UC Irvine
UC Irvine Previously Published Works

Title

Vascular characteristics of port wine birthmarks as measured by dynamic optical coherence tomography

Permalink

https://escholarship.org/uc/item/8s39w4ss

Journal

Journal of the American Academy of Dermatology, 85(6)

ISSN

0190-9622

Authors

Mehrabi, Joseph N
Holmes, Jon
Abrouk, Michael
et al.

Publication Date

2021-12-01

DOI

10.1016/j.jaad.2021.08.007

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Vascular characteristics of port wine birthmarks as measured by dynamic optical coherence tomography

Joseph N. Mehrabi, MS,[a] Jon Holmes, MS,[b] Michael Abrouk, MD,[c] Jordan V. Wang, MD, MBE, MBA,[d] Hyemin Pomerantz, MD,[d] Anton M. Palma, PhD, MPH,[e] Christopher B. Zachary, MBBS,[a] Roy G. Geronemus, MD,[d] Jill S. Waibel, MD,[f] and Kristen M. Kelly, MD[a,g]
Irvine, California; Maidenstone, United Kingdom; Miami, Florida; and New York, New York

Background: Port wine birthmarks (PWBs) are congenital capillary malformations. Vessel characteristics, such as diameter and depth, may impact presentation and outcomes. They can be imaged using dynamic optical coherence tomography, a high-resolution, noninvasive imaging method.

Purpose: We conducted a cross-sectional observational study to measure in vivo vascular characteristics as a function of PWB color.

Methods: Patients undergoing treatment for PWB were recruited from 3 sites. PWBs were classified by color. Dynamic optical coherence tomography images with calculations were obtained.

Results: One hundred eight patients were enrolled. Mean age correlated with PWB color, with birthmarks being lighter in younger patients and darker in older patients ($P < .01$). Mean superficial plexus depth was significantly shallower in purple PWBs than in pink PWBs. Color was not associated with significant differences in mean superficial vessel density or diameter. Among pink PWBs, each 10-year increase in age was associated with a 10.6-μm increase in superficial plexus depth. Among purple PWBs, each 10-year increase in age was associated with a 16.2-μm reduction in superficial plexus depth. In lesions without prior treatment, vessel density was 12.7% lower in purple PWBs than in pink PWBs.

Conclusion: Superficial vessels of purple PWBs were significantly closer to the epidermis than those of pink PWBs, which might impact optimal laser parameters. (J Am Acad Dermatol 2021;85:1537-43.)

Key words: capillary malformation; color; dynamic optical coherence tomography; imaging; port wine birthmark; vascular characteristics.

INTRODUCTION

Port wine birthmarks (PWBs) are congenital capillary malformations.[1] They begin as flat pink-to-red patches and can darken into red or purple lesions, which have associated nodules and tissue hypertrophy.[2,3] Laser treatment can be challenging, especially in adults. Vessel size, depth, and density of PWBs vary significantly between patients and even within individual lesions, which may contribute to variability in color and treatment response.[4-6] In accordance with the theory of selective photothermolysis,[7] laser treatment should be guided by vessel characteristics including vessel diameter and plexus depth. Knowledge of vessel characteristics

From the Department of Dermatology, University of California, Irvine[a]; Michelson Diagnostics Ltd, Maidenstone[b]; University of Miami Miller School of Medicine[c]; Laser & Skin Surgery Center of New York[d]; Institute for Clinical and Translational Science, University of California, Irvine[e]; Miami Dermatology, Laser Institute[f]; and Beckman Laser Institute, University of California Irvine.[g]

Funding sources: None.

IRB approval status: Approval was obtained from the institutional review boards at each of the 3 study sites: University of California, Irvine (2019-5222); Laser & Skin Surgery Center of New York; and Miami Dermatology, Laser Institute.

Accepted for publication August 3, 2021.

Reprint requests: Kristen M. Kelly, MD, Department of Dermatology, University of California, Irvine, 118 Med Surge 1, Irvine, CA 92697. E-mail: kmkelly@hs.uci.edu.

Published online August 11, 2021.
0190-9622

© 2021 by the American Academy of Dermatology, Inc. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.1016/j.jaad.2021.08.007

associated with different clinical presentations (including color) may guide treatment parameter selection.

Previous studies used punch biopsies to determine the vascular characteristics of PWBs and found relationships among darker birthmarks, larger vessel diameters, and advancing age.[4,5] Red PWBs had the shallowest vessels, and pink PWBs had the smallest vessels.[4,6]

Optical imaging has been used to study PWB vascular characteristics in a limited number of patients.[8-12] Optical coherence tomography (OCT) was adapted for dermatology in 1997[13] to diagnose nonmelanoma skin cancers and actinic keratoses, but also can have utility for the evaluation of cutaneous vascular lesions.[14] Unlike Doppler OCT, which is based on phase-sensitive imaging, dynamic OCT (D-OCT) is based on speckle variance analysis.[15] D-OCT can calculate the characteristics of cutaneous vasculature, such as superficial plexus depth and diameter, and the density of superficial blood vessels. D-OCT provides measurements to a depth of approximately 0.5 mm.

Doppler OCT and D-OCT have been used in small studies to evaluate PWBs. One study of 5 patients with PWB, in which Doppler OCT was used, reported vessel diameters ranging from 10 to 474 μm at depths between 108 and 672 μm.[10] In another study, 2 patients with PWB were observed to have vessels with diameters between 40 and 90 μm near the dermoepidermal junction and between 300 and 500 μm up to 1-mm deep.[11]

The advantage of D-OCT used in this study is the ability to capture images in a clinical setting and rapidly calculate vascular measurements, providing immediate feedback to the treating physician. Although many lesions may be deeper than 0.5 mm, treatment will be targeting the most superficial vessels encountered by the laser light, and it is likely that shallower vessels have a greater impact on PWB surface appearance and color. D-OCT of 28 patients with PWB identified vessel diameters ranging from 20 to 160 μm, with the largest vessels of 120-160 μm in diameter mostly at a depth of 500 μm.[9]

In this study, we utilized D-OCT at 3 cutaneous vascular birthmark treatment centers to measure in vivo vessel diameter, density, and superficial plexus depth in patients of all ages with PWBs. We hypothesized that pink, red, and purple PWBs would have different vessel diameters, densities, and superficial plexus depths.

CAPSULE SUMMARY

- Dynamic optical coherence tomography measured vascular characteristics of pink, red and purple port wine birthmarks (PWBs). Purple PWBs had shallower superficial plexuses than pink. Color was not associated with significant differences in vessel density or diameter.
- Vessel characteristics varied with age and history of treatment. All measured birthmarks had plexus depths targetable by pulsed dye laser.

METHODS

Patients presenting with PWB were recruited from 3 sites: the University of California, Irvine; Laser & Skin Surgery Center of New York; and Miami Dermatology and Laser Institute. Approval from the Institutional Review Board at each site was obtained and informed consent was collected from each patient and/or parent. Patient baseline demographic and clinical characteristics were extracted from medical records, including age in years (mean-centered), gender, Fitzpatrick skin type, PWB location, and previous treatment. Patients were consented and imaging performed immediately prior to laser treatment. Enrollment characteristics differed between sites, but patients of all ages seeking treatment for PWB were included. At the study visit, each patient's PWB was photographed and classified as pink, red, or purple by board-certified dermatologists.

A D-OCT scanner equipped with multibeam Swept-Source Frequency Domain OCT processing and software was used (VivoSight Dx, Michelson Diagnostics Ltd). The device has a field of view of 6 × 6 mm with a resolution of <5 μm axially and <7.5 μm laterally and a penetration depth of 1-2 mm. The normal D-OCT imaging mode captures 120 cross-sectional scans with a pixel size of 4.44 μm[16] in 30 seconds, from which 6 × 6 mm en face (horizontal) images can be viewed at any desired depth. In this device, the superficial plexus depth (the depth of the top of the plexus) is estimated by finding the depth at which the blood vessel density reaches 50% of the maximum. At this depth, modal vessel diameter and density were calculated by the device using proprietary algorithms. Calculated results were checked against manual measurements on sample images and found to be representative.

No skin preparation was performed prior to D-OCT. Although 1 or more sites in each PWB could be imaged, we only used imaging data from each patient's first OCT measurement visit. Superficial plexus depth and vessel diameter and density were calculated and recorded immediately after each

Abbreviations used:

D-OCT:	dynamic optical coherence tomography
OCT:	optical coherence tomography
PDL:	pulsed dye laser
PWB:	port wine birthmark

image capture. One or more sites were scanned with OCT in each patient in order to capture vascular characteristics of multiple portions of the same birthmark or of multiple isolated birthmarks. Each individual researcher determined the sites to be measured.

Demographic and clinical characteristics of study participants were described using univariate statistics overall and compared between participants with varying PWB colors using chi-squared and t tests for categorical and continuous variables, respectively. To account for multiple OCT measurements per participant, we performed linear mixed models with random intercepts for each subject, in order to assess the mean differences in superficial plexus depth and vessel density and diameter by PWB color at this depth. Mean differences were adjusted for age (mean-centered), sex, previous treatment, and the other outcomes. As a sensitivity analysis, we also tested whether associations between color and each vascular characteristic varied by age or treatment history by individually including interaction terms for color*age and color*treatment history. To correct for multiple comparisons for each pairwise comparison across PWB color, we applied Bonferroni correction with an α significance level of 0.05/3 = 0.017. All analyses were conducted using R v4.0.3 (R Core Team).

RESULTS

A total of 108 patients (40 men, 68 women; mean ± SE age, 32.3 ± 18.5 years) with PWB were enrolled (Table I). Of all PWB lesions scanned, 62.3% (n = 127) were located on the head; 14.2% (n = 29), on the upper extremities; 8.3% (n = 17), on the lower extremities; 7.8% (n = 16), on the trunk; and 7.8% (n = 15), on the neck. The PWB lesions were classified as pink in 27% (n = 29; mean age, 21.2 years); red in 45% (n = 49; mean age, 32.0 years); and purple in 26% (n = 30; mean age, 43.5 years). A sample en face OCT image taken at a depth of 300 μm is shown in Fig 1. Mean patient age correlated with PWB color, with PWB being predominantly pink in younger patients and more purple in older patients ($P < .01$). Fitzpatrick skin types ranged from I to IV. Of all the patients, 57% (n = 61) reported receiving previous PWB treatment.

Mean superficial plexus depth was marginally significantly shallower in purple PWBs than in pink PWBs (mean ± SE purple vs pink, 209.7 ± 13.7 μm vs 252.4 ± 10.2 μm; adjusted mean difference, −39.1 μm; 95% CI, −80.9 to 2.6). Although there was a trend toward increasing vessel diameter from pink to red to purple, PWB color was not associated with a statistically significant difference in mean diameter or density of the measured superficial vessels (Fig 2). Associations between color and superficial plexus depth, density, and diameter after controlling for age, sex, previous treatment, and other PWB characteristics are displayed in Supplemental Table I available via Mendeley at http://doi.org/10.17632/rgd8szmks7.1. No differences in superficial plexus depth were observed between pink and red PWBs (adjusted mean difference, 3.6 μm; 95% CI, −30.8 to 37.9). Similarly, purple and red PWBs had slightly larger diameters, but these differences were not significant (purple vs pink: 19.2 μm; 95% CI, −6.6 to 45.0; red vs pink; 13.8 μm, 95% CI, −7.4 to 34.9). PWB density was marginally lower between purple versus pink PWBs (purple vs pink: −5.5%, 95% CI, −11.2 to 0.3); and red versus pink PWBs (−4.4%; 95% CI, −9.1 to 0.3). Red PWBs were not observed to have statistically significant differences from pink or purple PWBs for vessel depth, diameter, or density.

The results of models separately including interaction terms between color*age and color*previous treatment are shown in Supplemental Table I. Among pink PWBs, each 10-year increase in age was associated with a 10.6-μm increase in superficial plexus depth. In contrast, among purple PWBs, each 10-year increase in age was associated with a 10.6 − 26.8 = 16.2-μm reduction in superficial plexus depth. This interaction was marginally significant ($P < .06$). No other significant interaction effects were found between color and age on vessel density or diameter.

In PWB with no previous treatment, purple PWBs had, on average, 12.7% lower vessel density than pink PWBs (P = .02). We observed that in patients with previous treatment, differences in vessel density between purple and pink PWBs, and likewise for red and pink PWB, disappeared; however, these interaction effects were not statistically significant (P = .12) (Fig 3).

DISCUSSION

This cross-sectional observational study outlines vascular characteristics of superficial plexus depth and vessel density and diameter observed in pink, red, and purple PWBs, as recorded in vivo by D-OCT. Purple PWBs are clinically more apparent and previously noted to be present in older individuals,[3] and this predominance with increased age was

Table I. Sample characteristics

Variable	Overall	Color			*P* value
		Pink	Red	Purple	
Total, n (%)	108 (100)	29 (27)	49 (45)	30 (28)	
Age (y), mean ± SD	32.3 ± 18.5	21.2 ± 17.0	32.0 ± 16.1	43.5 ± 17.4	<.01
Male, n (%)	40 (37)	13 (44)	18 (37)	9 (30)	.50
Previous treatment, n (%)	61 (57)	16 (55)	27 (55)	18 (60)	.97
No. of OCT measurements, mean (range)	1.9 (1-10)	1.4 (1-4)	2.2 (1-10)	1.9 (1-7)	.11

OCT, Optical coherence tomography.

Fig 1. Sample patient photograph and en face optical coherence tomography image of a red port wine birthmark with a dense network of medium vessels.

confirmed in our study. D-OCT demonstrated that purple PWBs are composed of the most superficial, and although there was overlap in vessel size for different color PWB, purple PWBs had, in general, the largest vessels (Fig 2). In contrast, pink PWBs were determined to have smaller blood vessels and a wide variation in superficial plexus depth. Waibel et al[9] examined D-OCT of PWBs and noted larger diameter vessels at shallower depths, which was consistent with the results of the present study.

Evaluating interaction between color and age indicated that shallower superficial plexus depths observed with purple lesions are driven by older patients. Perhaps with increasing age, larger vessels are present up into the superficial dermis, creating more apparent and darker color as well as potential nodularity. Evaluating the interaction between color and previous treatment, untreated purple PWBs had lower density compared to untreated pink PWBs, whereas treated lesions saw no difference in density in lesions with different color. Although these findings were not statistically significant, this may reflect the effectiveness of treatment on reducing vessel density or be the result of self-selection as individuals with more prominent lesions may be more likely to seek treatment. Further studies will be required.

To our knowledge, to date, this is the first study to use in vivo D-OCT to characterize the vascular characteristics of PWBs of differing colors in over 100 patients. This has been attempted in previous studies through ex vivo techniques, such as skin biopsy. The present study documents a correlation between color and age, with birthmarks in younger patients being lighter and those in older patients being darker, which is consistent with a prior study finding a strong relationship between age and the deepening of color in 100 patients.[5] However, another study did not identify the same relationship in a smaller cohort of 30 patients.[4] Larger vessel diameters were found in purple PWBs and smaller diameters in pink, which is consistent with previous histologic results[4,6]; however, this relationship was not observed to be statistically significant. This was possibly due to the power of the current study being too low. This previous study found that red lesions were shallower than purple and pink lesions, which was not consistent with our findings. We found that differences between both pink and purple PWBs

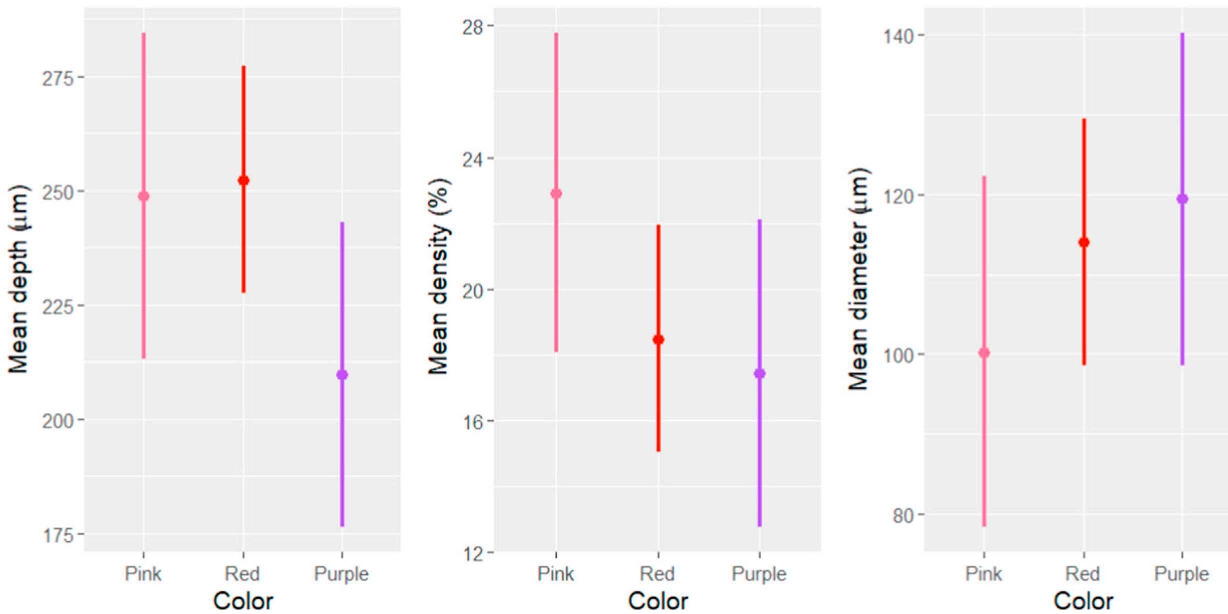

Fig 2. Mean port wine birthmarks superficial plexus depth, density and diameter sorted by color. The 95% CIs and *P* values are all adjusted for multiple pairwise comparisons across port wine birthmark color using Bonferroni correction.

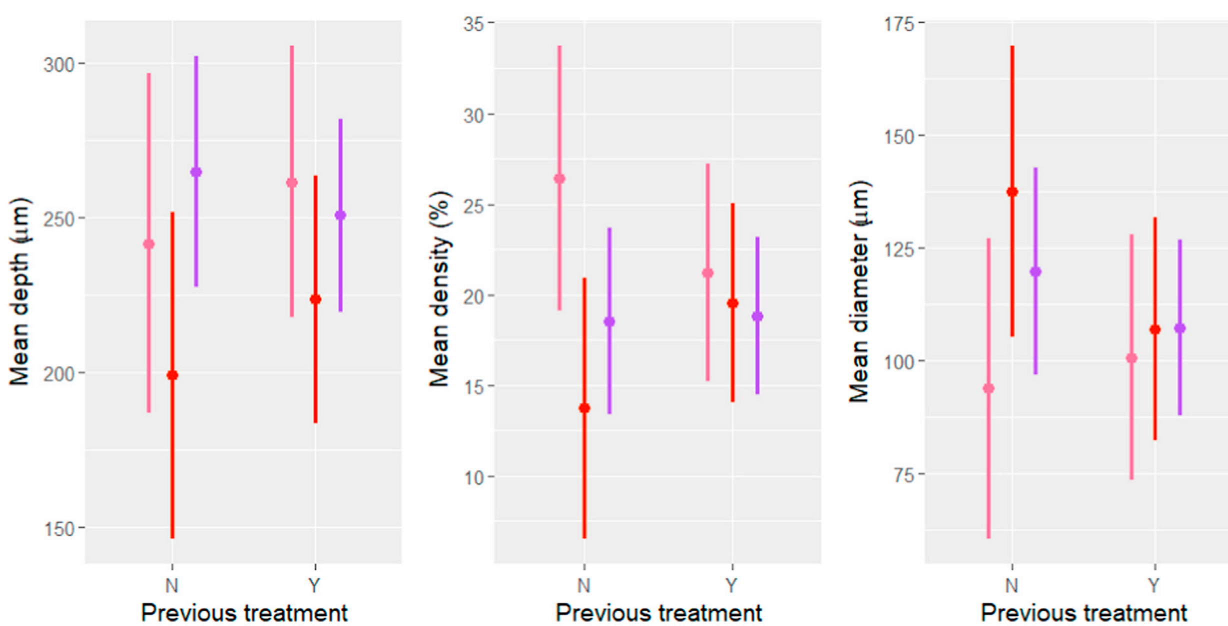

Fig 3. Mean port wine birthmarks superficial plexus depth, vessel density, and diameter sorted by previously treated and untreated lesions. The 95% CIs and *P* values are all adjusted for multiple pairwise comparisons across port wine birthmark color using Bonferroni correction. *N*, No; *Y*, yes.

were statistically insignificant compared with red PWBs. Although prior smaller studies noted mean vessel and lesional depth weakly correlated to PWB color,[4,5] the present study observed statistically significant differences in superficial plexus depth only between purple and pink PWBs.

The superficial plexus depths of the measured PWB in this study support pulsed dye laser (PDL) as a first-line treatment. PDL has a wavelength of 585-595 nm, and remains the gold standard therapy for the mitigation of PWB because of its ability to destroy superficial blood vessels.[17] Superficial plexuses measured in this study were observed to be located less than 600 μm below the skin surface, capable of being targeted by PDL. Although PDL may be effective, it is thought that the efficacy of laser treatment can be impacted by depths, densities, and diameters of blood vessels, and multiple treatments are often required because of a variety of factors, including inability to adequately target all lesional vessels and angiogenesis.[17-22] Additional therapeutic options must be found for birthmarks resistant to treatment.

Larger blood vessels are known to absorb more heat and require longer pulse durations for destruction.[23] Based on the D-OCT measurements of this study, different colors of PWB seemed to have varied vessel diameters with larger vessels associated more with darker purple PWBs and smaller vessels more prevalent in lighter pink PWBs. Therefore, darker PWBs may warrant the use of longer pulse durations, and shorter pulse durations may benefit pink PWBs. Further study is required.

Studies on measurements of the vessel characteristics of PWBs using reflectance confocal microscopy, an alternative skin imaging method that compromises the depth of penetration for higher image resolution,[24] have concluded that on average, larger (diameter $\approx$ 109 μm) and deeper (depth $\approx$ 106 μm) blood vessels were more resistant to laser therapy; however, vessel density was not a factor.[8] In treatment-responsive PWBs, PDL therapy resulted in reductions of vessel diameter and density and increased plexus depths.[8] In the present study, we did not observe statistically significant differences in vessel characteristics between treated and untreated PWBs.

The advantage of D-OCT used in this study is the ability to capture images in a clinical setting and rapidly calculate vascular measurements, providing immediate feedback to the treating physician. The limitations of this study include the number of subjects, likely contributing to the lack of statistical significance for some outcomes. Furthermore, many of the enrolled patients had undergone previous laser treatment, achieving substantial improvement in their lesions prior to their introductory D-OCT measurements, likely influencing their PWB vascular characteristics. Lesion thickness is an important consideration for treatment; however, it is unable to be measured using D-OCT and should not be extrapolated from calculations of superficial plexus depth. Moreover, measured vessel diameters and densities only reflect the means of such values in the superficial plexus and cannot represent the vascular characteristics of the full thickness of the lesion.

This study not only offers an insight into the vascular composition of different presentation of PWBs but also demonstrates that D-OCT offers the potential to assess the characteristics of vessels in PWBs. This can be used to guide laser parameter selection in real time at the bedside. Future studies could evaluate and compare the vascular characteristics of PWBs in different body locations (for example face versus extremity) and potentially shed further light on the degree of intrapatient and intralesional variability. Additionally, D-OCT could be used to evaluate the vascular characteristics of resistant PWBs and elucidate why PWBs of untreated infants are often more responsive to laser therapy. Finally, technologic advancements in D-OCT systems could provide measurements of deeper vessels for a more precise and thorough analysis.

CONCLUSION

D-OCT measurements of superficial plexus depth, density, and diameter of PWB vessels were found to vary depending on PWB color. The superficial aspect of purple PWBs was significantly closer to the epidermis than pink PWBs, which might impact laser parameter selection.

Equipment was provided to the University of California Irvine for this study by Michaelson Diagnostics, Ltd. The project described was also supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1 TR001414. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of interest

Drs Geronemus, Kelly, and Waibel have received investigative equipment from Michelson Diagnostics Ltd. Author Holmes is an employee of Michelson Diagnostics Ltd. Dr Kelly has received equipment and honoraria from, and serves as a consultant to, Sciton Inc. Dr Zachary serves on the advisory board of Allergan, Soliton, and Sofway. Drs Abrouk, Wang, Pomerantz, and Palma and Author Mehrabi have no conflicts of interest to declare.

REFERENCES

1. Chen JK, Ghasri P, Aguilar G, et al. An overview of clinical and experimental treatment modalities for port wine stains. *J Am Acad Dermatol*. 2012;67(2):289-304. e229.
2. Klapman MH, Yao JF. Thickening and nodules in port-wine stains. *J Am Acad Dermatol*. 2001;44(2):300-302.
3. Minkis K, Geronemus RG, Hale EK. Port wine stain progression: a potential consequence of delayed and inadequate treatment? *Lasers Surg Med*. 2009;41(6):423-426.
4. 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*. 1996;134(6):1039-1043.

5. Barsky SH, Rosen S, Geer DE, Noe JM. The nature and evolution of port wine stains: a computer-assisted study. *J Invest Dermatol*. 1980;74(3):154-157.
6. Fiskerstrand EJ, Svaasand LO, Kopstad G, Ryggen K, Aase S. 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(5):671-675.
7. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science*. 1983;220(4596):524-527.
8. Fu Z, Huang J, Xiang Y, et al. Characterization of laser-resistant port wine stain blood vessels using in vivo reflectance confocal microscopy. *Lasers Surg Med*. 2019;51(10):841-849.
9. Waibel JS, Holmes J, Rudnick A, Woods D, Kelly KM. Angiographic optical coherence tomography imaging of hemangiomas and port wine birthmarks. *Lasers Surg Med*. 2018;50(7):718-726.
10. Latrive A, Teixeira LR, Gomes AS, Zezell DM. Characterization of skin port-wine stain and hemangioma vascular lesions using Doppler OCT. *Skin Res Technol*. 2016;22(2):223-229.
11. Liu G, Jia W, Nelson JS, Chen Z. In vivo, high-resolution, three-dimensional imaging of port wine stain microvasculature in human skin. *Lasers Surg Med*. 2013;45(10):628-632.
12. Nelson JS, Kelly KM, Zhao Y, Chen Z. Imaging blood flow in human port-wine stain in situ and in real time using optical Doppler tomography. *Arch Dermatol*. 2001;137(6):741-744.
13. Olsen J, Themstrup L, Jemec GB. Optical coherence tomography in dermatology. *G Ital Dermatol Venereol*. 2015;150(5):603-615.
14. Sigsgaard V, Themstrup L, Theut Riis P, Olsen J, Jemec GB. In vivo measurements of blood vessels' distribution in non-melanoma skin cancer by dynamic optical coherence tomography: a new quantitative measure? *Skin Res Technol*. 2018;24(1):123-128.
15. Mahmud MS, Cadotte DW, Vuong B, et al. Review of speckle and phase variance optical coherence tomography to visualize microvascular networks. *J Biomed Opt*. 2013;18(5):050901.
16. Themstrup L, Ciardo S, Manfredi M, et al. In vivo, micro-morphological vascular changes induced by topical brimonidine studied by dynamic optical coherence tomography. *J Eur Acad Dermatol Venereol*. 2016;30(6):974-979.
17. Chang CJ, Kelly KM, van Gemert MJ, Nelson JS. Comparing the effectiveness of 585-nm vs 595-nm wavelength pulsed dye laser treatment of port wine stains in conjunction with cryogen spray cooling. *Lasers Surg Med*. 2002;31(5):352-358.
18. Greve B, Raulin C. Prospective study of port wine stain treatment with dye laser: comparison of two wavelengths (585 nm vs. 595 nm) and two pulse durations (0.5 milliseconds vs. 20 milliseconds). *Lasers Surg Med*. 2004;34(2):168-173.
19. Geronemus RG, Quintana AT, Lou WW, Kauvar AN. High-fluence modified pulsed dye laser photocoagulation with dynamic cooling of port-wine stains in infancy. *Arch Dermatol*. 2000;136(7):942-943.
20. Yung A, Sheehan-Dare R. A comparative study of a 595-nm with a 585-nm pulsed dye laser in refractory port wine stains. *Br J Dermatol*. 2005;153(3):601-606.
21. Welch AJ, van Gemert MJC, eds. *Optical-Thermal Response of Laser-Irradiated Tissue*. 2nd ed. Springer; 2011.
22. Laube S, Taibjee S, Lanigan SW. Treatment of resistant port wine stains with the V Beam pulsed dye laser. *Lasers Surg Med*. 2003;33(5):282-287.
23. Adamič M, Pavlović MD, Troilius Rubin A, Palmetun-Ekbäck M, Boixeda P. Guidelines of care for vascular lasers and intense pulse light sources from the European Society for Laser Dermatology. *J Eur Acad Dermatol Venereol*. 2015;29(9):1661-1678.
24. Rajadhyaksha M, Marghoob A, Rossi A, Halpern AC, Nehal KS. Reflectance confocal microscopy of skin in vivo: from bench to bedside. *Lasers Surg Med*. 2017;49(1):7-19.