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ORIGINAL ARTICLE

Histochemical Evaluation of the Vessel Wall Destruction and Selectivity After Treatment with Intense Pulsed Light in Capillary Malformations

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Nitroblue-tetrazolium chloride (NBTC);
Stacking;
Selective photothermolysis

Abstract
Background: Among the different approaches for improving the effectiveness in the treatment of Capillary Malformations type Port Wine Stain (CM type PWS) are the intense pulsed light sources. There are few clinical studies prove useful in the treatment of CM. Furthermore, no studies have been published yet demonstrating the histological effects of IPL in CM.
Objectives: To assess the histological effects of pulsed light in capillary malformations type port wine stain. We wanted to compare epidermal, dermal and vessel wall damage after treatment with different combinations of IPL parameters.
Material and methods: Fifty-five post-treatment biopsies were performed in 15 consenting patients with CM and stained with nitroblue-tetrazolium chloride (NBTC). Patients had not been treated previously.
Results: Fifteen patients with CM, with a median age of 39 years-old were enrolled in this study. In this series, the patients with the most severe epidermal damage were those with a darker phototype. Pink CM were especially resistant to treatment, even using high fluences, short pulse durations and stacking pulses. Longer intra- and interpulse delays were effective in purple CM, achieving adequate vessel destruction.
Conclusions: IPL devices provide a vast amount of treatment possibilities and further studies are necessary to optimize therapeutic approaches to CM. In this study we have observed the histological effects of different pulses on the MC type PWS.

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PALABRAS CLAVE
Malformaciones capilares;
Fluencia;
Histoquímica;
Luz pulsada intensa (IPL);
Múltiples pulsos;
Cloruro de nitroazul-tetrazolio (NBTC);
Pulsos consecutivos;
Fototermólisis selectiva

Evaluación histoquímica de la destrucción de la pared del vaso y la selectividad después del tratamiento con luz pulsada intensa en las malformaciones capilares

Resumen

Antecedentes: Entre las distintas estrategias para intentar mejorar la eficacia en el tratamiento de las malformaciones capilares tipo mancha en vino de Oporto (MC tipo MVO) están las fuentes de luz pulsada intensa. Existen hasta la fecha pocos estudios clínicos que avalen su utilidad en el tratamiento de las MC. Además, no disponemos de estudios histológicos que objetiven los efectos de la luz pulsada en la coagulación de estos vasos anómalos.

Objetivos: Evaluar los efectos histológicos de la luz pulsada en las MC tipo MVO. Intentamos comparar el daño epidérmico, dérmico y de la pared de los vasos después del tratamiento con distintos parámetros de IPL.

Material y métodos: Fueron realizadas 55 biopsias postratamiento en las MC de 15 pacientes. Las muestras fueron teñidas con cloruro de nitroblue tetrazolium.

Resultados: Quince pacientes (edad media: 39 años) fueron inscritos en este estudio. En esta serie los pacientes con mayor daño epidérmico fueron aquellos con un fototipo más alto (>IV). Las malformaciones de color rosa pálido eran especialmente resistentes al tratamiento, incluso con altas *fluencias*, duraciones de pulso corto y pulsos repetidos. Los pulsos de una mayor duración fueron especialmente eficaces en malformaciones capilares violáceas.

Conclusiones: Los equipos de IPL ofrecen una gran cantidad de opciones de tratamiento en las MC, sin embargo necesitamos conocer mejor sus efectos para realizar abordajes más eficaces y seguros. En este estudio hemos podido observar los efectos histológicos de los distintos pulsos sobre las MC tipo MVO.

Introduction

Capillary malformations type port wine stain (CM type PWS) constitute congenital anomalies that are believed to represent errors in vascular development during embryogenesis.[1–4]

Currently, pulsed dye laser (PDL) remains the *gold standard* treatment for CM.[1,4–6] However, 25–50% of treated lesions do not demonstrate significant improvement.[7] Other options have been used in resistant lesions, such as alexandrite, neodymium-YAG, dual wavelength lasers[8] and, more recently intense pulsed light (IPL) sources.[3]

IPL systems emit non-coherent broadband light with wavelengths in the 515–1200 nm range[6] and, theoretically, this spectrum matches the absorption coefficient and thermal relaxation time of a broader range of vessels within CM.[9] Similar to laser systems (though less selective than them), this technology seems to respect the principle of selective photothermolysis (SP),[3,10] which consists in the preferential absorption of light by oxy/deoxy-hemoglobin and the subsequent conversion into thermal energy, leading to the selective coagulation of blood vessels.[11] Specific output wavelengths depend on the cutoff filters used,[6] which optimize absorption of the target chromophores, reduce the strong absorption of melanin and prevent adverse effects such as erythema, blistering and crusting.[12] Recently, the optimized pulsed light sources (OPL) have been developed, providing a dual-band output spectrum from 500 to 670 nm and 870 to 1200 nm, which are even more selective to oxy/deoxy-hemoglobin. Theoretically, the use of OPL reduces the risk of epidermal damage, by displacing the interval between 610 and 870 nm, characterized by melanin's absorption peak.[13]

IPL systems allow the individual selection of multiple parameters, such as: wavelength, pulse duration, fluence, multipulse mode and intrapulse time delay.[12] On the other hand, we can also use only one pulse or multiple pulses, with different delays, creating a multiplicity of possible combinations.

Although IPL sources have been increasingly used in the treatment of CM, there are very few clinical studies regarding their effectiveness.[5,11] Furthermore, we have not found any published histochemical studies describing the histological effects of IPL treatment of CM. We aim to describe the vascular, epidermal and dermal tissue damage in CM treated with IPL with variations in multiple parameters using nitroblue-tetrazolium chloride (NBTC) histochemical staining of biopsies taken from the CM immediately after treatment.[4,14–17]

Material and methods

Fifteen adult patients with CM were enrolled in this study. Four men and eleven women were treated with the Ellipse Flex (Ellipse®, Denmark) IPL device that can provide two different spectrums of polychromatic light, according to the different cut-off filters used: between 555 and 950 nm (VL-2®) and 530 and 750 nm (PR®). For epidermal protection, a cold-air cooling system (Cryos5™, Zimmer Medizinsysteme GMbH, Neu-Ulm, Germany) and an ultrasound gel were used. Different wavelengths, fluences, pulse durations, inter- and intradelay times with stacking pulses[18,19] and multiple passes were used to treat an average of four CM test areas per patient (range 2–7). The hospital's ethics committee approved the study. Fully informed written consent was obtained from all patients before the first

Table 1 Characteristics of the IPL device and the parameters used.

Technical characteristics	
Wavelength/emission spectrum	555–950 nm 530–750 nm
Light characteristics	Polychromatic, incoherent
Shape of spot	Rectangular
Spot size (cm^2)	5.0 (10 × 48 mm)
Cooling	Cold-air cooling and ultrasound gel
Pulse duration (ms)	2.5–8
Fluence range (J/cm^2)	5.3–19.4
Number of minipulses in one pulse	1–4
Number of pulses	1–7
Delay (inter-/intrapulses)	1[a]–60 s/1.5– 200 ms

[a] Interpulse delays of 1 s correspond to stacking pulses.

treatment. A technical description of the IPL device and the parameters used in the study are shown in Table 1.

Post-treatment biopsies of each of the 55 test areas were performed, 10 min after a single IPL treatment using a 3-mm punch. We chose slightly sun-exposed skin areas in order to minimize epidermal damage and hair-free areas to avoid melanin light absorption, since melanin behaves as a competitive chromophore to oxyhemoglobin.[9,20] Biopsies were frozen in liquid nitrogen and the tissue sections were stained with NBTC, as described in previous studies.[4,14,16] This enzyme is a redox indicator that is reduced by NADPH-diaphorase. This enzymatic activity stops immediately after cell death and is present in endothelial cells, fibroblasts and smooth muscle fibers but not in the dermal connective matrix. Therefore, only the viable tissue stains blue, allowing an easy differentiation from thermally damaged tissue.

Three parameters were evaluated: vessel wall destruction, epidermal and dermal damage, using a semiquantitative score: 0, absent; 1, minimal; 2, moderate; and 3, extensive tissue damage. Two dermatologists and one dermatopathologist evaluated all slides blindly. We always chose the majority agreement among the three observers, and in case of total disagreement, we suited the particular case to achieve a consensus agreement.

Results

The median age of the 15 patients was 39 years-old (21–80 years) and most biopsies were taken from the retroauricular area (47%) and back (33%). The areas within the treated CM were all flat, their color varied from pink to purple and the vessels had varying diameters (Table 2). The histochemical findings are illustrated in Table 3, showing how variations in each IPL parameter can modify the capacity of vessel wall destruction and production of epidermal and dermal collateral damage.

In this series, the patients with the most severe epidermal damage were those with a darker phototype (patients 2, 5 and 7). In both cases, in spite of intense epidermal cooling, severe epidermal damage was noted with high fluences (around 19 J/cm^2), but also with lower fluences (11 and 13 J/cm^2) and a long pulse duration (8 ms) in case 7. Indeed, a significant proportion of the applied energy was absorbed by melanin in the basal layer of the epidermis, and the amount of energy that reached the dermis was insufficient to achieve enough heat to cause vessel wall destruction (Fig. 1).

Patients with pink lesions were difficult to treat, as expected. In case 15, stacking a multipulse train of short pulses with high fluences obtained more vessel wall damage but with intense epidermal (but not dermal) damage.

Table 2 Characteristics of the patients and capillary malformations treated.

Patients			Capillary malformations			
	Sex	Age	Location	Color	Vessels caliber	Phototypes (Fitzpatrick)
1	Female	53	Shoulder	3	2	III
2	Female	45	Back	2	2	IV
3	Female	80	Retroauricular	3	2	III
4	Female	29	Arm	1	1	II
5	Male	27	Retroauricular	3	2	IV
6	Male	42	Retroauricular	2	2	III
7	Female	38	Face	2	2	IV
8	Female	38	Retroauricular	2	2	II
9	Male	59	Back	3	3	III
10	Female	57	Retroauricular	2	2	III
11	Female	54	Back	3	2	II
12	Female	31	Back	2	1	II
13	Female	21	Retroauricular	2	2	II
14	Female	39	Back	2	1	III
15	Male	38	Back	1	1	III

*Color of capillary malformation (colorimetric scale)[28]: pink (1); red-violaceous (2) or purple (3).
**Vessel caliber: thin 10–50 μm (1); medium 50–200 μm (2) or large ≥200 μm (3).

Table 3 IPL parameters used and histopathological findings seen with the NTBC histochemical stained biopsies.

Patients	IPL Parameters					Histopathological findings		
	Wavelengths (nm)	Pulses[a] (number)	Fluence (J/cm^2)	Pulse duration (ms)	Delay time: inter-/intrapulses	Vessel wall damage	Epidermal damage	Dermal damage
1	555–950	1	19	8	–/–	3	0	2
	555–950	2	19	8	60 s/–	3	0	2
	530–750	1	8.9	8	–/–	2	0	0
	530–750	1	5.3	2.5	–/–	1	1	0
	555–950	1	7.9	2.5	–/–	1	0	0
2	555–950	1 (3 mp)	19.4	2.5	–/200 ms	1	2	0
	555–950	1 (3 mp)	19.4	2.5	–/100 ms	0	3	0
	555–950	1 (3 mp)	19.4	2.5	–/50 ms	0	3	0
3	555–950	1	15	8	–/–	2	1	1
	555–950	2	15	8	1 s/–	3	2	3
	555–950	2	15	8	30 s/–	3	1	1
	555–950	1	13	8	–/–	1	2	0
	555–950	2	13	8	1 s/–	2	3	1
	555–950	2	13	8	30 s/–	1	3	1
4	530–750	1 (3 mp)	9	2.5	–/1.5 ms	0	0	0
	530–750	7 (3 mp)	9	2.5	1 s/1.5 ms	0	0	0
5	555–950	1	19	8	–/–	3	2	1
	555–950	2	19	8	60 s/–	3	3	3
	555–950	1	14	8	–/–	2	2	1
	555–950	2	14	8	60 s/–	2	3	1
6	555–950	1 (4 mp)	19.3	2.5	–/1.5 ms	2	2	0
	555–950	1 (4 mp)	19.3	2.5	–/200 ms	1	1	0
7	555–950	1	19	8	–/–	2	3	2
	555–950	2	19	8	1 s/–	3	3	3
	555–950	2	19	8	60 s/–	2	3	2
	555–950	1	13	8	–/–	1	3	0
	555–950	2	11	8	60 s/–	1	3	0
8	530–750	1	5.3	2.5	–/–	1	0	0
	555–950	1	7.9	2.5	–/–	1	0	0
	555–950	1 (3 mp)	19.4	2.5	–/50 ms	2	1	1
	555–950	1 (3 mp)	19.4	2.5	–/200 ms	1	1	1
	555–950	3	7.9	2.5	1 s/–	2	0	0
	555–950	1 (2 mp)	13.4	2.5	–/5 ms	3	0	0
	555–950	1 (3 mp)	19.4	2.5	–/5 ms	3	1	1

Table 3 (*Continued*)

Patients	IPL Parameters					Histopathological findings		
	Wavelengths (nm)	Pulses[a] (number)	Fluence (J/cm^2)	Pulse duration (ms)	Delay time: inter-/intrapulses	Vessel wall damage	Epidermal damage	Dermal damage
9	555–950	1 (3 mp)	19.3	2.5	–/1.5 ms	3	3	3
	555–950	1 (3 mp)	19.3	2.5	–/200 ms	3	2	2
10	555–950	1	19	8	–/–	3	0	2
	555–950	2	19	8	60 s/–	3	2	2
	555–950	1	16	8	–/–	2	1	1
	555–950	2	16	8	60 s/–	3	2	2
11	555–950	1 (4 mp)	19.3	2.5	–/1.5 ms	3	1	2
	555–950	1 (4 mp)	19.3	2.5	–/200 ms	3	0	1
12	555–950	1	19	8	–/–	1	0	0
	555–950	2	19	8	1 s/–	3	1	1
	555–950	2	19	8	60 s/–	3	1	1
13	555–950	1 (4 mp)	17.5	2.5	–/5 ms	3	0	1
	555–950	2 (4 mp)	17.5	2.5	1 s/5 ms	3	2	3
	555–950	1 (4 mp)	17.5	2.5	–/200 ms	1	0	0
14	555–950	1	14	8	–/–	2	0	1
	555–950	1	19	8	–/–	3	2	2
	555–950	2	19	8	60 s/–	3	2	3
	530–750	1	9.9	8	–/–	2	0	0
15	555–950	1 (3 mp)	19.2	2.5	–/2.5 ms	0	0	0
	555–950	2 (3 mp)	19.2	2.5	1 s/2.5 ms	2	2	0
	555–950	3 (3 mp)	19.2	2.5	1 s/2.5 ms	2	3	0

[a] 1 s, Interpulse delays of 1 s correspond to stacking pulses; mp, minipulses. The histopathological findings are described using a semiquantitative scale (0–3), regarding: vessel wall damage (0, absent; 1, focal intravascular coagulation; 2, moderate destruction of vessel walls; 3, intense destruction of vessel walls), epidermal damage (0, absent; 1, focal epidermal damage; 2, moderate epidermal damage; 3, full epidermal necrosis) and dermal damage (0, absent; 1, focal dermal damage with perivascular collagen denaturation; 2, moderate dermal damage with a limited coagulation area; 3, intense dermal damage with an extensive coagulation area).

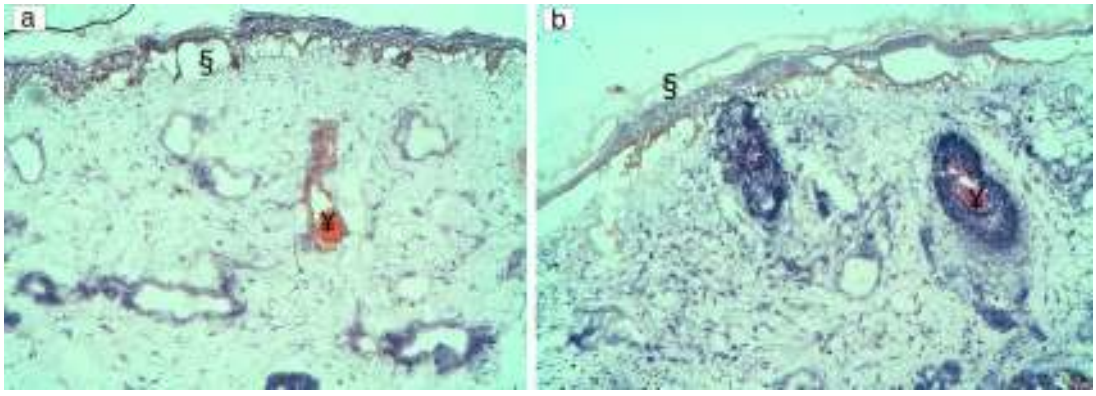

Figure 1 In patients with a darker phototype, severe epidermal damage with full epidermal necrosis (§) was noted with (1a) high fluences (19.4 J/cm^2) in patient 2 and (1b) lower fluences: 13 J/cm^2 in patient 7. In these patients, focal intravascular coagulation was also seen (¥). No dermal damage was observed.

In case 4, using the shorter pulse duration (2.5 ms) and a total energy of 9 J/cm^2 per pulse, even with seven stacking pulses, we could not obtain the necessary heating to cause any vascular or tissular alteration (Fig. 2). However, in case 12 both multiple and stacking pulses obtained more intense vessel wall damage than using only one pulse, without significant collateral damage.

On the other hand, in a purple CM lesion treated with a fluence of 15 J/cm^2 and a pulse duration of 8 ms, we found complete vessel wall destruction with less dermo-epidermal damage using multiple passes (interdelay time: 30 s) instead of stacking pulses (case 3).

In some patients, using multiple pulses instead of a single pulse, improved treatment efficacy in CM with thin vessels. This happened in case 12 (fluence of 19 J/cm^2 and pulse duration of 8 ms) and case 15 (fluence of 19.2 J/cm^2 and pulse duration of 2.5 ms), where two pulses achieved a selective destruction of the vessel wall. In case 15, when we used 3 pulses (with the same referred parameters), the same level of vessel walls destruction was obtained (as with two pulses), but with more dermo-epidermal damage.

Intrapulse delay adjustments may also become extremely important to achieve complete destruction of vessel walls, with less collateral damage. Decreasing the intrapulse delay time, we obtained more efficient vessel walls destruction, without substantial increase of the dermo-epidermal damage (cases 8 and 13). Both patients had CM with medium caliber vessels. They were treated with high fluences (19.4 J/cm^2 in case 8 and 17.5 J/cm^2 in case 13) and intrapulse delay times were reduced from 50 to 5 ms (case 8) and from 200 to 5 ms (case 13). In case 8, however, an even more selective result was acquired, maintaining the referred parameters (including the 5 ms intrapulse delay) and decreasing the total fluence (from 19.4 to 13.4 J/cm^2), without any dermo-epidermal damage.

In some purple CM containing vessels with a medium to large vessel caliber, increased intrapulse delay times led to complete destruction of vessel walls, with less collateral damage. In cases 9 and 11, for example, increasing intrapulse delay times from 1.5 to 200 ms resulted in complete vessel wall destruction with slightly less epidermal and dermal tissue damage (Fig. 3).

In patients 1, 4, 8 and 14, who were treated with the PR® handpiece (530–750 nm), the settings used seemed insufficient to destroy the vessel walls effectively, even when using a shorter pulse duration (2.5 ms) with the highest fluences allowed by the device. As mentioned above, no vascular

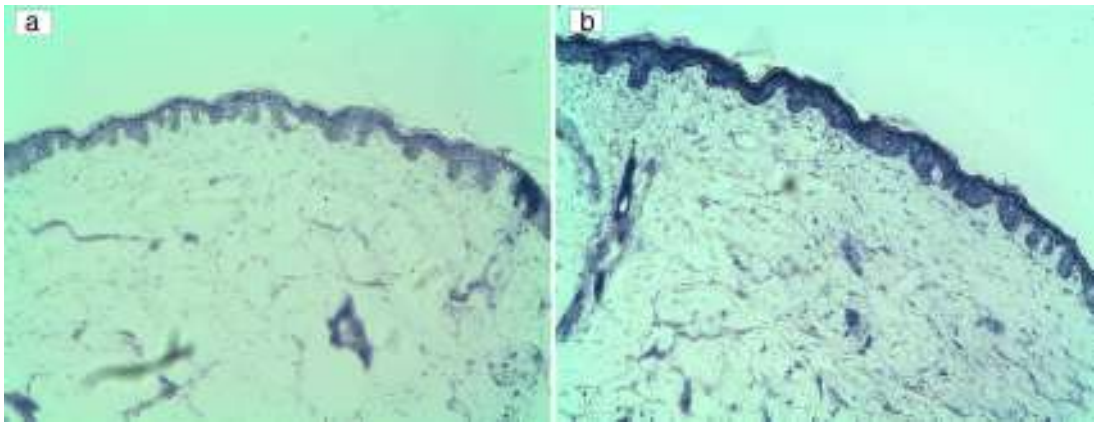

Figure 2 A pink CM (patient 4) was treated using a short pulse duration (2.5 ms) and a total energy of 9 J/cm^2 per pulse without causing any vascular or dermal–epidermal damage neither with (2a) one pulse nor after (2b) seven stacking pulses.

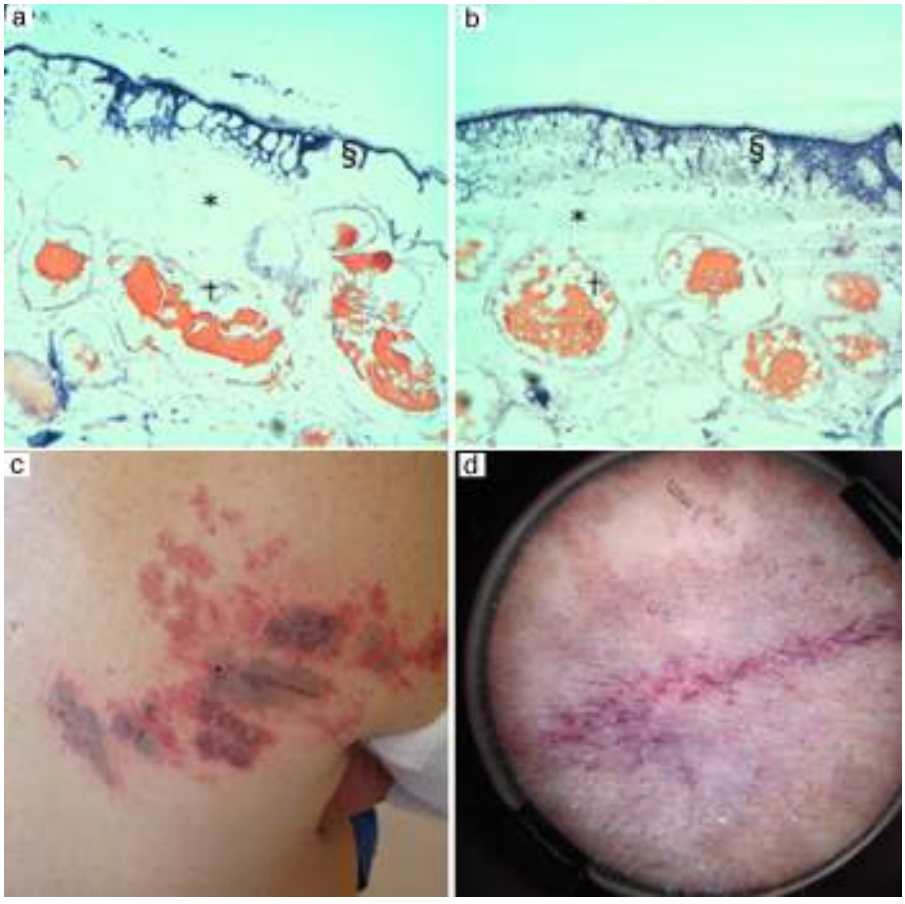

Figure 3 A purple CM (patient 9) was treated with a fluence of 19.3 J/cm^2 and a pulse duration of 2.5 ms with delay times of (a) 1.5 ms and (3b) 200 ms. Intense destruction of the vessel walls (†) was achieved with both delay times. The shorter delay time (2a) lead to intense epidermal (§) and dermal (*) tissue damage in comparison to the moderate epidermal and dermal damage observed with the longer delay time (b). (c) Note the permanent gray in the central area (*) following treatment with high fluences (19.3 J/cm^2) and short pulse (2.5 ms). (d) In dermoscopy we observe intense vessel coagulation.

damage was observed in patient 4, even when stacking pulses.

Discussion

Although PDL is still the first choice for the treatment of CM, second generation IPL devices have improved their selectivity and have been increasingly used.[5,9,21] Currently reported clearing rates range between 6% and more than 90%,[21–24] which may be due to the IPL devices' wide wavelength spectrum (a second absorbance peak of oxyhemoglobin at 555 nm and a third around 900 nm).[5] IPL is a less studied technique, in which multiple parameters (fluence, wavelength spectrum, pulse duration, intra- and interpulse delay times) may be adjusted, conditioning an endless amount of therapeutic options.

In the present study we have been able to show the histochemical SP of IPL, even with multipulse treatments, according to the Verkruysse model[25] first demonstrated with PDL treatments. Using multipulses of IPL, higher energy thresholds are reached in the vessel walls with less collateral damage.[17,18,25]

Single and multiple pulses

Multiples pulses produce an accumulative increase in temperature in blood vessels, leading to thermal coagulation.[18] In order to maintain SP the caliber and density of the vessel walls must be taken into account.

Interpulse delay times (multiple passes and stacking pulses)

Multiple passes with large interpulse delays (30–60 s) seem to heat the vessel walls slowly, being less aggressive than stacking pulses. This may be an advantage for patients who did not respond to a single-pulsed treatment. This treatment modality is especially useful in CM in the presence of clusters of vessels with a larger caliber, which have a longer thermal relaxation time (TRT), because it allows cooling between pulses.[17] In these cases, stacking pulses may lead to overheating and consequent collateral dermal damage.

On the other hand, in patients with CM with sparse and thin vessels, short interpulse delay times or stacking pulses could be safer in fair-skinned patients and allow the necessary temperature increase to achieve thermal coagulation

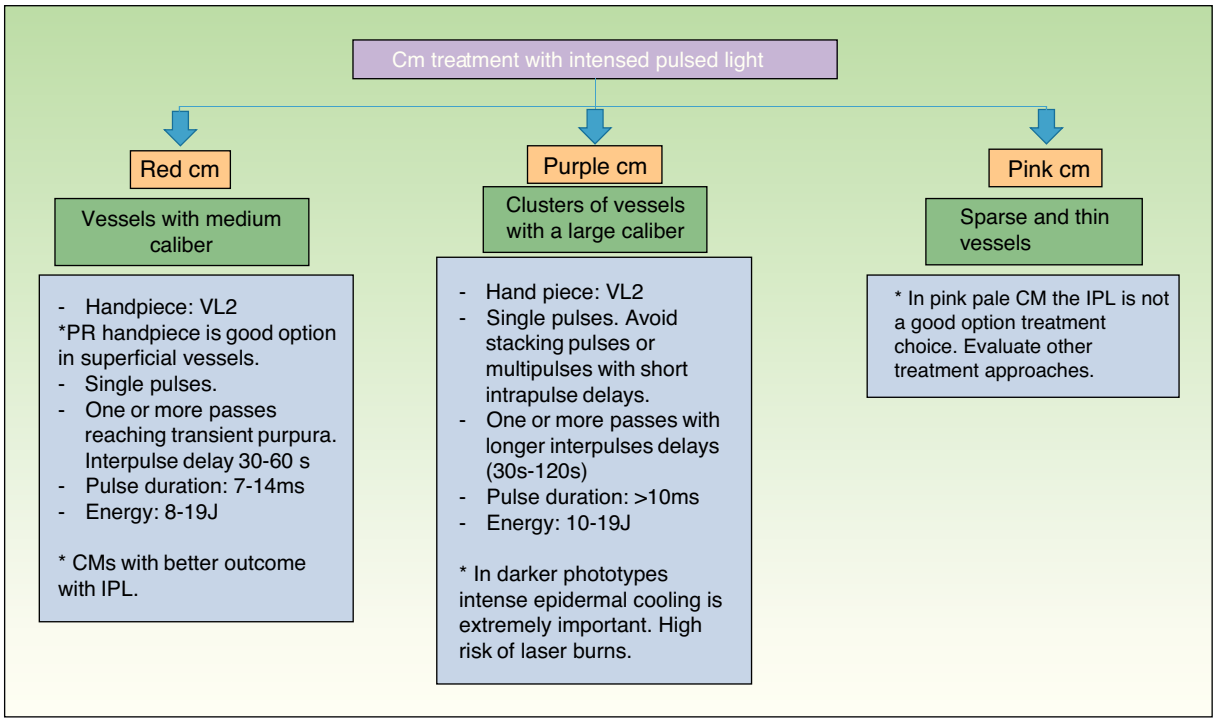

Figure 4 Treatment algorithm with intense pulsed light in capillary malformations type Port wine stain according to the caliber and depth of their vessels.

and consequent vessel wall destruction, without collateral damage.

Intrapulse delay times

Managing intrapulse delays is also extremely important, taking into account the color of the CM, which depends on the vessel density and caliber.[15] Again, long intrapulse delays are safer in the presence of high vessel density and larger caliber. Short intrapulse delays are appropriate to treat CM lesions with low vesselsdensity and small diameter and therefore with less chromophore to absorb light energy.[17]

Darker phototypes and epidermal damage

The risk of epidermal injury is especially seen in patients with darker phototypes, due to considerable melanin absorption of IPL light, mainly in the basal layer.[5] Besides epidermal damage, these patients also showed a poor response to treatment, with insufficient vessel heating and thermal coagulation because most of the energy was retained in the epidermis. Intense epidermal cooling is extremely important in these patients to allow light energy penetration in the dermis.[26]

The therapeutic approach to the CM depends on the caliber, capillary density and depth of the vessels as well as the patient's skin type (Fig. 4).

This study has many limitations, such as the heterogeneity of the selected patients (regarding the skin phototype of the patients or the color and location of the CM) and, above all, the amount of different IPL setting combinations used. However, our aim was only to start to understand the potential improvements we could achieve with the IPL device by individualizing the selection of parameters through these first histochemical observations. These observations may allow for a future comparative and controlled study in which a single or only a small number of parameters are changed when treating comparable lesions/patients in regards to the color and location of the CM and the patient's skin phototype.

In IPL treatment of CM, the goal is to take full advantage of the energy of the emitted light to selectively damage only the vessels without collateral damage. The final outcomes are conditioned by many factors. With regard to the patient, physicians should consider: the vessel size, density and depth; the quantity of red blood cells inside the vessels (i.e., the target chromophore); and the epidermal pigmentation of the patient.[9,18] Regarding the IPL device and the operator, factors that influence our results are: the wavelength spectrum of the IPL device, the light energy, the pulse duration, the possibility of using single or multiple pulses with variable inter- and intrapulse delay times and choosing adequate cooling strategies.[18]

Conclusions

In this study we explored the potential of an IPL device in treating CM and the huge number of possible treatment parameter combinations that can be used. IPL intra- and interpulse delay changes produce very different final results. Our findings suggest that patients with thin vessels should be treated in a more aggressive way. Perhaps with the recent introduction of next-generation intensed pulsed lights with shorter pulses can improve the treatment of this group

of lesions. On the other hand, physicians treating CM in patients with darker phototypes should choose an adequate cooling strategy to avoid epidermal damage.

Ethical responsibilities

Protection of people and animals. The authors declare that this research has not been conducted experiments on humans or animals.

Confidentiality of data. The authors declare that they have followed the protocols of the workplace on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the informed consent of patients and/or subjects referred to in Article consent. This document is in the possession of the corresponding author.

Conflict of interests

The authors declare no conflict of interest.

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