

ORIGINAL ARTICLES

## Selectivity of Photothermolysis in the Treatment of Port Wine Stains Using Multiple Pulses With a Pulsed Dye Laser

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**Abstract.** *Background.* In 25 % to 50 % of patients with port wine stains treated with pulsed dye laser (even in multiple sessions), only a partial improvement is obtained that is often unsatisfactory for the patient. Various factors have been proposed to explain the resistance to treatment, such as the presence of deep, small-caliber, or very thick vessels that cannot be coagulated with a single laser pulse. Certain mathematical models suggest that multiple pulses would be effective in coagulating those vessels without losing the selectivity of photothermolysis, since more energy could be delivered to the target structures without damaging the surrounding tissue. The aim of this study was to assess the efficacy and selectivity of photothermolysis with multiple laser pulses.

*Methods.* Twelve patients with port wine stains were included in the study. Various areas of each lesion were treated with pulsed dye laser (Candela V-beam, 595 nm) using different parameters. Immediately afterwards, the treated areas were biopsied.

*Results.* The risk of exceeding an appropriate dose was greater with small spot size, long pulse duration, short intervals between pulses, and high fluences. Heat damage was less with spots of 10 mm, short pulses, intervals of 1 minute between pulses, and low fluences. In selected cases, multiple pulses were effective and selective where single pulses did not successfully coagulate the vessels.

*Conclusions.* Our results indicate the enormous possibilities of multiple laser pulses for the treatment of refractory vascular lesions. In addition, we have addressed the variables defining the complex thermodynamic interaction between the laser and the cutaneous tissue and indicated the safest parameters. We discuss the possible usefulness of this approach with other lasers and skin diseases.

**Key words:** port wine stain, nevus flammeus, capillary malformation, pulsed dye laser, multiple pulses.

### SELECTIVIDAD DE LA FOTOTERMÓLISIS EN EL TRATAMIENTO DE LAS MANCHAS EN VINO DE OPORTO MEDIANTE MÚLTIPLES PULSOS DE LÁSER DE COLORANTE PULSADO

**Resumen.** *Introducción.* En un 25-50 % de los pacientes con manchas en vino de Oporto tratadas con láser de colorante pulsado (incluso con múltiples sesiones) obtenemos un resultado parcial y frecuentemente insatisfactorio para el paciente. Se han propuesto varios factores que explicarían esta resistencia al tratamiento, como la presencia de vasos profundos, de pequeño calibre o muy gruesos, a los que un solo pulso de láser no logra coagular. Existen modelos matemáticos que sugieren que los multipulsos serían eficaces para coagular estos vasos sin perder la selectividad de la fototermólisis, ya que permiten administrar más energía sobre las estructuras diana, sin lesionar el tejido circundante. El objetivo de este estudio es evaluar la eficacia y la selectividad de la fototermólisis al usar multipulsos.

*Métodos.* Se seleccionaron 12 pacientes con manchas en vino de Oporto. Se trataron varias zonas de cada lesión con láser de colorante pulsado (V-beam 595 nm, Candela®) y distintos parámetros. Inmediatamente después se biopsiaron las zonas tratadas.

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**Resultados.** El riesgo de sobredosificar fue mayor con tamaños del haz pequeños, pulsos largos, intervalo breve entre pulsos y fluencias elevadas. El daño térmico fue menor con tamaños del haz de 10 mm, pulsos cortos, intervalo de 1 minuto entre los pulsos y fluencias bajas. En casos seleccionados, los multipulsos eran eficaces y selectivos, ahí donde el pulso único no lograba coagular los vasos.

**Conclusiones.** Nuestros resultados ponen de manifiesto las enormes posibilidades que los multipulsos ofrecen para el tratamiento de las lesiones vasculares refractarias. Asimismo, definimos los parámetros que intervienen en este complejo proceso termodinámico de interacción del láser con el tejido cutáneo, se señalan cuáles son más seguros y apuntamos su utilidad con otros láseres y patologías cutáneas.

**Palabras clave:** mancha en vino de Oporto, *nevus flammeus*, malformación capilar, láser de colorante pulsado, multipulsos.

## Introduction

### Therapeutic Failure: The Problem

Failure of treatment of port wine stains with pulsed dye laser (PDL) occurs in between 25% and 50% of patients. In these patients, the lesions do not resolve after several laser sessions using a single pulse per unit surface treated.<sup>1</sup>

Several factors have been suggested to explain this resistance to treatment.

### Presence of Deep-lying Vessels

Vascular coagulation is induced to a depth of about 370  $\mu\text{m}$ . At the deepest levels, less energy is available, and, moreover, the most superficial vessels “cast a shadow” over the deeper ones, absorbing most of the light energy (Figure 1).<sup>2-4</sup>

### Presence of Small Vessels<sup>3</sup>

The thermal relaxation time (TRT) of small vessels is short ( $\tau = d^2/8\alpha$ ), and so they cool quickly. In addition, they do not contain much oxyhemoglobin and so absorb little energy. As a result, not much heating occurs and the increase in temperature is insufficient to destroy the vascular wall. Treatment of such vessels requires much higher fluences.

### Presence of Very Thick Vessels<sup>5</sup>

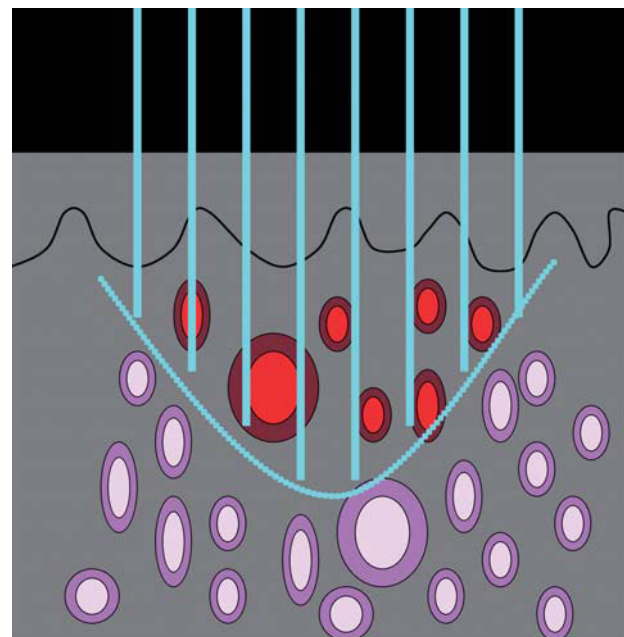
In very thick vessels, the light cannot penetrate the whole vessel thickness because it is absorbed in the upper part of the vessel, leading to incomplete heating of the vessel wall. Port wine stains with deep and thick vessels are very difficult to treat.<sup>6,7</sup>

## Rationale for Multiple-Pulse Treatment

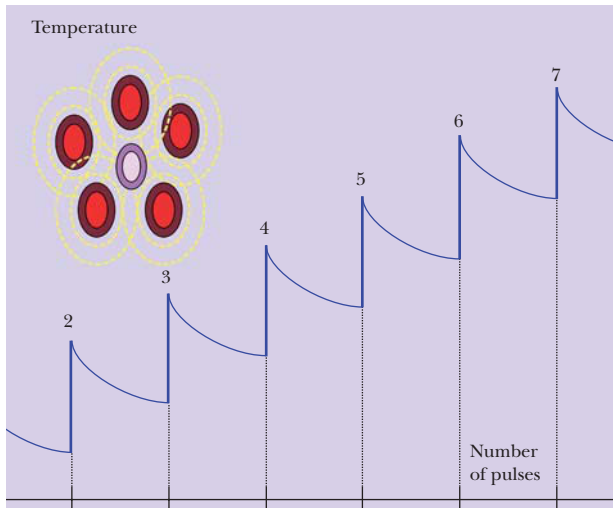
Mathematical models suggest that multiple pulses might be effective for coagulating these problematic vessels without losing the selectivity of photothermolysis. These models have been proposed by Verkrusse et al<sup>8</sup> and Anderson and Dierick.<sup>2,9</sup>

### Verkrusse Model<sup>8</sup>

Using a theoretical model, Verkrusse et al<sup>8</sup> showed that multiple pulses favor an increased temperature in the deepest-



**Figure 1.** “Shadow” effect of the superficial vessels on the deep-lying ones.



**Figure 2.** When there is a central vessel surrounded by a number of other vessels, the central vessel is the one that undergoes the smallest temperature increase after firing the laser as it is “in the shadow” of the surrounding ones. In addition, heat loss in the central vessel occurs more slowly than in the surrounding vessels due to heat transfer from these vessels to the central one. Thus, if several pulses are administered in succession, the temperature after each pulse increases more quickly in the central vessel than in the surrounding ones. According to the model of Verkruijsse et al.,<sup>8</sup> peak equivalent temperatures were attained in the seventh pulse. Gradual increase in temperature of the central vessel occurs after several pulses in succession, thanks to heat transfer from adjacent vessels.

Taken from Verkruijsse et al.<sup>8</sup>

lying vessels and also those most optically hidden by surrounding vessels (Figure 2). These investigators established the hypothesis that multiple pulses would yield better outcomes in the treatment of port wine stains with this type of vessel (clustered vessels, vessels of the deep dermis).

A single high-fluence laser pulse does not cause the same thermal damage as multiple lower-energy pulses. Verkruijsse et al.<sup>8</sup> suggested that thermal damage is greater in the second case.

### Anderson and Dierickx Model<sup>2,9</sup>

The effective fluence is greater in the case of multiple pulses, according to the mathematical model postulated by Anderson and Dierickx.<sup>2,9</sup> Thus:

$$\text{Effective fluence} = \text{number of pulses} \times \text{fluence applied}$$

This means that, for a constant pulse duration, if multiple pulses with a lower fluence are used, thermal damage equivalent to that produced by a single higher-energy pulse

can be achieved. For example, if an area of skin is treated with 16 pulses, the total energy delivered is twice the fluence used. Clinically, applying multiple pulses at energies below the purpuric threshold should induce at least as much clearance as that achieved with single pulses at purpuric fluences.

## Methods

Twelve patients with port wine stains were selected for the study. After obtaining their informed consent, several areas were treated with PDL (V-beam 595 nm, Candela Iberica S.A., Madrid, Spain). The pulse parameters were as follows: wavelength, 595 nm; number of pulses, 1 to 3; interval between pulses, 0.75 seconds to 1 minute; pulse duration, 1.5, 6, and 10 ms; fluences, 6.5 (spot diameter, 10 mm), 10, and 12 J/cm<sup>2</sup> (spot diameter, 7 mm) (Table 1).

Immediately afterwards, a 4-mm biopsy was taken from each of the treated areas (31 biopsies in total). The pieces were transported in saline solution and frozen sections were prepared. The biopsy samples were stained with nitroblue tetrazolium, which is a redox compound that is reduced by nicotinamide adenine dinucleotide (NADH) diaphorase to form an intensely blue precipitate (diformazan). Only viable cells express this enzyme. It is therefore possible to clearly distinguish between healthy tissue, which is stained blue, and unstained necrotic tissue after exposure to laser light. Endothelial cells, fibroblasts, hair follicles, sweat glands, and smooth muscle show high NADH-diaphorase activity, whereas the stroma of connective tissue does not. Epidermal damage was considered as elongation and/or basal keratinocyte ballooning or focal areas of weaker staining.<sup>10-13</sup>

## Results

Table 2 shows the clinical characteristics, age, and parameters of the laser pulses used. In total, 3 venous-capillary patterns were observed in the biopsies taken: thick vessels close to one another, and more widely spaced small-diameter vessels. We present below the results for patients 1, 2 and 3, as examples of the 3 forms of reaction observed according to the thickness and depth of the vessels (Figure 3). For each venous-capillary pattern, similar histological findings were reported in the biopsies of the other 9 patients.

### Patient 1

Two pulses in succession induced greater coagulation than a single pulse, although the photothermolytic process lost selectivity and epidermal damage was apparent. In addition, an improved clinical response was observed (Figure 4).

**Table 1.** Patients Who Underwent Biopsy; Age, Site, and Color of the Port Wine Stain, and Laser Parameters

Patient	Site	Age, y	Color	Parameters
1	Back of the ear	23	Wine red	1 pulse: 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
2	Back of the ear	31	Violaceous	1 pulse: 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
3	Back of the ear	30	Wine red	1 pulse: 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 7 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				3 pulses (1.5 Hz): 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
4	Back of the ear	20	Wine red	1 pulse: 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
5	Back of the ear	36	Pink	1 pulse: 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
6	Back of the ear	42	Wine red	1 pulse: 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
7	Back of the ear	33	Violaceous	1 pulse: 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
8	Back of the ear	33	Violaceous	1 pulse: 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
9	Back of the ear	46	Violaceous	1 pulse: 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
				3 pulses (1.5 Hz): 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
10	Back	55	Violaceous	1 pulse: 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
				3 pulses (1.5 Hz): 7 mm, 10 ms, 10 J/cm <sup>2</sup> , 30-20
11	Back	27	Pink	1 pulse: 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
				3 pulses (1 min): 10 mm, 10 ms, 6.5 J/cm <sup>2</sup> , 30-20
12	Back	53	Wine red	1 pulse: 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20
				2 pulses (1.5 Hz): 7 mm, 6 ms, 12 J/cm <sup>2</sup> , 30-20

## Patient 2

A 10-mm spot size was used. Both the successive pulses as well as those separated by 1 minute proved safe and caused greater coagulation than a single isolated pulse. In this case, the vessels were small in diameter and so there was less delay in cooling (Figure 5)

## Patient 3

The first pulse induced good coagulation. When 3 successive pulses were applied, diffuse nonspecific thermal damage was observed. In this case, the vessels were large, with a high vascular density, and so more time was required for cooling. Radial patterns of thermal damage were observed

**Table 2.** Factors Determining Treatment Response

<b>Laser Factors</b>
Wavelength
Energy or fluence
Pulse duration
Spot size
Cooling system
Delay between application of cooling and laser pulse
Interval between pulses, if more than 1 applied
<b>Patient Factors</b>
Thickness of epidermis
Pigmentation (melanic or not)
Depth of target tissue
Thickness of vessel wall
Vessel diameter
Total vascular area
Amount of chromophore (hemoglobin in vessel)
Presence or not of dermal fibrosis
Thermal relaxation time of tissue

due to propagation of heat in concentric waves from the center of each vessel. The loss of selectivity was associated with anomalous healing and pigmentary changes. Given that 3 pulses were applied with 1 minute between each, greater coagulation was achieved than with a single pulse, but without losing selectivity because cooling between pulses can occur, and so the dermal and epidermal structures are not damaged (Figure 6).

## Discussion

Multiple pulses are a promising alternative in the treatment of port wine stains that do not respond well to conventional treatment with a single pulse in view of their characteristics (high vascular density, very narrow or very deep vessels). This technique has yet to be fully optimized.

According to the findings of this study, the efficacy of multiple pulses depends on 2 types of factor: laser-dependent factors and patient-dependent factors (Table 2). With regard to the laser parameters, the following should be assessed.

## Fluence<sup>14</sup>

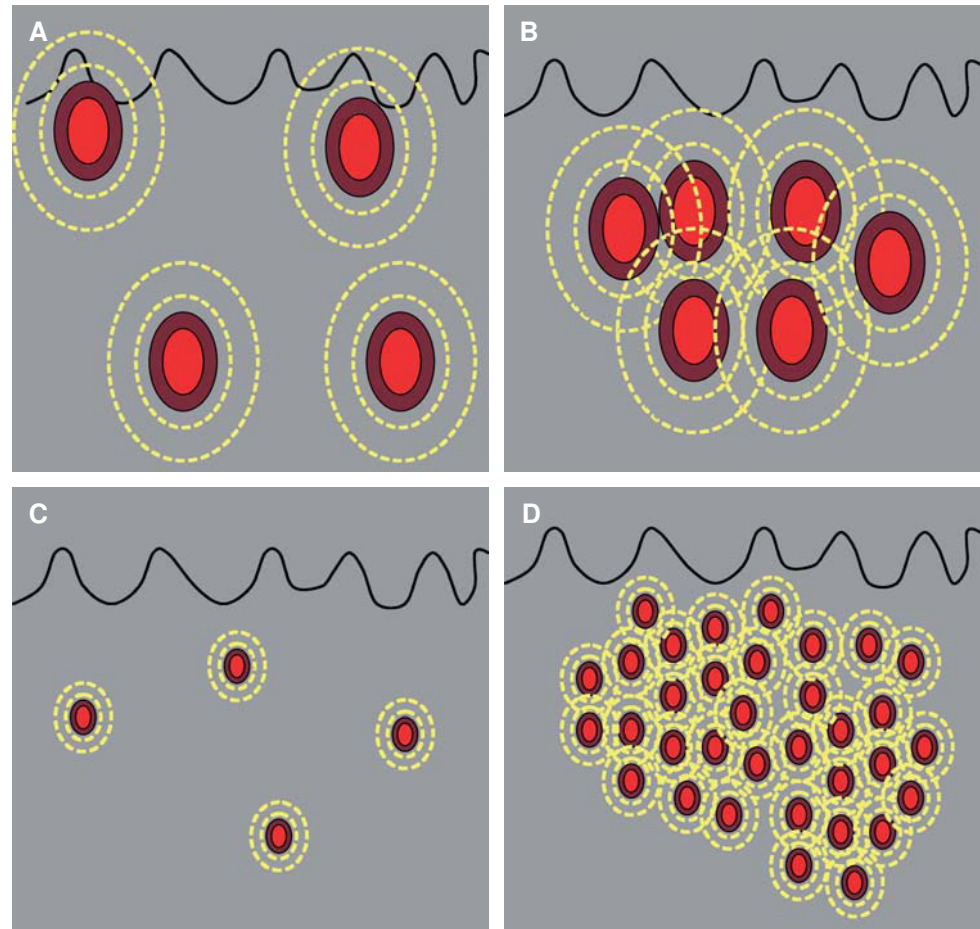
The objective is to use the minimum fluence that is useful for attaining the best outcome, with acceptable side effects. When single pulses are used, high fluences may be required, making photothermolysis less selective with the corresponding risk of overdosing and damage to other structures. The objective of multiple pulses is to increase the selectivity of the laser. Instead of delivering all the energy in a single pulse, with the associated risks, the energy is spread among lower-energy pulses. The total cumulative fluence is greater than after a single pulse, but adjacent structures are spared. Use of energies at or below the purpuric threshold seems safe and, in fact, extensive positive experience has been reported for such strategies in the treatment of facial telangiectases.<sup>15</sup>

## Interval Between Pulses

The interval between pulses can be crucial, as reflected by Patient 3. Successive pulses led to necrosis, and 3 pulses separated by a minute gave rise to effective and selective coagulation because the temperature increased rapidly when short intervals between pulses were used and cooling that might help preserve the adjacent structures did not occur. According to the theory of thermal diffusion, the purpuric threshold increases with increasing interval between pulses. However, some studies done in skin have found a decrease in the purpuric threshold and greater vascular damage the longer the interval between pulses.<sup>16</sup> This discrepancy can be explained by a biological phenomenon such as induction of the formation of methemoglobin after the first pulse and the theoretical increase in susceptibility of the lesion induced by the second pulse. Other investigators have suggested that the state of anoxia that occurs in endothelial cells after the first pulse is responsible for greater susceptibility to subsequent pulses.

## Number of Pulses

With administration of multiple pulses, an increase in temperature in a target vessel can be achieved if the surrounding vessels are also heated at the same time. This mechanism of linear propagation of heat in the form of concentric rings from the peripheral vessels towards the central one is responsible for a continuous increase in peak temperature at the end of each laser pulse, and the same increase in temperature in the surrounding dermis according to the model of Verkruysse et al.<sup>8</sup> Dermal heating endangers the selectivity the photothermolytic process and makes the use of cooling systems essential. Further studies are required to define the ideal number of pulses with the appropriate



**Figure 3.** Thermal damage according to the anatomy of the vascular lesion. Thermal damage is greater with higher vascular density and vessel diameter and lower with lower vascular density and smaller diameter vessels.

parameters in order to achieve good outcomes without overexposure.

### Spot Size

For equal fluence, the larger the spot size, the deeper the penetration (because the effect of light dispersion is reduced). A large spot size improves propagation through the tissue, providing deeper penetration and better heating of the deepest-lying vessels. For this reason, lower fluences are required, less epidermal damage occurs, and the extent of purpura is limited, making possible a second pulse. In our experience, the safest spot size is 10 mm. Probably, the fact that lower fluences—not equivalent to those that can be used with a 7-mm spot size—were used contributed to this safety.

### Pulse Duration

The longer the pulse duration, the more time the skin structures need for cooling. However, for the same fluence,

shorter pulse durations correspond to greater destructive capacity.

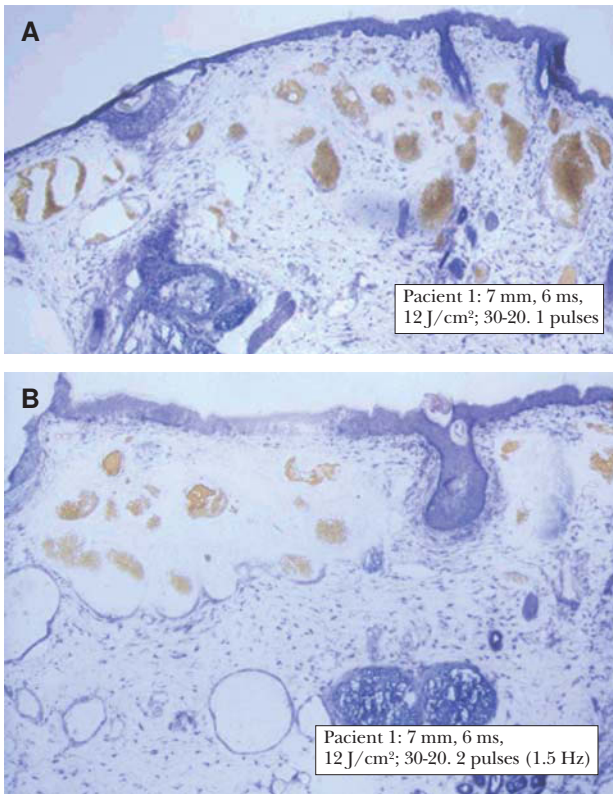
### Cooling Parameters

It is also important to consider cooling parameters, although these were maintained constant in our study. The safety of the procedures increases with increased cooling, but at the expense of lower efficacy. Cooling protects the epidermis, although if very high fluences are used, that protection is insufficient.<sup>17</sup> The area that is most damaged is at the center of the spot.<sup>18</sup> The risk is greater in children and at certain anatomic sites, such as the neck.

The parameters used depend on the patient. The following sections describe the parameters that should be taken into account to ensure patient safety during multiple pulse procedures.

### Vessel Size and Vascular Density

The larger the vessel size and the higher the vascular density, the more time is required for cooling. The interval between

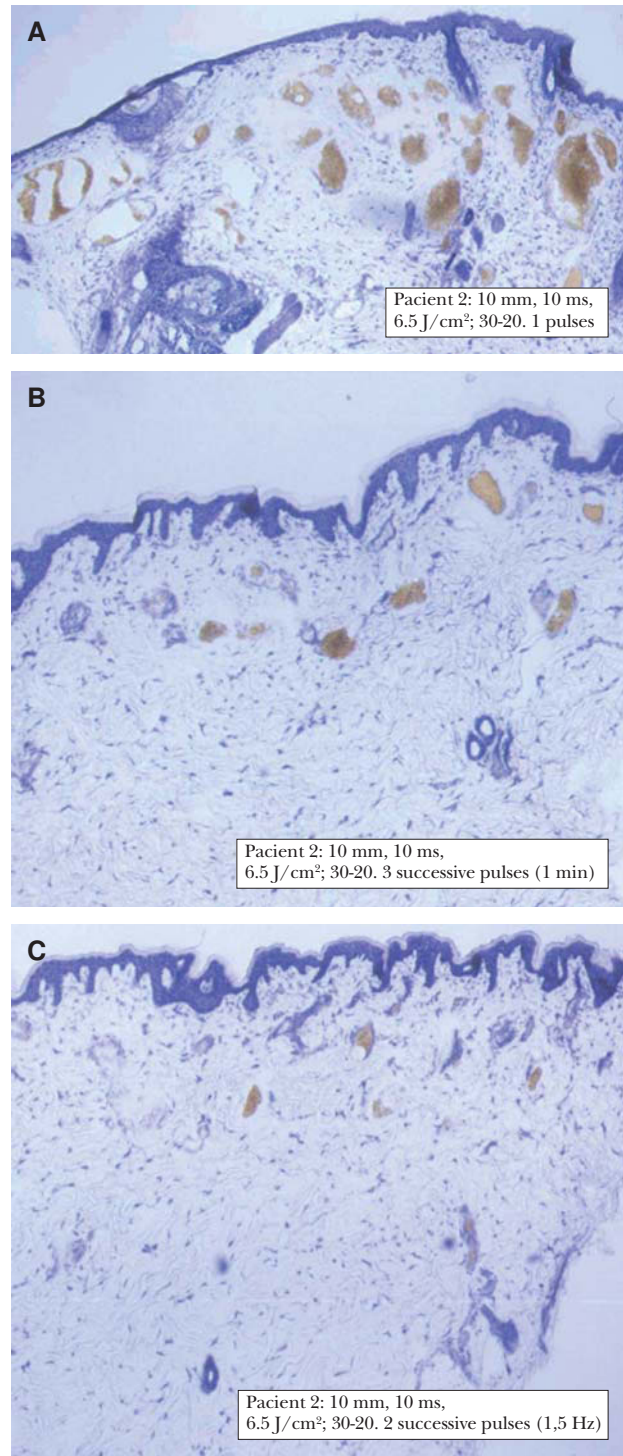


**Figure 4.** Patient 1. A: A pulse caused marked coagulation of the dermal vessels. The surrounding tissue stains with nitroblue tetrazolium, providing an indication of its viability. Photothermolysis is seen to be selective. B: With 2 pulses, coagulated vessels and inviable surrounding tissue are seen following the form of the thermal waves.

pulses should be lengthened to maintain the selectivity of photothermolysis. This is the case for patients 1 and 3, whose vessels were thick and abundant. Concentric waves of necrosis occurred with the successive pulses (1.5 Hz). In patient 3, pulses were separated by 1 minute, allowing effective and safe treatment. It may be concluded that multiple pulses are a safer technique if they are used with small-diameter vessels and in lesions with low vascular density (Patient 2)

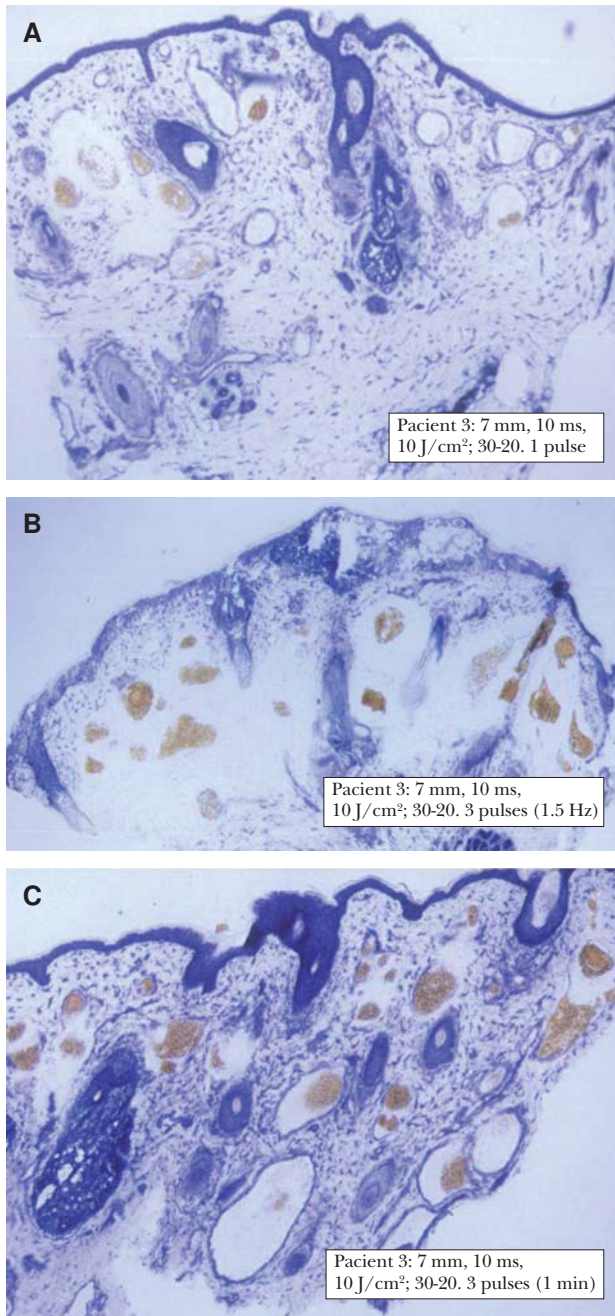
### Amount of Hemoglobin in the Vessels

It is worth considering the amount of hemoglobin in the vessels. The PDL directly induces vasodilation in healthy blood vessels.<sup>19</sup> This vasodilation is substantial (up to 3 times in venules and 4 times in arterioles), almost immediate (within 200 ms),<sup>8</sup> and larger for larger energies. The vessels of port wine stains have limited innervation, and their vasodilatory response is slower and late; even so, vasodilation has been observed, at least in the deepest-lying vessels.



**Figure 5.** Patient 2. A single pulse hardly has any effect on a small number of narrow vessels. The most effective coagulation, without loss of selectivity, is achieved with 3 successive pulses separated by 1 minute.

Likewise, it has also been observed that multiple pulses lead to reactive hyperemia after the second pulse,<sup>8</sup> as was also the case in our patients. These phenomena lead to an



**Figure 6.** Patient 3. A single pulse hardly has any effect on a small number of narrow vessels. The most effective coagulation, without loss of selectivity, is achieved with 3 successive pulses separated by 1 minute.

increase in the number of erythrocyte-filled capillaries. Thus, after the second pulse, subsequent pulses cause deeper vascular damage. In addition, coagulation of vessels causes vascular collapse, and the chromophore disappears. By using consecutive pulses, each pulse induces greater coagulation. For this reason, it might be thought that repeated pulses are safer than a single pulse with a higher fluence.

## Depth of the Vessels

The depth of the damage is greater with multiple pulses and, as has been explained earlier, with increased spot size. The depth of coagulation is greater with an interval between pulses of 1 minute than with successive pulses (0.88 mm for a triple pulse over a 1-minute period; 0.66 mm for 3 consecutive pulses at 1.5 Hz; and 0.77 mm for a single pulse). The maximum depth and maximum effect are attained at the center of the spot.

In summary, with multiple pulses, we can affirm that the risk of overexposure is greater with smaller spot sizes,<sup>20</sup> longer pulses (6-10 ms), short interval between pulses (1.5 Hz), and high fluences. Thermal damage is less, and so the approach can be used more safely with spot sizes of 10 mm, short pulses (1.5 ms), 1-minute intervals between pulses, and low fluences. However, we should remember that for equal fluences, shorter pulse durations correspond to greater destructive capacity.

Further studies are still required to elucidate the technical and biologic characteristics of multiple-pulse techniques, and the optimum time between pulses to optimize the clinical outcomes.

## Conflicts of Interest

The authors declare no conflicts of interest.

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