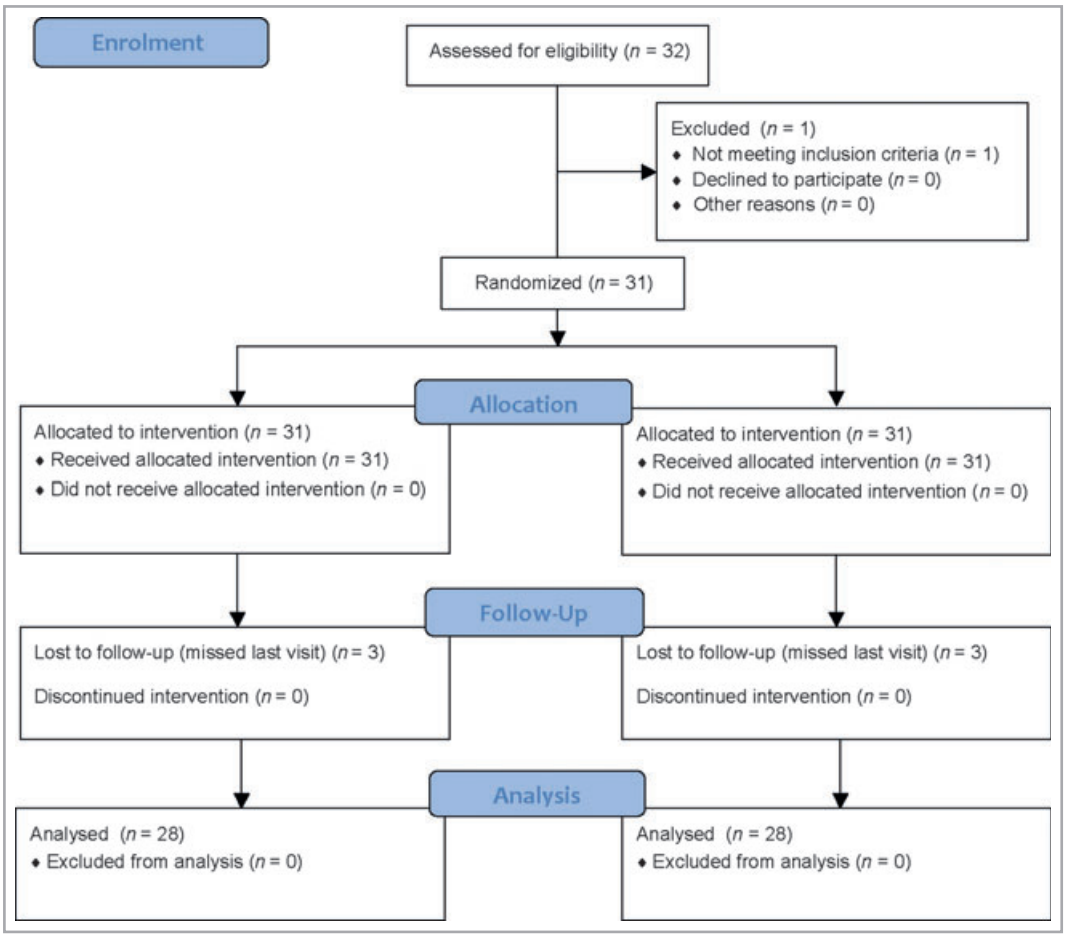

Fig 1. Flow diagram showing the flow of participants in the study including dropouts according to CONSORT guidelines.

The treatment area was divided into three parts. Depending on the lesion site, different dividing procedures were employed: when the PWS was located unilaterally or nonsymmetrically on the extremities or trunk, it was divided through the sagittal or transversal axis but through a transversal axis when located unilaterally on the face. For each laser procedure (FPDL, diode laser alone, ICG+DL), two treatment areas of 2 × 2 cm were chosen (Fig. 2b) so that six to nine laser spots could be applied for each procedure. The Centre for Clinical Studies generated the randomization list by a computer using the software PASS 2008 (NCSS, Kaysville, UT, U.S.A.) so that patients were consecutively allocated to ICG+DL treatment in the right/above or the left/below part of the treatment area. Sealed envelopes were used to conceal the random allocation sequence.

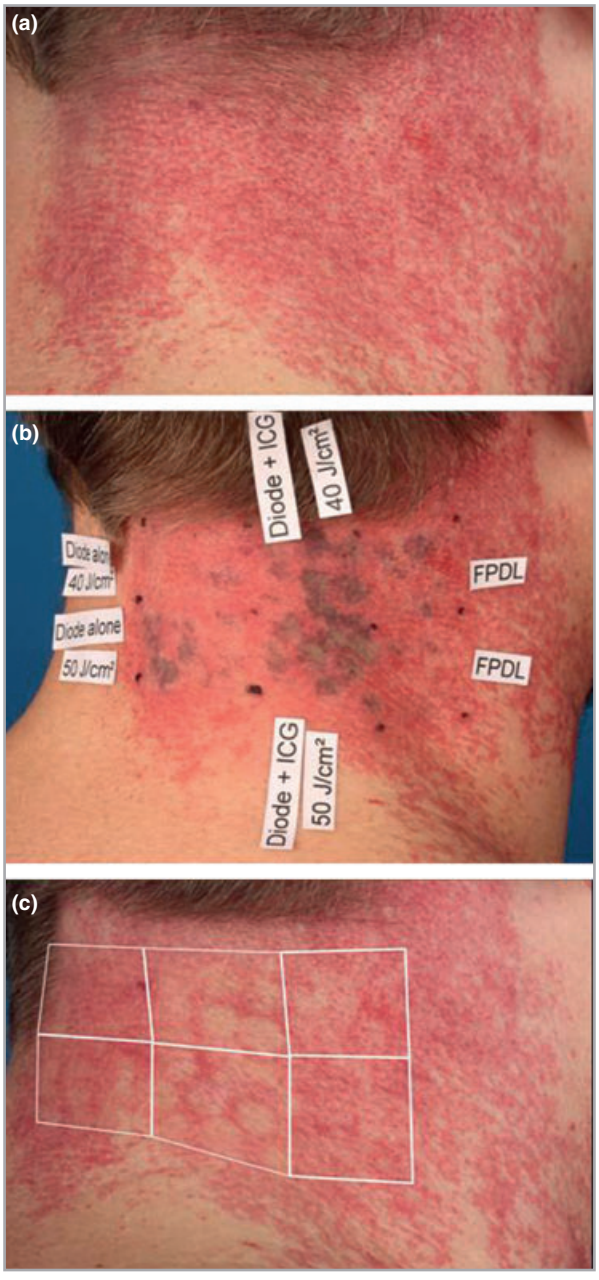

Fig 2. Clinical image (a) prior to, (b) immediately after and (c) 3 months after study treatment. Vessel clearance is most pronounced after indocyanine green (ICG)-augmented diode laser treatment. There is no difference between 40 and 50 J cm^{-2}. There was minor clearance after diode laser therapy alone, which was applied for safety reasons before the application of ICG. The flashlamp-pumped pulsed dye laser (FPDL) failed to be effective here.

Two parts of the treatment area were randomized to the two procedures: FPDL and ICG+DL. First, the FPDL (λ_{em} = 585 nm, 6 J cm^{-2}, 7 mm spot size, 0·45 ms pulse duration; 10–20% overlapping technique; Cbeam™, Candela Laser Corp., Wayland, MA, U.S.A.) was applied. Afterwards, the diode laser (λ_{em} = 810 nm, 20–50 J cm^{-2}; 7 mm spot size; 10–25 ms pulse duration; LightSheer XC, Lumenis, CA, U.S.A.) was used in close proximity. Diode laser without intravenous ICG application was applied for safety reasons and in terms of a 'negative control' in the third part of the treatment area. As the diode laser has a quadratic spot size of 12 mm^2, we used a paperboard stencil to obtain 7mm spots. In the next step, the patient received an intravenous access into the median cubital vein. ICG (Pulsion Medical Systems AG, Munich, Germany) was dissolved in water for injection (50 mg ICG in 10 mL aqua ad iniectabilia) at a concentration of 5 mg mL^{-1} and immediately injected intravenously as a bolus. The ICG concentration administered was 2 mg kg^{-1} body weight (b.w.). After intravenous application, ICG remains within the intravascular space because of its binding to plasma proteins, and in 80% to globulins, mainly α-lipoproteins (molecular weight 200 kDa).[11] ICG fluorescence was visualized using a commercial fluorescence imaging system (IC-View®; Pulsion Medical Systems, Munich, Germany) consisting of a digital video recorder with an integrated near-infrared (NIR) light source (energy 0·16 W, wavelength 780 nm) and recorded on video tape (BASF, Ludwigshafen, Germany). The object lens of the camera was covered with a filter (835 nm) to collect NIR radiation and reject visible light. The half-life is approximately 2·5–3 min, and intravenous ICG does not enter the enterohepatic circulation.[17] Because of its fast pharmacokinetics, intravenous ICG allows administration and light irradiation in one session. Because of the short half-life time of ICG in plasma, diode laser treatment (λ_{em} = 810 nm, 7 mm spot size; LightSheer XC) was started 2–3 min after intravenous ICG application. The first eight patients were treated with 10, 20, 30, 40 and 50 J cm^{-2} (pulse duration: 5, 10, 15, 20, 25 ms). As only fluence rates of 40 and 50 J cm^{-2} induced any visible clearance in the 6-week follow-up, no lower fluence rates were used in the following treatments. The FPDL has an integrated spray cooling system to protect the epidermis, whereas the diode laser has an integrated cooling system within the handpiece. In addition, a thin layer of water-based ultrasound gel was applied to the treatment area before ICG+DL.

Assessments and response evaluation

After the screening visit, patients received the treatment within 2 weeks (visit 1). Follow-up visits were done 1 week

post-treatment for screening of side-effects (visit 2), and 6 weeks (visit 3) and 12 weeks post-treatment (visit 4) for evaluation of side-effects and efficacy. At each visit, results were photo documented (Fig. 2). Photographs of all treatment sites were taken under standardized conditions with the same camera [Canon Digital Camera EOS D30, Canon Macro Lens, EF-50 mm 1 : 2·5, and lens mounted ring lite (MR-14EX); all Canon, Tokyo, Japan]. Before therapy, the cosmetic appearance of the treatment area was evaluated by means of a continuous scale from 0 to 10 (0, very poor cosmetic appearance; 10, very good cosmetic appearance) by the patients. Pain during treatment was documented for each FPDL as well as ICG+DL treatment on a visual analogue scale (VAS) ranging from 0 to 10. At each visit, the clearance rate of the treatment area was rated by the patients as follows: no clearance, slight clearance (< 25%), moderate clearance (25–50%), good clearance (51–75%), or excellent clearance (> 75%). Side-effects (hypopigmentation, hyperpigmentation, atrophy, scar, hypertrophic scar, keloid formation, infection) and therapy sequelae (blistering, purpura or crusting) in the treated areas were assessed by the investigators (A.K., P.B.).

Physician-based assessment and response evaluation were carried out on the basis of photo documentation by two independent, blinded investigators. If the investigators documented different values, the mean was calculated. The cosmetic appearance of the treatment area and the clearance rate were evaluated for each visit as described above.

Histology and immunohistochemistry

Punch biopsies were taken before (n = 8), and 1 week (n = 10) and 3 months (n = 5) after laser treatment (ICG+DL) in eight adult patients with a PWS in a cosmetically less important area. After disinfection of the skin, 4-mm punch biopsies were performed under local anaesthesia with prilocaine 1% (1–2 mL). The wound was closed with nylon sutures. The tissue was fixed in 2% formalin, embedded in paraffin and prepared for paraffin sections (2 μm). Sections were stained with haematoxylin and eosin for routine histology. Endothelial cells were stained immunohistochemically with the endothelial marker CD34 (QBEnd/10; Leica Inc., Newcastle upon Tyne, U.K.) to evaluate the impact of ICG+DL on blood vessels.[12] Histological scores in terms of tissue destruction (epidermis, dermis, subcutis) and vessel destruction (depth of destructed vessels, diameter of destructed vessels) were rated using 20-fold magnification in eight regions of interest. The biopsies were analysed by two experienced dermatopathologists (M.L., P.B.).

Outcome measures

The primary outcome parameter was the proportion of eligible patients successfully completing the study. Secondary outcome parameters were adverse events and local reactions during the application of the study medication as well as during and after study therapy, and clearance rates of PWS and cosmetic outcome after 6 weeks and 3 months evaluated by the patients and the two blinded observers.

Quality assurance and monitoring

The study protocol was established by the Department of Dermatology as the scientific study centre in cooperation with the Centre for Clinical Studies at the University Hospital Regensburg. Data were closely monitored onsite according to Good Clinical Practice criteria. Additionally, all data were checked for completeness and plausibility in the Centre for Clinical Studies by in-house monitors as well as by an independent organization.

Statistical methods

Continuous data are summarized as mean and SD or as median values and interquartile ranges (first to third quartile). Categorical data are expressed as frequency counts and percentages. For the primary endpoint, we used a one-sided binomial test with a target significance level $\alpha = 0{\cdot}05$. Based on the number of patients successfully completing this pilot study, the following decisions were made. If, out of 33 patients, 21 or fewer patients complete the study successfully, then the hypothesis that H_0 is $P \leq 0{\cdot}5$ is not rejected. In this case, the planned main confirmatory study would not be considered feasible. If, out of 33 patients, 22 or more patients complete the study successfully, then the hypothesis that H_0 is $P \leq 0{\cdot}5$ is rejected in favour of the hypothesis that H_1 is $P \geq 0{\cdot}75$. In this case, the planned main confirmatory study would be considered feasible. Furthermore, exact two-sided confidence intervals for the point estimate are reported. The sample size was calculated by means of the primary endpoint by using the software PASS 2008 (NCSS).

Inter-rater agreement was assessed by means of Cohen's kappa for categorical outcomes and by means of intraclass correlation for continuous outcomes.

For clinical ratings, repeated measures two-way analysis of variance (ANOVA) was used to investigate the interaction and significance of the differences between the type of treatment (ICG and FPDL) and the factor time. Normality assumptions were verified by means of skewness and kurtosis.

To assess differences in the clearance ratings, a Wilcoxon signed-rank test between both treatments was done. As all secondary endpoints were analysed in a purely exploratory manner, no adjustments for multiple testing were done and a P-value < 0·05 was considered statistically significant. All calculations were made with the software package SPSS Statistics 19·0 (SPSS Inc., Chicago, IL, U.S.A.).

Results

Patient characteristics

During the study period, 32 patients were randomized. Baseline characteristics of the study population are summarized in Table 1. Thirty-one patients received laser treatment and were

Table 1 Patient characteristics (n = 32)

Characteristics	
Age (years), median (range)	33 (14–66)
Sex, n (%)	
Male	16 (50)
Female	16 (50)
Ethnicity, n (%)	
Caucasian	32 (100)
Location of PWS, n (%)	
Head/neck	18 (56)
Trunk	10 (31)
Extremities	4 (13)
Colour of PWS, n (%)	
Pale red	4 (13)
Red	14 (44)
Dark red	9 (28)
Bluish	5 (16)
Skin type (according to Fitzpatrick), n (%)	
I	1 (3)
II	13 (41)
III	16 (50)
IV	2 (6)
Menopausal status of women, n (%)	
Premenopausal	7 (44)
Postmenopausal	9 (56)

PWS, port-wine stain.

included in the first outcome evaluation (visit 3). Three patients missed the last visit (visit 4) so that 28 patients completed the study and were included in the final outcome evaluation.

Twenty-eight patients had received multiple pulsed dye laser treatments in the past, two patients had previously received intense pulsed light treatment and three patients argon laser therapy. Some of these patients had been treated with several laser devices. Four patients had received no previous therapy in the study area, and one patient had received radiotherapy during infancy.

Feasibility

Twenty-eight out of 32 patients (88%) finished the study in accordance with the protocol. One patient was excluded after randomization because of concomitant epilepsy with a risk of seizures during the painful therapy. Three patients missed the last visit without giving any reason. In the study protocol, feasibility was defined as a significant difference in the rate of patients completing the study to a constant of 50%. Calculating a one-sided binominal test against the proportion $P = 0{\cdot}5$ results in a P-value $P \leq 0{\cdot}001$ with a two-sided 95% confidence interval ranging from 0·71 to 0·96. Thus, the null hypothesis that at least 50% of patients drop out of the study prematurely can be rejected.

Safety

For safety analyses, all patients who received at least one treatment (n = 31) were included. No serious adverse events occurred, but two adverse events were observed. One patient experienced strong site-specific pain during ICG+DL (8 on a 10-point scale) and one patient developed an atrophic scar measuring 5 mm in diameter. The scar corresponded to one of the laser spots in the ICG+DL treatment area at a fluence rate of 50 J cm^{-2} and 25 ms pulse duration. Further side-effects were burning (FPDL: 58%; ICG+DL: 68%), oedema (FPDL: 3%; ICG+DL: 10%) and purpura (FPDL: 71%; ICG+DL: 42%). For safety reasons, diode laser treatment alone was applied before the intravenous administration of ICG or in terms of a 'negative control'. No side-effects were observed after diode laser treatment alone. Side-effects related to the intravenous administration of ICG or the dye itself did not occur.

Pain and treatment preference

To analyse pain ratings, all patients receiving the treatments (n = 31) were included. During ICG+DL, all patients (n = 31) experienced pain within the treatment area, whereas during FPDL therapy 21 patients (68%) experienced pain. On a 10-point scale indicating pain during treatment, the mean score was 5·8 (SD 2·1) for ICG+DL and 1·6 (SD 1·8) for FPDL. Twenty-six out of 31 patients (84%) rated ICG+DL as more uncomfortable than FPDL treatment; three out of 31 (10%) regarded FPDL treatment as more uncomfortable and two out of 31 (7%) perceived no differences between the two therapies. Nevertheless, at the last visit, 18 out of 28 (64%) of patients stated that they would prefer ICG+DL as a future treatment, five out of 28 (18%) would prefer FPDL therapy and five out of 28 (18%) each rated both therapies as equal.

Efficacy

To evaluate differences in efficacy between ICG+DL and FPDL therapy, the clearance rate as well as the cosmetic appearance of PWS were taken into account at visit 3 (n = 31) and at visit 4 (n = 28) (Fig. 3). On the Likert scale of PWS clearance, Cohen's kappa ranged from 0·59 to 0·72 between the two investigators and from 0·21 to 0·42 between patients and each investigator. The interclass correlation coefficients for the ratings of the cosmetic appearance (VAS 0–10) between the two independent investigators were very high (0·74–0·84), whereas the coefficients between patients and each investigator were in the zero range (–0·10 to 0·25).

The investigators rated the clearance of ICG+DL as $1{\cdot}30 \pm 1{\cdot}29$ compared with $0{\cdot}89 \pm 0{\cdot}99$ after FPDL treatment (scale: 0, no clearance to 4, excellent clearance; 3 months after treatment; $P = 0{\cdot}114$). The means of the investigators' ratings at visits 3 and 4 show a preference for ICG+DL over FPDL, although the difference does not reach conventional levels of statistical significance (visit 3: $P = 0{\cdot}119$; visit 4: $P = 0{\cdot}114$). However, the rating of vessel clearance by the patients showed a significant difference both at visit 3 ($P = 0{\cdot}002$) and visit 4 ($P = 0{\cdot}003$). The patients rated the clearance of ICG+DL as $1{\cdot}71 \pm 1{\cdot}24$ compared with $0{\cdot}89 \pm 0{\cdot}88$ after FPDL treatment (3 months after treatment).

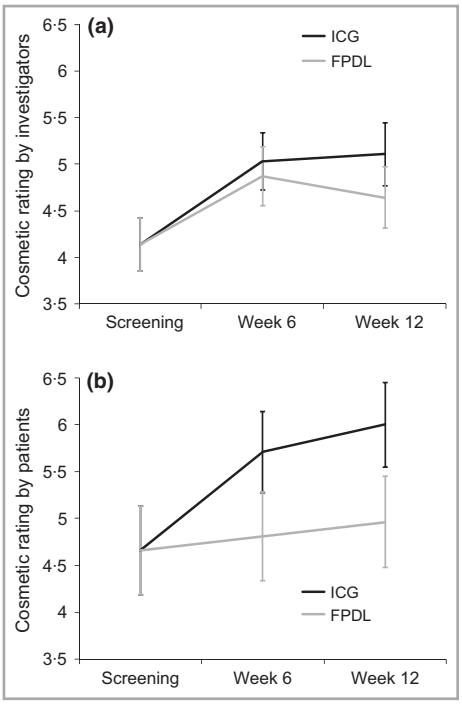

Fig 3. Cosmetic rating prior to and after laser treatment. (a)The mean of cosmetic rating on a visual analogue scale (ranging from 0 to 10) by two independent investigators. Both treatments induced significantly better cosmetic appearance than at baseline ($P < 0{\cdot}001$). No significant difference between the two treatments was found. (b) Patients' cosmetic ratings on a visual analogue scale (ranging from 0 to 10). Both treatments had significantly improved the cosmetic appearance at visit 4 (week 12) compared with baseline ($P = 0{\cdot}004$). Patients rated the cosmetic appearance after indocyanine green (ICG)-augmented diode laser treatment at visit 4 as significantly better than after flashlamp-pumped pulsed dye laser (FPDL) treatment ($P = 0{\cdot}006$). Error bars represent the standard error of the mean.

Diode laser treatment alone did not result in any significant clearance rates 12 weeks after treatment.

The ratings of the cosmetic appearance at screening, visit 3 and visit 4 are shown in Table 2. Since skewness and kurtosis ranged between −1 and 1, normal distribution can be assumed for this analysis. Taking the mean of the two investigators' cosmetic ratings, repeated measures ANOVA showed a significant effect only for the factor time ($F_{2,54} = 16{\cdot}27$; $P < 0{\cdot}001$), indicating a better cosmetic rating at study visits than at baseline for both treatments. No significant difference could be found for the factor treatment ($F_{1,27} = 1{\cdot}16$; $P = 0{\cdot}291$) as well as for the interaction of 'time * treatment' ($F_{2,54} = 1{\cdot}73$; $P = 0{\cdot}198$). Nevertheless, a tendency towards better results of ICG+DL can be seen (Fig. 3a). Regarding the cosmetic rating by the patients, repeated measures ANOVA showed a significant effect for the factor treatment ($F_{1,27} = 9{\cdot}08$, $P = 0{\cdot}006$), the factor time ($F_{2,54} = 6{\cdot}74$, $P = 0{\cdot}004$) and the interaction of 'time * treatment' ($F_{2,54} = 5{\cdot}13$, $P = 0{\cdot}01$), indicating better cosmetic results for ICG+DL than for FPDL therapy as well as a constant time effect. The significant interaction is due to a high effect from screening to visit 3 and a lower effect from visit 3 to visit 4 (Fig. 3b).

Histological analysis

Before laser treatment, blood vessels were histologically detectable from the papillary dermis down to the subcutaneous fat in haematoxylin and eosin staining as well as in CD34 staining. The deepest blood vessel was found 2·6 ± 0·8 mm beneath the horny layer. The number of vessels in the different microvascular segments was 24·2 ± 11·2 for capillaries (< 20 μm diameter), 9·3 ± 4·8 for precapillaries and postcapillary venules (21–100 μm diameter) and 4·6 ± 6·1 for arterioles and collecting venules (> 101 μm diameter).

Table 2 Mean value (SD) of cosmetic appearance rated by two blinded investigators and the patients on a scale from 0 (very bad cosmetic appearance) to 10 (very good cosmetic appearance) and repeated measures analysis of variance (ANOVA)

	Patient	Blinded investigator 1	Blinded investigator 2
Screening			
ICG	4·66 (1·64)	4·16 (1·74)	4·13 (1·74)
FPDL	4·66 (1·64)	4·16 (1·74)	4·13 (1·74)
Visit 3			
ICG	5·71 (2·41)	4·87 (1·73)	5·19 (1·89)
FPDL	4·81 (2·64)	5·0 (1·90)	4·74 (1·79)
Visit 4			
ICG	6·0 (2·40)	5·04 (1·88)	5·18 (1·96)
FPDL	4·96 (2·57)	4·71 (1·88)	4·96 (2·57)
Repeated measures two-way ANOVA			
Treatment	$F_{1,27} = 9{\cdot}08$; $P < 0{\cdot}01$	$F_{1,27} = 1{\cdot}16$; $P = 0{\cdot}291$	
Time	$F_{2,54} = 6{\cdot}74$; $P < 0{\cdot}01$	$F_{2,54} = 16{\cdot}27$; $P < 0{\cdot}001$	
Treatment * time	$F_{2,54} = 5{\cdot}13$; $P = 0{\cdot}01$	$F_{2,54} = 1{\cdot}73$; $P = 0{\cdot}198$	

ICG, indocyanine green; FPDL, flashlamp-pumped pulsed dye laser.

One week after laser treatment, intact and destroyed blood vessels were detectable from the papillary dermis down to the subcutaneous fat. The destroyed blood vessels showed endothelial swelling with fibrinoid necrosis, variable mixed inflammatory infiltrate, surrounding erythrocyte extravasation, perivascular oedema and a lack of CD34 staining (Fig. 4). The deepest destroyed blood vessel was found 1·8 ± 0·6 mm beneath the horny layer. One week after laser treatment, the number of destroyed vessels in the different microvascular segments was 3·5 ± 2·8 for precapillaries and postcapillary venules (21–100 μm diameter) and 3·0 ± 2·2 for arterioles and collecting venules (> 101 μm diameter). No capillaries (< 20 μm diameter) were destroyed at that time. Minor to moderate epidermal damage (crust formation, keratinocytic apoptosis and necrosis) could be detected in four out of 10 biopsies, which also showed dermal damage (degenerated collagen, loss of elastic tissue, swollen and hyalinized collagen fibrils). The epidermal damage was not dose-dependent, but dermal damage was more pronounced after the application of higher fluence rates (30 vs. 40 J cm^{-2}).

Three months after laser treatment, destroyed blood vessels were no longer detectable, neither after haematoxylin and eosin staining nor after CD34 staining (Fig. 5). The number of intact blood vessels in the different microvascular segments 3 months after laser treatment was 33·25 ± 5·8 for capillaries (< 20 μm diameter) and 3·0 ± 3·5 for precapillaries and postcapillary venules (21–100 μm diameter), whereas larger blood vessels could not be detected (> 101 μm diameter) (Fig. 5). Thus, the number of capillaries even increased after ICG+DL. Neither epidermal nor dermal damage was detectable.

Discussion

The rationale for performing this prospective randomized pilot study is the unique findings of two consecutive animal trials on the impact of laser treatment on blood vessels. In the first work, we showed that smaller blood vessels (diameter < 29 μm) are rather resistant to FPDL treatment.[9] The subsequent use of ICG+DL therapy in the same animal model induced the irreversible photocoagulation of blood vessels of all diameters including blood vessels of the capillary segment.[12]

In the present work, we showed that ICG+DL therapy is suitable for coagulating blood vessels of vascular malformations in humans (Fig. 2). The sample size for the present study was calculated with respect to the primary endpoint 'feasibility' and, therefore, statistically significant efficacy findings should not automatically be expected. Nevertheless, the patients rated ICG+DL as significantly more effective than FPDL treatment (1·70 ± 1·24 vs. 0·89 ± 0·88 after FPDL treatment ($P < 0·005$; scale: 0, no clearance to 4, excellent clearance; 3 months after treatment; $P = 0·114$). In addition, the patients assessed the cosmetic appearance of their PWS as significantly better after ICG+DL (Table 2; Fig. 3b). Therefore, it is hardly surprising that 18 (64%) patients stated at their last visit that – if they could choose one of the study treatments in future – they would prefer ICG+DL. However, blinding of patients was not possible as they experienced the different laser procedures (FPDL vs. intravenous application of ICG followed by diode laser irradiation). As most patients had high expectations of the new treatment option and as they had to put a certain effort into participating in this pilot study (five visits), this has probably influenced the scores they gave regarding clearance rate and cosmetic outcome. In social psychology, this effect is called 'effort justification'.[18] It might also be an explanation for the low agreement between patient- and investigator-rated efficacies. In addition, the fact that the majority of patients (28/32) has had FPDL treatment in the past might be a source of bias in patients' self-assessment of clinical responses, as well.

Histological analysis showed three major findings regarding the impact of ICG+DL therapy: (i) whereas medium-sized blood vessels (21–100 μm diameter) and in particular larger blood vessels (> 101 μm diameter) respond very well to ICG+DL, smaller blood vessels (< 20 μm) turned out to be resistant; (ii) 3 months after treatment, an increased number of capillaries indicated compensatory neoangiogenesis in this microvascular segment; and (iii) ICG+DL treatment induced only minor to moderate epidermal damage and dose-dependent collateral

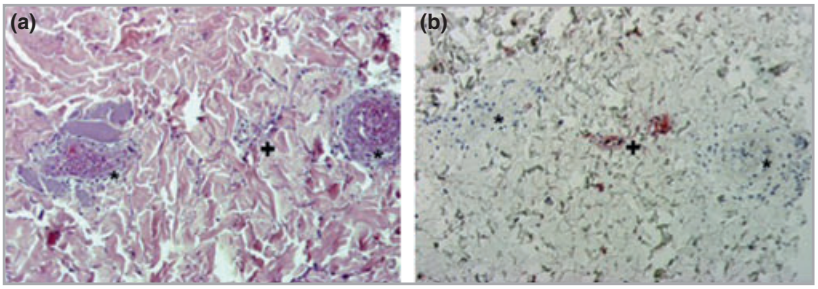

Fig 4. Histological aspect 1 week after indocyanine green-augmented diode laser treatment. Smaller blood vessels (+) were left unaffected, whereas thicker blood vessels (*) showed pronounced thermal damage, even when located at the same depth within the dermis. (a) In haematoxylin and eosin staining, alterations such as endothelial swelling with fibrinoid necrosis, mixed inflammatory infiltrate, erythrocyte extravasation and perivascular oedema were observed. Thermal damage was limited to blood vessels. (b) Destroyed blood vessels (*) were negative in CD34 staining, whereas intact smaller vessels (+) remained positive.

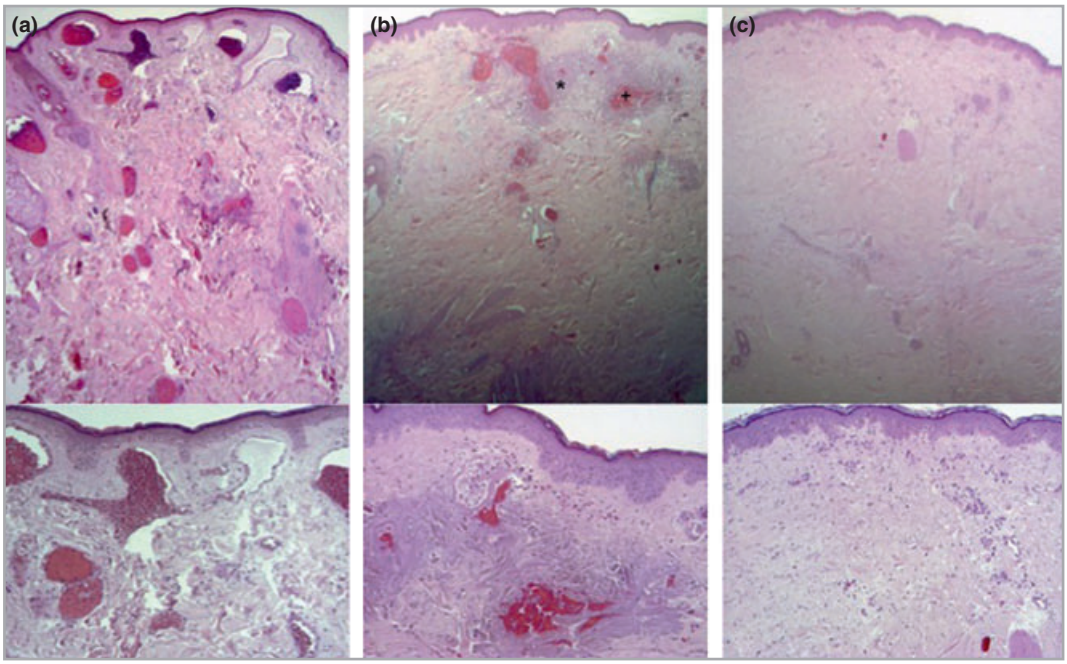

Fig 5. Histological slides of one patient (a) prior to, and (b) 1 week and (c) 3 months after indocyanine green (ICG)-augmented diode laser treatment. (a) Before treatment, multiple dilated dermal vessels were detectable. (b) One week after ICG-augmented diode laser treatment, blood vessels showed pronounced endothelial swelling with fibrinoid necrosis, mixed inflammatory infiltrate, erythrocyte extravasation, and perivascular oedema. Epidermal damage was missing, but collateral collagen damage was detectable. (c) Three months after ICG-augmented diode laser treatment, massive rarefication of larger blood vessels (> 20 μm) was obvious. Smaller blood vessels (< 20 μm) were frequently visible. Restitution ad integrum of the unspecific dermal damage was detectable. Upper panels, overview; lower panels, 10-fold magnification.

dermal damage; both types of damage were completely reversible within 3 months after treatment.

Our observation that smaller blood vessels are less susceptible to laser treatment is in accordance with the literature regarding other laser procedures. Sivarajan and MacKay[19] used videomicroscopy for examining any changes of the capillary structure of 22 PWS after FPDL treatment. The authors proved that vessels with a diameter > 50 μm can be sufficiently treated, whereas smaller vessels appeared resistant to laser treatment. Fiskerstrand *et al.*[4] analysed histological changes due to FPDL treatment in 51 patients with PWS, proving a positive correlation between vessel diameter and susceptibility to laser irradiation. Blood vessels still viable after treatment showed a median diameter of only 14 μm. Svaasand *et al.*[20] used mathematical modelling based on clinical observations of FPDL treatments of PWS and found out that smaller vessels (measuring 4–30 μm in diameter) require higher fluence rates for sufficient photocoagulation than larger vessels (measuring 40–60 μm in diameter).

The preliminary animal study using ICG+DL irradiation showed sufficient photocoagulation even in smaller blood vessels,[12] which seemed to be in contrast to present histological results. However, in the animal study, we observed a reduction in perfused blood vessels, which depended on ICG concentration as well as on pulse duration. The most effective ICG concentration was 4 mg kg^{-1} b.w.; in this treatment arm, even the smallest blood vessels were photocoagulated. Using 2 mg kg^{-1} b.w., the coagulation rate of smaller blood vessels decreased even in the animal model. Therefore, use of a higher ICG concentration would probably also enhance the coagulation rate of smaller blood vessels in humans. As we observed unspecific collateral damage in the animal model using the higher ICG concentration, we started with a reduced ICG concentration in the present clinical pilot study to avoid unspecific thermal damage and consecutive scars for our patients.

The compensatory neoangiogenesis of the small vessel compartment (< 20 μm) 3 months after treatment has also been described in the literature for other lasers. Edström *et al.*[21] analysed the histological effects of FPDL treatment on the vessel architecture of PWS in 30 patients. The authors showed a significant decrease in the number of vessels only in the vessel compartment > 20 μm. In addition, they documented an increase in blood vessels with smaller diameters (diameter < 10 μm) after one single treatment. When analysing the histological effects of argon laser treatment of PWS in 28 patients, Finley *et al.*[22] found that large vessels responded well to argon laser therapy, whereas the number of smaller blood vessels tripled. The persisting and newly developed small blood vessels observed in our histological analysis may explain the lack of clinical clearance according to the investigators' assessment. The fact that particularly small vessels contribute to the skin colour and to the cosmetic appearance of PWS is described in the literature. Verkruysse *et al.*[23] calculated reflectance spectra for visible light from normal skin and PWS skin using a Monte

Carlo algorithm that was applied to a multilayered skin model. The authors found that PWS appear redder when consisting of many small vessels than when consisting of fewer large vessels. Optically, several small vessels act similarly to one large vessel as theoretically shown by Verkruysse *et al.*[23]

In general, ICG is a well-tolerated medicinal product. None of our patients experienced any drug-related adverse events. However, ICG must be injected intravenously so that a venipuncture with possible side-effects is necessary. Such side-effects occur rarely. Especially in patients where the standard laser treatment results in no further PWS clearance of their PWS, the new treatment option might be an alternative with a low risk of side-effects.

In conclusion, we showed for the first time that ICG+DL treatment represents a new and safe therapeutic modality for the treatment of PWS. Furthermore, first evidence with regard to the efficacy of ICG+DL therapy is promising. In the present setting, 64% of treated patients would prefer ICG+DL over FDPL treatment. Follow-up studies are planned to evaluate the efficacy of higher ICG concentrations, optimal laser parameters and the necessary number of treatment sessions. The fluence rate should only be increased with care because of a reversible dose-dependent collateral damage observed in histological analysis. With the use of the approved mathematical model,[12,24] the increase in temperature and the amount of thermal damage can be predicted for different settings. We are convinced that the use of a diode laser is of great advantage because of its wavelength of 810 nm that allows deeper penetration into the dermis and less melanin absorption than FPDL therapy.[25] However, a sufficiently large number of patients need to be assessed in order to achieve sufficient power to detect clinically significant differences between ICG+DL and FPDL.

What's already known about this topic?

- We showed in an animal model that indocyanine green (ICG)-augmented diode laser therapy is capable of destroying even small blood vessels (vessel diameter < 20 μm) effectively.

What does this study add?

- To the best of our knowledge, this is the first report on the use of ICG-augmented diode laser therapy of port wine stains in 31 patients.
- The treatment was shown to be safe, and the first evidence with regard to the efficacy of this new therapy option is promising.

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References

1 Alper JC, Holmes LB. The incidence and significance of birthmarks in a cohort of 4641 newborns. *Pediatr Dermatol* 1983; **1**:58–68.
2 Viator JA, Au G, Paltauf G *et al.* Clinical testing of a photoacoustic probe for port wine stain depth determination. *Lasers Surg Med* 2002; **30**:141–8.
3 Shafirstein G, Baumler W, Lapidoth M *et al.* A new mathematical approach to the diffusion approximation theory for selective photothermolysis modeling and its implication in laser treatment of port-wine stains. *Lasers Surg Med* 2004; **34**:335–47.
4 Fiskerstrand EJ, Svaasand LO, Kopstad G *et al.* Photothermally induced vessel-wall necrosis after pulsed dye laser treatment: lack of response in port-wine stains with small sized or deeply located vessels. *J Invest Dermatol* 1996; **107**:671–5.
5 Klein A, Baumler W, Landthaler M, Babilas P. Laser and IPL treatment of port-wine stains: therapy options, limitations, and practical aspects. *Lasers Med Sci* 2011; **26**:845–59.
6 Wlotzke U, Hohenleutner U, Abd-El-Raheem TA *et al.* Side-effects and complications of flashlamp-pumped pulsed dye laser therapy of port-wine stains. A prospective study. *Br J Dermatol* 1996; **134**:475–80.
7 Scherer K, Lorenz S, Wimmershoff M *et al.* Both the flashlamp-pumped dye laser and the long-pulsed tunable dye laser can improve results in port-wine stain therapy. *Br J Dermatol* 2001; **145**:79–84.
8 Renfro L, Geronemus RG. Anatomical differences of port-wine stains in response to treatment with the pulsed dye laser. *Arch Dermatol* 1993; **129**:182–8.
9 Babilas P, Shafirstein G, Baumler W *et al.* Selective photothermolysis of blood vessels following flashlamp-pumped pulsed dye laser irradiation: *in vivo* results and mathematical modelling are in agreement. *J Invest Dermatol* 2005; **125**:343–52.
10 Tan OT, Murray S, Kurban AK. Action spectrum of vascular specific injury using pulsed irradiation. *J Invest Dermatol* 1989; **92**:868–71.
11 Flock ST, Jacques S. Thermal damage of blood vessels in a rat skin-flap window chamber using indocyanine green and a pulsed alexandrite laser: a feasibility study. *Lasers Med Sci* 1993; **8**:185–96.
12 Babilas P, Shafirstein G, Baier J *et al.* Photothermolysis of blood vessels using indocyanine green and pulsed diode laser irradiation in the dorsal skinfold chamber model. *Lasers Surg Med* 2007; **39**:341–52.
13 Fox IJ, Brooker LGS, Heseltine DW *et al.* New dyes for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturations. *Am J Physiol* 1956; **187**:599.
14 He YL, Tanigami H, Ueyama H *et al.* Measurement of blood volume using indocyanine green measured with pulse-spectrophotometry: its reproducibility and reliability. *Crit Care Med* 1998; **26**:1446–51.
15 Iijima T, Aoyagi T, Iwao Y *et al.* Cardiac output and circulating blood volume analysis by pulse dye-densitometry. *J Clin Monit* 1997; **13**:81–9.
16 Hope-Ross M, Yannuzzi LA, Gragoudas ES *et al.* Adverse reactions due to indocyanine green. *Ophthalmology* 1994; **101**:29–33.
17 Jacob M, Conzen P, Finsterer U *et al.* Technical and physiological background of plasma volume measurement with indocyanine green: a clarification of misunderstandings. *J Appl Physiol* 2007; **102**:1235–42.
18 Aronson E, Mills J. The effect of severity of initiation on liking for a group. *J Abnorm Soc Psychol* 1959; **59**:177–81.
19 Sivarajan V, MacKay IR. The relationship between location, color, and vessel structure within capillary vascular malformations. *Ann Plast Surg* 2004; **53**:378–81.

20 Svaasand LO, Fiskerstrand EJ, Kopstad G *et al*. Therapeutic response during pulsed laser treatment of port-wine stains: dependence on vessel diameter and depth in dermis. *Lasers Med Sci* 1995; **10**:234–43.
21 Edström DW, Hedblad MA, Ros AM. Flashlamp pulsed dye laser and argon-pumped dye laser in the treatment of port-wine stains: a clinical and histological comparison. *Br J Dermatol* 2002; **146**:285–9.
22 Finley JL, Barsky SH, Geer DE *et al*. Healing of port-wine stains after argon laser therapy. *Arch Dermatol* 1981; **117**:486–9.
23 Verkruysse W, Lucassen GW, van Gemert MJ. Simulation of color of port wine stain skin and its dependence on skin variables. *Lasers Surg Med* 1999; **25**:131–9.
24 Shafirstein G, Buckmiller LM, Waner M *et al*. Mathematical modeling of selective photothermolysis to aid the treatment of vascular malformations and hemangioma with pulsed dye laser. *Lasers Med Sci* 2007; **22**:111–18.
25 Whang KK, Byun JY, Kim SH. A dual-wavelength approach with 585-nm pulsed-dye laser and 800-nm diode laser for treatment-resistant port-wine stains. *Clin Exp Dermatol* 2009; **34**:e436–7.