Photothermally Induced Vessel-Wall Necrosis After Pulsed Dye Laser Treatment: Lack of Response in Port-Wine Stains With Small Sized or Deeply Located Vessels

Eli Janne Fiskerstrand,*‡ Lars O. Svaasand,§ Gunnar Kopstad,†‡ Kristin Ryggen,* and Steinar Aase‡

Departments of *Dermatology and †Pathology, University Hospital, Trondheim; ‡Institute of Morphology, University of Trondheim; and §Norwegian Institute of Technology, Trondheim, Norway

The optimal treatment of port-wine stains is laser-induced selective photothermolysis. Lesion color and location and the age of the patient are reported to influence the therapeutic outcome. This study was initiated to analyze the outcome not only by the clinical response of lightening, but also in terms of photothermally induced necrosis to the vessel wall. Punch biopsy specimens were taken from 51 patients before treatment. Post-treatment biopsies were taken after exposure to a pulsed dye laser (585-nm wavelength, 0.45-ms pulse length) with an irradiant fluence of 6.5 J/cm^2. Vessel diameter, depth, and wall thickness were measured in all histologic slides. The viability of the vessel walls was evaluated using an enzyme histochemical method. Port-wine stains with good blanching had significantly more superficially located vessels than the moderate and poor responders ($p < 0.000$). The moderate and good responding lesions consisted of moderate-sized vessels with diameters of 38 ± 17 μm and 38 ± 19 μm (mean ± SD), respectively. The lesions showing poor blanching had significantly smaller vessels, with a diameter of 19 ± 6.5 μm ($p < 0.000$). Analyses of the post-treatment specimens showed that coagulated vessels were superficially located and of moderate size, whereas the viable vessels were small with a median diameter of 14 μm. The probability of coagulation correlated with the thickness of the vessel wall. These data indicate that the therapeutic outcome of port-wine stains can be improved by using the lesional vessel parameters to select the optimal laser wavelength, pulse duration, and dose. ***Key words: photothermolysis/enzyme histochemistry/morphometric measurements. J Invest Dermatol 107:671–675, 1996***

Port-wine stains (PWS) consist of ectatic vessels in the superficial vascular plexus of dermis (Barsky *et al*, 1980). Reported results on the treatment of PWS with pulsed dye lasers (PDL) demonstrated that blanching of the lesion was dependent on patient age, lesion color, and location (Tan *et al*, 1989; Reyes and Geronemus, 1990; Renfro and Geronemus, 1993; Fitzpatrick *et al*, 1994), but published studies have not compared the therapeutic outcome with relevant morphometric parameters such as vessel diameter, wall thickness, and depth in the dermis. A recent study analyzing the vascular parameters in test sites before laser treatment (585-nm wavelength, 0.45-ms pulse, 5.25–6.50 J/cm^2 fluence) found the best response in PWS with moderate-sized vessels located above 300 μm in the dermis (Fiskerstrand *et al*, 1996). The average diameter and depth from the dermoepidermal junction were 40 μm and 200 μm, respectively. PWS with more deeply located (about 300 μm depth) vessels of moderate size demonstrated moderate blanching, whereas PWS with an average diameter less than 20 μm generally responded very poorly. Hoehenleutner *et al* (1995) analyzed histochemically stained biopsy specimens from 14 patients with PWS treated with the PDL, but the mean vessel coagulation diameter was not reported, and the response of extremely small-sized vessels was not discussed.

The objective of this study was to examine the influence of vessel morphology on vessel-wall viability after laser exposure.

MATERIALS AND METHODS

Only macular PWS were submitted, excluding vascular malformations such as salmon patch and telangiectatic lesions. After giving their informed consent, 51 patients were included consecutively in the study from May 1992 to October 1995. Test areas of 3 cm^2 in the most homogeneously colored parts of the lesions were selected for treatment.

Laser Specifications The laser was a PDL emitting at 585 nm with a 0.45-ms pulse length and a 5-mm diameter spot size (Cynosure LPDL-5; Cynosure Inc., Bedford, MA). Three test sites were exposed to slightly overlapping pulses with fluences from 5.25 to 6.50 J/cm^2. The distance between the individual test sites was 5 mm. The pulse energy was measured with a thermopile energy meter (Ophir model DGX, Monitor F-150-APH; Ophir Optronics Ltd., Jerusalem, Israel) with ±5% accuracy.

Histologic Preparations Three-millimeter pre-treatment punch biopsy specimens were taken from all patients within 5 mm of the test site exposed to 6.50 J/cm^2. In a subgroup of 14 patients, one punch biopsy was taken from the test site exposed to 6.50 J/cm^2 15–20 min after laser treatment. All specimens were taken under local anesthesia with 1% xylocaine and no

Manuscript received March 1, 1996; revised July 10, 1996; accepted for publication July 23, 1996.

Reprint requests to: Dr. Eli Janne Fiskerstrand, Department of Dermatology, University Hospital of Trondheim, 7006 Trondheim, Norway.

Abbreviations: NBTC, nitroblue tetrazolium salt; PDL, pulsed dye laser; PWS, port-wine stains.

0022-202X/96/$10.50

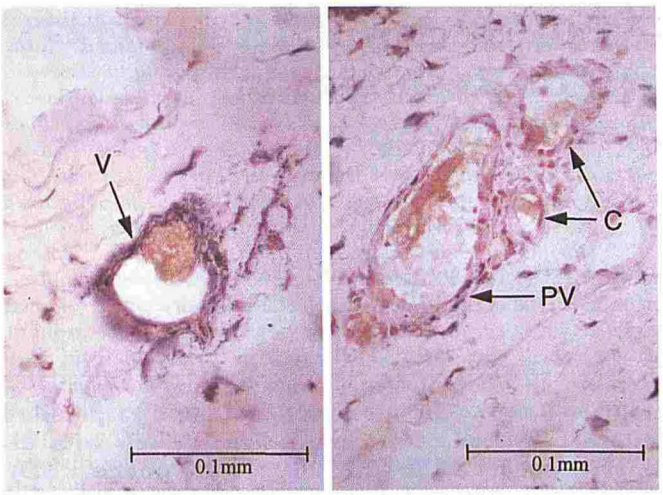

Figure 1. Absence of blue formazan granules identifies laser-induced thermal damage. Viable vessels (V) containing intracellular NADH-diaphorase in the vessel wall will, in the presence of nitroblue tetrazolium salt, precipitate small blue formazan granules. Partly viable vessels (PV) show scattered blue staining in parts of the wall, whereas coagulated vessels (C) completely lack blue formazan granules.

vasoconstrictor. The pre-treatment samples were fixed in 10% neutral buffered formalin, and the histologic examination was done on standard 4-μm–thick sections embedded in paraffin. The slides were stained with hematoxylin-eosin-saffron and periodic-acid Schiff stains. The post-treatment specimens were snap-frozen in liquid nitrogen and were then stored at -80 (± 5)°C for 1–4 mo before processing.

Histochemical Staining Vessel viability was examined by the histochemical reduction of nitroblue tetrazolium salt (NBTC) by nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase). In the presence of NBTC, active tissue sections with viable cells containing NADH-diaphorase will precipitate formazan, consisting of small, blue, protein-bound amorphous particles that are insoluble in both lipid and water. The lack of intracellular formazan granules can be used to determine the regions of photothermally induced necrosis, and the NBTC/NADH-diaphorase histochemical reaction has been shown to be specific for the exact definition of laser-induced thermal damage (Neumann *et al*, 1991). The histochemical procedures were performed as described in detail by Neumann *et al* (1991), with the following modifications. The unfixed frozen biopsy specimens were cut into sections 5 μm thick and then incubated with test solution for 15 min. The sections were washed in distilled water and stained with nuclear red for 4 min. After being rinsed in water, the sections were dehydrated in increasing concentrations of alcohol (80%, 96%, and 100%). Finally the slides were passed through xylene and then mounted in Histokitt Corbit Balsam number 1025/250 (Karl Hecht Glaswarenfabrik, Sondenheim, Germany). By light microscopy, the NBTC staining pattern was uniform and reproducible in all sections. Diffusion of the dye into the mounting medium was not observed.

Morphometric Analysis The histologic slides were examined with a computer-assisted image analysis program (Vidas 2.5; Kontron Elektronik GmbH, Eching, München, Germany). In all slides, the total dermal area and the number, perimeter, and depth of vessels were measured to a depth of 0.8 mm from the dermoepidermal junction. Vessel diameter and area were calculated from the vessel perimeter under the assumption of a circular cross-section of the intact vessel, and the percentage vascular area was obtained from the vessel and dermal areas.

The viability of the vessel wall was evaluated by the staining pattern. The vessels were classified as coagulated when NBTC staining was absent, partially viable when there was scattered staining in parts of the walls, and viable when the walls were completely covered by NBTC stain **(Fig 1)**.

Clinical Evaluation The colors of the test areas were compared with and numbered according to the Pantone Color System, and the different colors were then grouped into pink, red, dark red, and purple stains. The degree of blanching was recorded 6–8 wk after laser exposure. The method of evaluation was a slight modification of that described by Garden *et al* (1988). The color of the nearby normal skin was designated as 100% lightening. More than 75% lightening was assessed as good, 25–75% as moderate, and less than 25% as poor. Pre-treatment color and post-treatment blanching were evaluated by a dermatologist and a medical assistant; later they were re-evaluated by a second dermatologist from photographs taken before treatment and 6–8 wk after laser exposure. There was a 94% overall agreement between the classifications done by the different observers.

Statistical Analysis Statistical analysis was performed with the SPSS statistical program (SPSS Inc., Chicago, IL). The morphometric parameters from the 51 pre-treatment slides were normally distributed. The data from the 14 post-treatment slides were highly skewed except from the distribution of vessel-wall thickness. Skewed data are presented with medians (25–75th percentile). Student's t test and the Mann-Whitney test were used to compare differences between groups. Logistic regression analysis was used to study possible interactions of vessel diameter, vessel depth, and vessel-wall thickness on the outcome of laser therapy. Differences were considered significant at $p < 0.05$.

RESULTS

The Clinical Response of Lightening Is Dependent on Vessel Depth and Diameter Twenty-two patients (43%) achieved good clinical lightening, 21 (41%) had moderate, and eight (16%) had poor lightening of the test areas. **Table I** shows that the vessels of the moderate and good responding PWS were of moderate size, whereas the vessels of the poor responders were significantly smaller. The test sites with good clinical response had significantly more superficially located vessels than those of the moderate and poor responding lesions. The thickness of the vessel wall was in the range of 4–5 μm for all response groups. There was a tendency toward larger vessel-wall thickness with increasing depth in the dermis. This corresponds to the fact that the good responders, who had the most superficial vessels, also had those

Table I. Pre-Treatment Biopsy Specimens: Clinical Response of Lightening Is Determined by Vessel Diameter and Depth

Measurement (μm; mean ± SD)	Poor Lightening (n = 8)	Moderate Lightening (n = 21)	Good Lightening (n = 22)	Two-Tailed p Values (95% CI)[a]		
				Poor *vs* Moderate	Moderate *vs* Good	Poor *vs* Good
Diameter	19 ± 7	38 ± 17	38 ± 19	0.000 (−27.9; −10.4)	0.97	0.000 (−28.4; −9.7)
Depth	280 ± 46	315 ± 89	202 ± 56	0.7	0.000 (66.2; 159.1)	0.002 (31.9; 122.1)
Wall thickness	5.3 ± 1.8	5.4 ± 2.1	4.5 ± 1.7	0.76	0.01 (0.23; 2.3)	0.348

[a] CI, confidence interval.

Table II. Pre-Treatment Biopsy Specimens: Vessel Diameter, Depth, and Wall Thickness Vary With Different Locations of the PWS

Measurement (μm; mean ± SD)	Face (n = 14)	Neck (n = 7)	Truncus (n = 13)	Upper Part of the Extremities (n = 16)
Diameter	38 ± 16	47 ± 18	25 ± 13[a]	36 ± 21
Depth	352 ± 87[b]	229 ± 60	224 ± 62	230 ± 61
Wall thickness	5.0 ± 1.7	5.7 ± 1.6	4.1 ± 0.6	5.5 ± 2.4
Response[c]	1, 10, 3	0, 1, 6	4, 4, 5	3, 6, 8

[a] Significantly different from face, p = 0.03 (95% confidence interval [CI] 1.10; 23.7); and neck, p = 0.02 (95% CI −32.5; −3.0).
[b] Significantly different from neck, p = 0.002 (95% CI 54.2; 207.4); truncus, p = 0.000 (95% CI 67.7; 187.7), and extremities; p = 0.000 (95% CI 68.4; 175.4).
[c] Numbers of lesions with response of poor, moderate, and good, respectively.

with the thinnest walls, but the relation between wall thickness and vessel depth varied with anatomic site of the PWS **(Table II)**.

Vessel Depth Varies With Location of the PWS, and Lesion Color Is Determined by the Diameter and Depth of the Vessels The vessel parameters showed some variation in different lesion locations **(Table II)**. The PWS located in the face were characterized by significantly more deeply located vessels than the lesions situated elsewhere on the body. Of a total of 14 facial lesions, only three (21%) obtained good blanching. In comparison, the percentages of good responding lesions on the neck, trunk, and upper part of the extremities were 86%, 39%, and 47%.

The color of the test sites was dependent on the dermal blood fraction, i.e., on the relative vascular area as determined in the biopsy specimens. The vascular area was positively correlated with vessel diameter (Pearson r = 0.8, p < 0.000), but not with the number of vessels. The pink lesions had the smallest vessels (p < 0.01), with diameters of 15 ± 4.5 μm (mean ± SD). All of the pink lesions achieved poor blanching of the test areas. The red lesions with relatively superficial vessels, at a mean depth of 212 ± 57.6 μm (p < 0.05), obtained the best results, with a 67% good response rate. The purple lesions had the deepest vessels, with a mean depth of 318 ± 101.4 μm, and only four of 12 (33%) achieved good blanching.

The Degree of Photothermally Induced Vessel-Wall Necrosis Is Determined by Vessel Diameter, Depth, and Wall Thickness Post-treatment biopsy specimens were taken from PWS of 14 patients. The samples were prepared for histochemical staining, allowing us to differentiate between viable and intact vessel walls. **Figure 1** illustrates the criteria for classification of vessels as completely coagulated, partly viable, or viable. A total of 265 vessels were examined.

Table III shows that the median diameters of the coagulated and partly viable vessels were approximately the same, i.e., 55.8 μm and 51.5 μm, respectively. The diameters of the viable vessels, however, were only about 25% of the values of the other two groups (median 14.4 μm). The coagulated vessels were significantly more superficially located, whereas there were no differences in depth of the viable and partly viable vessels. **Figure 2** shows the distribution of the vessels in relation to vessel diameter and depth. None of the vessels smaller than 12 μm, and only five of the 49 vessels of 20 μm or smaller, were coagulated **(Fig 2*a*)**. Most of the coagulated vessels were located less than 400 μm from the dermoepidermal junction. **Figure 2*b*** demonstrates that a relatively large proportion of the partly viable and viable vessels either had diameters less than 20 μm or had a depth greater than 400 μm from the dermoepidermal junction. We conclude that the small and deeply located vessels were beyond the reach of coagulation.

There was no correlation between vessel diameter and depth, but because of a weak correlation between wall thickness and depth (r = 0.2, p = 0.005), we used logistic regression analysis to identify a possible effect of wall thickness on vessel viability. By forward stepwise regression analysis, vessel depth (p = 0.0000, r = 0.30) and vessel diameter (p = 0.0000, r = −0.22) were defined as the most important factors for vessel-wall coagulation. Vessel-wall thickness, however, was an independent, though less significant factor for wall coagulation (p = 0.0016, r = 0.14). The results indicate that the vessel-wall thickness is of greater importance for coagulation in small vessels than in large ones. The mean wall thickness of the coagulated vessels with diameters less than or equal to 20 μm located in the upper 400 μm from the dermoepidermal junction was significantly smaller than that of the partly viable and viable vessels of the same size, i.e., 2.1 ± 1.1 μm (mean ± SD) compared with 5.1 ± 2.0 μm (p = 0.005, confidence interval −4.92; −0.95), with a mean difference of −2.8 μm.

Figure 3 demonstrates the effect of vessel diameter and depth on vessel-wall viability in histochemically stained specimens, showing both PWS with good clinical lightening and poorly responding PWS.

Vessel Diameter Is Enlarged as a Result of Photothermolysis The mean vessel diameter was two to three times larger in the specimens taken after laser exposure than in the pre-treatment biopsies. **Figure 4** demonstrates the effect of photothermolysis on vessel diameter and shows the cumulative percentage of vessel diameter in the pre- and post-treatment specimens.

DISCUSSION

Our study shows that the clinical response of lightening in PWS is dependent on vessel depth, diameter, and wall thickness. The poorest lightening was achieved in lesions with a small vessel diameter of 19 ± 7 μm (mean ± SD) **(Table I)**. These lesions were characterized by a homogeneously deep, saturated pink color. Darker colors with larger vessel diameter showed moderate or good response depending on the depth of the vessels. The demonstration of the poorest response in pink lesions by the 585-nm PDL is in good agreement with the studies of Taieb *et al* (1994) and Holy and Geronemus (1992). The 577-nm, 0.36-ms PDL has given excellent results on pale pink lesions (Tan *et al*, 1989; Reyes and Geronemus, 1990). Analytic modeling of the influence of wave-

Table III. Post-Treatment Biopsy Specimens: Vessel-Wall Coagulation Is Dependent on Vessel Diameter, Depth, and Wall Thickness

Measurement (μm)[a]	Coagulated (C) (n = 142)	Partly Viable (PV) (n = 71)	Viable (V) (n = 52)	Two-Tailed p Values[b]		
				C *vs* PV	PV *vs* V	C *vs* V
Diameter	56 (37.7–101)	52 (27.0–84.8)	14 (11.6–30.1)	0.137	0.000	0.000
Depth	150 (76.5–293.6)	380 (214.9–583.3)	361 (120.9–596)	0.000	0.590	0.000
Wall thickness	4.6 (3.3–5.7)	5.9 (3.9–7.0)	4.7 (3.6–5.7)	0.000	0.081	0.105

[a] Median (25–75th percentile).
[b] Mann-Whitney nonparametric test.

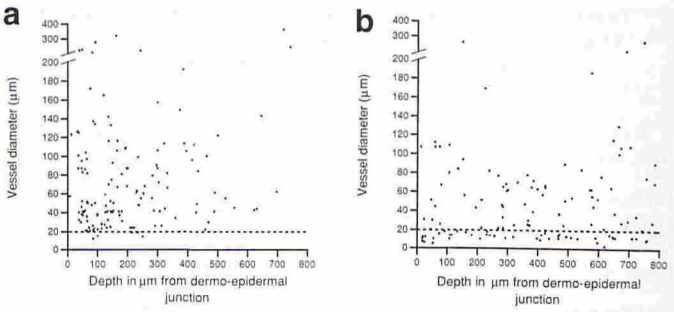

Figure 2. Coagulated vessels are more superficially located and have larger diameters than the partly viable or viable vessels. *(a)* Distribution of depth from the dermoepidermal junction and diameter of the coagulated vessels. *(b)* Distribution of depth and diameter of the partly viable and viable vessels.

length on PWS with different dermal blood content confirms that 577 nm is the optimal wavelength for treatment of pink PWS (Van Gemert *et al*, 1995). The escape of the smallest vessels from thermal necrosis is proved by the results of the histochemical analyses. Only 10% of the 49 vessels smaller than 20 μm, and none of the vessels smaller than 12 μm, were coagulated. Smaller vessels require higher fluence because the amount of energy needed to heat the vessel wall then becomes a substantial fraction of the absorbed energy. The primary sites of heat generation are the erythrocytes, and the maximum temperatures within the vessel wall and the perivascular structures are therefore determined by heat conducted from the lumen during the laser pulse. The thermal diffusion length, L (m), is given approximately by the square root of the product of the pulse duration Δt (s) and a tissue parameter χ (m^2/s), commonly referred to as the thermal diffusivity. The diffusion length in the case of a 0.45-ms pulse in a tissue with thermal diffusivity of 1.2×10^{-7} m^2/s is therefore:

$$L \approx \sqrt{\Delta t \chi} \Rightarrow 7\ \mu m$$

Thus, the optical energy density absorbed within the cross-sectional area, $\pi d^2/4$, of a vessel with lumen diameter d is also distributed over the cross-sectional area of the heated vessel wall, i.e., approximately πdL during the pulse. The required optical dose will therefore increase strongly with decreasing vessel diameter when the cross-sectional area of the lumen becomes smaller than the heated area of the vessel wall, i.e., when:

$$d \approx 4L = 4\sqrt{\Delta t \chi} \Rightarrow 28\ \mu m$$

This threshold diameter can be reduced by reducing the length of the laser pulse, but the pulse should, of course, not be smaller than the time required for the heat to diffuse across the entire wall thickness, i.e., about 0.2 ms in the case of a wall 5 μm thick (Svaasand *et al*, in press). Garden *et al* (1988) compared the degree of blanching in PWS after exposure to a 577-nm PDL with pulse lengths of 20 and 0.36 ms. The longer pulse duration produced the best degree of lightening. In the case of a 20-μs pulse, heat diffusion into the vessel wall will be insufficient to produce necrosis in the full thickness of the wall. Studies should be performed, however, to clarify the possible benefits of exposure times in the range of 0.2 to 0.36 ms on selected groups of patients with pink lesions proved to have small vessels.

Hoehenleutner *et al* (1995), analyzing histochemically stained specimens from 14 patients with PWS treated with the PDL at fluences of 6–8 J/cm^2, found that vessels larger than 200 μm were incompletely coagulated, whereas our data gave no evidence for a maximum coagulation diameter. In fact, we found the largest vessels among the completely coagulated ones, though it must be emphasized that the vessel diameter was enlarged as a result of laser exposure. Analyses of the pre-treatment biopsies showed very few vessels larger than 100 μm **(Fig 4)**. Laser-induced vasodilatation has been demonstrated recently, and possible mechanisms were discussed in the study of Kaoutzanis *et al* (1995).

This study shows a smaller wall thickness in coagulated vessels **(Table III)**. At present, it is not known whether permanent vessel destruction relies on thermolysis to the entire thickness of the wall. The mathematical modeling of Svaasand *et al* (in press) has demonstrated that complete thermal necrosis to the entire wall by the 585-nm, 0.45-ms PDL will be achieved only if the wall thickness is less than 5 μm. An increase in pulse length to 1.5 ms will result in a temperature distribution above the threshold value of thermal denaturation, i.e., 60°C, to the full thickness for walls up to 12 μm thick. The benefits of longer pulse duration have also been discussed recently by Dierickx *et al* (1995), but the study investigated the relation between thermal relaxation time and vessel size and did not discuss the importance of wall thickness. Dierickx *et al* concluded that pulse durations on the order of 1–10 ms allow destruction of 30–150-μm vessels while sparing the capillaries.

The coagulated vessels were superficially located **(Table III)**, and relatively few vessels were located more than 400 μm from the dermoepidermal junction **(Fig 2*a*)**. This was reflected by significantly more superficially located vessels in the good responding PWS compared with the vessels of the lesions with moderate and poor lightening **(Table I)**. Hoehenleutner *et al* (1995) found a mean

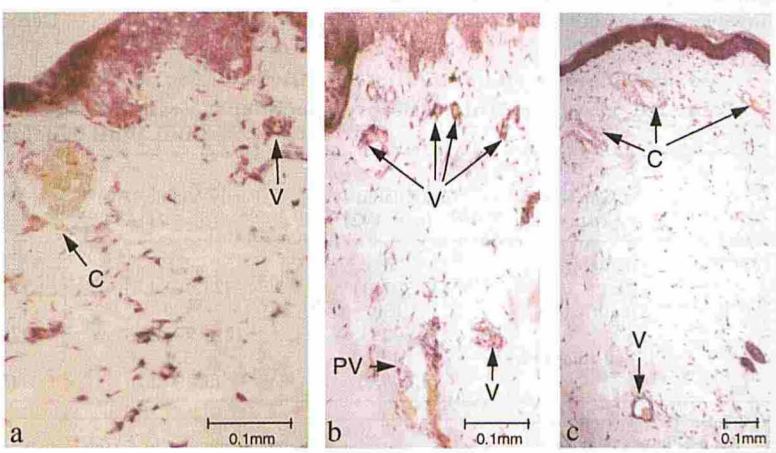

Figure 3. Incomplete coagulation of small or deeply located vessels after PDL treatment. Histochemically prepared slides from *(a)* good responder, showing a small, superficially located, viable vessel (V); the larger vessel is coagulated (C). *(b)* Poor responder with many small, viable vessels (V) and a more deeply located, larger, partly viable vessel (PV). *(c)* Good responder with superficially located large vessels that are completely coagulated (C). The viable vessel (V) is located deeper than 800 μm from the dermoepidermal junction.

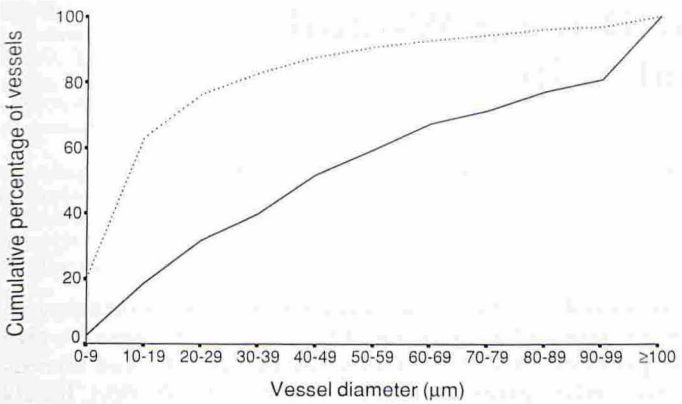

Figure 4. Heat-induced vessel damage results in vasodilation. The cumulative percentage of vessel diameter in the pre- and post-treatment biopsy specimens: *dotted line,* vessel diameter in the pre-treatment specimens; *solid line,* vessel diameter in the post-treatment specimens.

vessel coagulation depth of 370 ± 170 μm (mean ± SD). The higher fluences used in the referenced study might explain the deeper penetration. Tan *et al* (1990) analyzed the influence of wavelength on penetration depth in six adult PWS patients using 577 and 585 nm at a pulse duration of 360 μs. It was demonstrated that the 585-nm wavelength induced vessel damage to a depth of 1.16 ± 0.056 mm (mean ± SEM), compared with 0.72 ± 0.021 for the 577-nm PDL. The observed difference in penetration depth was explained by a smaller absorption coefficient in oxyhemoglobin at 585 nm, allowing light penetration deeper into the tissue. The reasons for the limited penetration depth in our study might include the possibility of self-shielding of the vessels. Histologic slides give limited information about the spatial orientation of the dermal microvasculature. Vessels might be located on top of each other without shielding the entire surface of the underlying ones. Vessel density, however, was not higher in those patients who achieved moderate lightening of their PWS. Theoretically, it should be possible to increase the penetration depth by choosing a wavelength that corresponds to a smaller absorption coefficient of oxyhemoglobin, as proposed by Van Gemert *et al* (1995).

To improve the therapeutic outcome of PWS, lesional vessel morphology should be used to select the appropriate laser parameters. The vessel diameter and depth can be predicted to some extent by the color and location of the lesions. There still is a need, however, for a noninvasive tool to anticipate the vascular morphology in PWS. Studies comparing the results of spectrophotometry with histologic parameters will clarify the ability of this method to provide information about vessel diameter and depth.

We gratefully acknowledge the expert technical assistance given by bioengineers Eli M. Johannesen and Borgny Ytterhus at the Institute of Morphology.

REFERENCES

Barsky SH, Rosen S, Geer DE, Noe JM: The nature and evolution of port wine stains. A computer-assisted study. *J Invest Dermatol* 74:154–157, 1980

Dierickx CC, Casparian JM, Venugopalan V, Farinelli WA, Anderson RR: Thermal relaxation of port-wine stain vessels probed *in vivo*: the need for 1–10 millisecond laser pulse treatment. *J Invest Dermatol* 105:709–714, 1995

Fiskerstrand EJ, Svaasand LO, Kopstad G, Dalaker M, Norvang LT, Volden G: Laser treatment of port wine stains; therapeutic outcome in relation to morphological parameters. *Br J Dermatol* 134:1039–1043, 1996

Fitzpatrick RE, Lowe NE, Goldman MP, Borden H, Behr KL, Ruiz-Esparza J: Flashlamp-pumped pulsed dye laser treatment of port wine stains. *J Dermatol Surg Oncol* 20:743–748, 1994

Garden JM, Polla LL, Tan OT: The treatment of port wine stains by the pulsed dye laser, analysis of the pulse duration and long term therapy. *Arch Dermatol* 124:889–896, 1988

Hoehenleutner U, Hilbert M, Wlotzke U, Landthaler M: Epidermal damage and limited coagulation depth with the flashlamp-pumped pulsed dye laser: a histochemical study. *J Invest Dermatol* 104:798–802, 1995

Holy A, Geronemus RG: Treatment of periorbital port wine stains with the flashlamp-pumped pulsed dye laser. *Arch Ophthalmol* 110:793–797, 1992

Kaoutzanis MC, Peterson JW, Anderson RR, McAuliffe DJ, Sibilia RF, Zervas NT: Basic mechanism of *in vitro* pulsed-dye laser-induced vasodilation. *J Neurosurg* 82:256–261, 1995

Neumann R, Knobler R, Pieczkowski F, Gebhart W: Enzyme histochemical analysis of cell viability after argon laser-induced coagulation necrosis of the skin. *Am Acad Dermatol* 25:991–998, 1991

Renfro L, Geronemus RG: Anatomical differences of port-wine stains in response to treatment with the pulsed dye laser. *Arch Dermatol* 129:182–188, 1993

Reyes BA, Geronemus RG: Treatment of port wine stains during childhood with the flashlamp-pumped pulsed dye laser. *J Am Acad Dermatol* 23:1142–1148, 1990

Svaasand LO, Fiskerstrand EJ, Kopstad G, Norvang LT, Svaasand EK, Nelson JS, Berns MW: Therapeutic response during pulsed laser treatment of port wine stains; dependence on vessel diameter and depth in dermis. *Lasers Med Sci* 10:235–243, 1995

Taieb A, Touati L, Cony M, Leaute-Labreze C, Mourtureux P, Renaud P, Boineau D, Maleville J: Treatment of port wine stains with the 585 nm flashlamp-pumped pulsed tunable dye laser: a study of 74 patients. *Dermatology* 188:276–281, 1994

Tan OT, Morrison P, Kurban AK: 585 nm for the treatment of port-wine stains. *Plast Reconstr Surg* 86:1112–1117, 1990

Tan OT, Sherwood K, Gilchrest BA: Treatment of children with port wine stains using the flashlamp-pulsed tunable dye laser. *N Engl J Med* 320:416–421, 1989

Van Gemert JC, Welch AJ, Pickering JW, Tan OT, Gijsbers GHM: Wavelengths for laser treatment of port wine stains and telangiectasia. *Lasers Surg Med* 16:147–155, 1995