The Non-invasive Assessment of Vascular Anomalies

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Abstract

The Non-invasive Assessment of Vascular Anomalies

Capillary Vascular Malformations (CMs) or Port Wine Stains are a congenital abnormality of the dermal vasculature that results in the skin having a pink to purple colouration. This is due to the ectasis of the dermal capillaries within the malformation. Laser treatment has been used to treat CMs for a number of years. However, only a minority of the malformations are completely cleared by laser treatment and the majority improve but remain visible.

The poor response of CMs to laser treatment is believed to be due to a number of factors; the vessels within the CM may be of the wrong size or depth to be treatable with currently available lasers. Also, the flow through these vessels, the amount of competing chromophores in the skin that will absorb the incident laser light and the type of capillary ectasia may influence the effectiveness of the laser.

This thesis examines the development of the videomicroscopy as a non –invasive tool to examine the vessel structure of CMs in vivo. A number of studies have been undertaken including:

1. The use of colour filtering as an adjunct to videomicroscopy
2. The development and validation through a biopsy study of a Depth Measuring Videomicroscope (DMV)

3. The description of vessel change following a single laser treatment using DMV
4. The relationship between location and colour of a CM and vessel structure
5. The effect of prolonged laser treatment on vessel structure
6. The effect of using new generation Pulsed dye lasers on CM vessel structure and their efficacy.

Conclusion:

Colour filtering appears to reduce the artefact from the reflection of light from the skin surface. The development of the Depth Measuring Videomicroscope (DMV), however, reduces this reflection and colour filtering is not required. The DMV can be used to measure the diameter and depth of Capillary Vascular Malformations (CM) in vivo and this has been validated against biopsy measurements using a Bland and Altman Test. Following laser treatment the larger and more superficial capillaries are successfully treated leaving the deeper ($p<0.02$) and smaller vessels ($p<0.001$). This occurs both after a single laser treatment and prolonged treatment.

To improve the treatment of CMs resistant to standard pulsed dye laser treatment the capillary characteristics of resistant CMs were studied prior to treatment with newer generation pulsed dye lasers. Although, the optimum treatment parameters for a particular malformation could not be predicted from this study, 595nm wavelength, 1.5 ms pulse duration and 14 j/sqcm fluence appeared to be the most successful settings.

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Publications

This thesis has generated the following manuscripts:

Chapter 3 Sivarajan V, Mackay IR. Developments in the videomicroscopic assessment of Capillary Vascular Malformations. Progress in Biomedical Optics and Imaging: Proceedings of Laser Florence 2002; 4(35), 71-82

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Chapter 5 Sivarajan V, Mackay IR. The Relationship between Location and Vessel Structure within Capillary Vascular Malformations. Ann Plast Surg. 2004 Oct; 53(4): 378-81

Chapter 6 Sivarajan V, Mackay IR. The Depth Measurement Videomicroscope (DMV): A non-invasive tool for the assessment of Capillary Vascular Malformations. Lasers Surg Med. 2004; 34(2): 193-7

Chapter 7 Sivarajan V, Mackay IR. The Non-invasive Assessment of Vessel Characteristics in Capillary Vascular Malformations Exposed to Five Pulsed Dye Laser Treatments. Plast Reconstr Surg 2005 Apr 115(15): 1245-52

Chapter 8 Sivarajan V, Maclaren WM, Mackay IR. The Effect of Varying Pulse Duration, Wavelength, Spot Size and Fluence on the Response of Previously Treated Capillary Vascular Malformations to Pulsed Dye Laser Treatment. Ann Plast Surg. 2006 Jul; 57(1):25-32

Copies of these manuscripts can be found attached to this thesis.

Declaration

I declare that the material in this thesis is composed by myself and that the vast majority was carried out by myself. I received help with the statistical analysis in Chapter 8 and the histological measurements in chapter 4. I hold the degree of MB ChB (Edin) 1997. I have not submitted this work for the award of any other degrees. Much of this thesis has been published, with the recommendation of my supervisor Mr Awf Quaba, in peer-reviewed journals.

CHAPTER 1

Historical Perspective

In 1917 Einstein defined the properties of electromagnetic energy in terms of the quantum processes of absorption, spontaneous emission and stimulated emission[1]. Under normal conditions electrons exist in their most stable energy state (ground state) unless stimulated by a quantum (defined amount) of energy, in which case they are excited to a higher energy state. Under these conditions electrons decay to their ground state, a process of spontaneous decay and release a non-coherent photon of energy. If, however, an electron in an excited state interacts with another photon of energy identical to the photon that caused the excited state it will decay to its ground state with the release of a coherent photon of energy.
This process is the basis of laser energy theory in that laser light is coherent in direction, time, phase and energy. It is also monochromatic (composed of only one wavelength of light), of very low divergence and has a high power density. It is these specific properties of laser light that make it useful for medical applications[2].

Despite the theoretical basis of laser technology being known for some time it was not till 1960 that Maiman[3] published the results of the first practical laser and not till 1963 that Leon Goldman[4] published the preliminary results of a ruby laser on tissue. Within a couple of years the advent of the argon laser improved the

treatment of vascular skin lesions and this was further improved with the advent of the continuous wave dye laser in the 1980's[5].

Vascular Lesions

Capillary Vascular malformations or Port wine Stains are congenital vascular abnormalities which consist of ectatic dermal capillaries and give the skin a pink, red or purple colouration. In 1980 Mulliken and Glowacki presented their work examining vascular anomalies histologically and grouped childhood lesions into two main categories of haemangiomas and malformations based on the characteristics of their endothelial cells[6]. Haemangiomas, which do not tend to be present at birth and have a female preponderance, show marked hypercellularity of their endothelial cells during their proliferative phase; and fibrosis and diminished cellularity during their involuting phase. This is distinct from vascular malformations, which tend to be present at birth, have equal sex distribution and do not involute, and whose endothelial cell turnover is normal. Vascular malformations therefore tend to grow steadily with the child. Vascular anomalies may have any combination of arterial, venous, capillary and lymphatic components or fistulae. Capillary Vascular Malformations (CMs) or Port Wine Stains are vascular malformations consisting of mainly dilated capillaries within the dermis. Capillary Vascular Malformations are the most commonly isolated vascular malformations with an incidence of between 1-3 per thousand live births[7]. They may, however, co-exist with other vascular malformations as mixed deformities (capillary-venous

malformations for example). They can also occur as part of a syndromal illness such as Sturge Webber, where involvement of meningeal vessels in the malformation can lead to seizures and mental retardation[8]. Any CM surrounding the eye can lead to glaucoma, due to the involvement of ophthalmic vessels.

Capillary Vascular Malformations cause considerable psychological distress for both the child and parents[9-15]. Previous questionnaire studies by Augustin et al and Lanigan et al and a structured interview study by Malm et al all demonstrate that patients with CMs tended to have a higher level of emotional stress and negative body image compared to the normal population. Patients tended to feel stigmatised by their malformation and stated that this affected their ability in social interaction. Indeed, in a minority of patients formal psychosocial intervention may be warranted[9]. Strauss et al found that the expectations of patients attending for treatment was high and this could contribute to negative feelings regarding treatment if the expected improvement was not attained[16]. To reduce this effect patients should be thoroughly counselled prior to treatment and appreciate that full clearance of the lesion is rare.

Studies by Hansen et al and Troilius et al found that patients undergoing laser treatment had an improvement in their psychosocial state and improved self-esteem following treatment[14,17]. Hansen et al found, however, that up to a quarter of patients undergoing laser treatment were dissatisfied with the results and men tended to be less satisfied than women. When the impact on the psychosocial wellbeing of children undergoing treatment was studied by Troilius et al[13] and Van

der Horst et al[15], they found that adolescents tended to perform worse than their normal cohorts and were more likely to suffer from poor relationships at school and depression. This, they argue supports the preference to treat patients at a younger age. Strauss et al, however, points out that the effect of repeated General Anaesthesia and the need for repeated hospital visits when very young may itself lead to psychological morbidity[16].

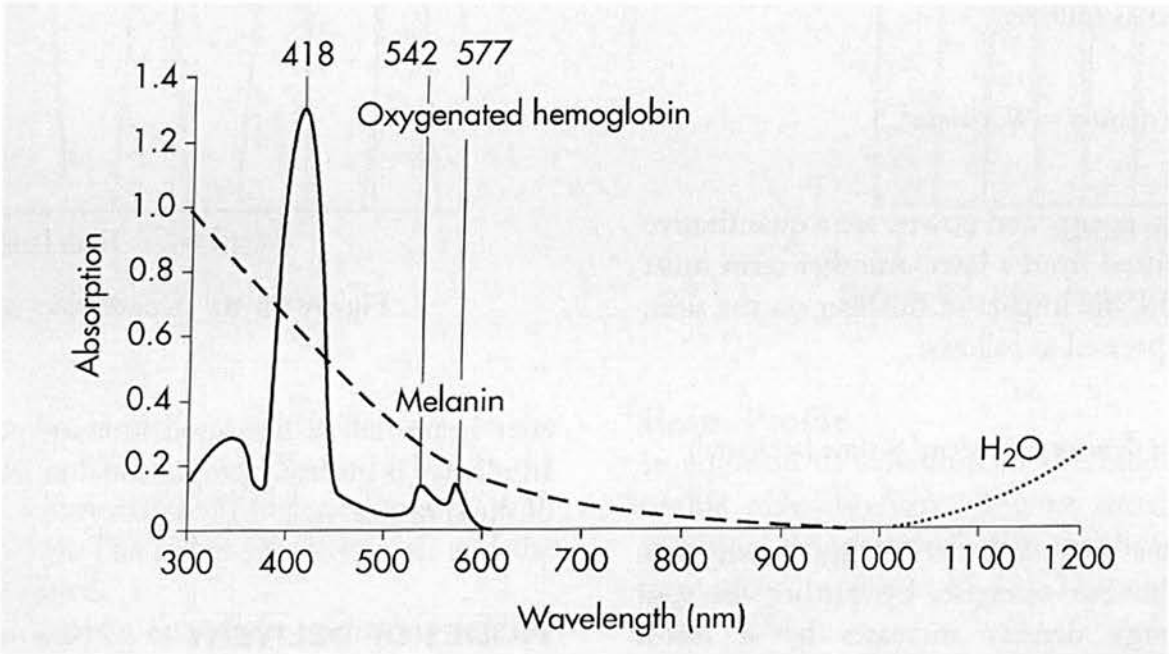
Prior to the advent of laser therapy the treatment of CMs consisted mainly of camouflage makeup and resection, although novel techniques such as tattooing and radiotherapy were tried[18]. The results of these therapies were poor, so that with the advent of the argon laser patients were given their first treatment, which realistically could lighten the colour of their lesion without the need for extensive scarring[5,19-22]. Although the results with the argon laser were good in some patients, the vast majority saw little improvement and a high rate of hypertrophic scarring and textural change approaching 40%[23].

In 1983 Anderson and Parish published their theory of Selective Photothermolysis[24]. This stated that to improve the effects of laser light on a target the wavelength of the laser should be matched to the absorption spectrum of the target. For example when treating vascular lesions the laser light should match a peak in the absorption spectrum of oxyhaemoglobin (figure 1). They also proposed that to minimise collateral damage to the surrounding skin and hence minimise the chances of scarring that the laser light should be pulsed to match the thermal relaxation time of the target tissue, that is the time taken for the target to dissipate

half of its thermal energy. For example, to cause thermal damage to a CM vessel that vessel must be heated to 70 ^{0}C by heating the oxyhaemoglobin it contains with the laser light[25]. To achieve this the laser light pulse duration must be less than the thermal relaxation time of the vessel to prevent energy diffusion into the surrounding skin and prevent scarring or pigment change.

The heating of a target, in this case capillaries within the skin, requires a specificity of the laser light to the target. This specificity is dependent upon the wavelength of the incident laser. From figure 1, the absorption spectra for the common endogenous skin chromophores can be seen. To obtain treatment via Selective photothermolysis requires that the wavelength of the incident laser be better absorbed by the target chromophore than any other. To achieve true Selective Photothermolysis this ratio of absorption should be around 10 times more selective for the target than the surrounding structures, although in practice 2 times the selectivity can be used.

Figure 1. Absorption Spectra for the common skin chromophores



Following the theory of Selective Photothermolysis, the first pulsed dye laser was made commercially available in 1984[26]. This had a wavelength of 577nm and a pulse duration of 360 micrometers. Early results with this laser indeed confirmed a higher efficacy and reduced complication rate when compared to the continuous wave Argon Laser. With this laser, the treatment of younger children became a reality and it was hoped that early treatment might provide better response and less psychological upset for the child[27].

The initial use of 577nm wavelength for the treatment of Capillary Vascular Malformations resulted in many patients experiencing incomplete clearance of their lesion. In an effort to improve the results obtained Tan et al used a 585nm pulsed dye laser[28]. This improvement with a longer wavelength was believed to be due to increase tissue penetration[28], increased vessel wall penetration[29] and overcoming the effect of shielding of deeper vessel by those more superficial[30]

Laser-Tissue Interaction.

An incident laser beam will interact with any substance in a number of ways[1,2]. The light may be reflected, transmitted, scattered or absorbed. The process of reflection can be seen by a laser that strikes a shiny surface and reflects away. This process of a specular reflection is dangerous and can be seen if a high power laser, such as a Co2 laser is allowed to reflect off a shiny wall. The reflection may also be diffuse if the incident structure has a roughened surface and this is much less dangerous. Transmission of the laser occurs when the incident beam passes through the substance unchanged. This can be seen in practice when a polythene film is used on the skin to prevent hair vaporisation during a hair removal treatment.

For the purposes of treating skin for vascular lesions the two important interactions are absorption and scattering. The absorption of the laser is dependent upon the concentration of target chromophore within the skin. Therefore, newly treated red capillary vascular malformations contain a high proportion of superficial ectatic

capillaries and therefore require a relatively low fluence to achieve a successful lightening. Whereas, this same CM after a number of treatments will have less target vessels within the range of the laser and a higher fluence is required to maintain response.

When the laser beam enters the skin it is scattered due to the effect of both the target vessels and other chromophores within the skin, for example melanin. The processes of absorption and the scattering cause the fluence incident on any point within the skin to be less the deeper it is from the skin surface. To compensate for this a wider spotsize of the incident laser can be used. This causes any point within the skin to have a higher incidence of scattered and transmitted laser light[31].

The effect of the incident laser light upon the target can vary depending upon the pulse duration of the laser[32,33]. For any laser this can be split into three main categories of photochemical damage, thermal damage and mechanical damage.

Certain compounds, such as haematoporphrins, are photosensitive and thus can be activated by laser light. The compounds can be given to a patient and then a treatment performed with a laser matched to the absorption spectrum of the compound[2]. This laser may have little or no effect on the surrounding tissue but cause destruction to any tissue containing the compound. This is thus known as photochemical damage.

Laser light striking non-photosensitised skin can cause damage by either thermal means or through mechanical damage. The pulse duration and power density of the incident laser is important in defining how the tissue behaves. For very short pulse durations and continuous wave lasers with high power densities the laser causes instant vaporisation of the target and causes shock waves to radiate from the target, thus causing damage by a mechanical effect[33]. This has been demonstrated with pulse dye lasers with a pulse duration of 20 microseconds[33]. This photodisruption causes widespread damage to the tissue and, if used on the skin for a vascular lesion can cause complications such as pigment change and scarring due to the effect on surrounding structures[24,34-36].

When longer pulse duration lasers are used, with a pulse duration similar to the thermal relaxation time of the target and a relatively low peak power density then heating of the target ensues. It is this interaction that is exploited in the theory of Selective Photothermolysis. The effect on the target is dependent upon the peak temperature reached. If the temperature is kept below 60 ^{0}C then little damage occurs to the target. At temperatures between 65 – 90 ^{0}C then coagulative necrosis occurs within the target due to protein denaturation. Above 90 ^{0}C vaporisation occurs and photomechanical damage can occur[1].

For the purpose of treating Capillary Vascular Malformations, therefore, a laser is chosen at a wavelength suitable for the absorption spectrum of the target, at a pulse duration and power density that will cause heating of the target to between 65 – 90 ^{0}C. For CM's the most often used laser is the Pulsed Dye Laser.

Poor Response to Laser Treatment

Despite the advent of the pulse dye laser working on the principle of Selective photothermolysis, the reality of treatment is that only a minority, approximately 15% can expect full clearance of their lesion[37]. In the remainder the CM will pale during treatment before becoming relatively stagnant. The majority of this improvement tends to occur within the first five treatments. Further long-term treatment of these lesions may lead to further modest improvement in their colour[38], although at the expense of much inconvenience to the patient and the possibility of complications. At present it is not possible to predict which lesions will respond better to laser treatment and which may be expected to clear completely[39].

The cause of this non-response to laser treatment in some lesions has been extensively investigated. It is believed that the response of a CM to laser treatment is due to factors related to the malformation itself and factors related to the laser. The improvement of colour within the lesion relies on the reduction in the number of ectatic capillaries within the area. By the principle of Selective Photothermolysis, this reduction is dependent on the laser imparting a sufficient amount of energy to a particular vessel to cause coagulative necrosis of that vessel and its replacement by fibrosis[24]. From the previous section it can be seen that this is related to the diameter of the vessel and also its' depth from the skin surface.

Previous biopsy studies by Fiskerstrand and Hohenleutner have suggested that following laser treatment the larger and more superficial vessels tend to be removed leaving smaller and deeper vessels untreated[40,41]. However, a novel experiment by Dierickx et al that has allowed the thermal relaxation time of CM vessels to be probed in vivo has suggested that these vessels require pulse durations in the order of 1 – 10 milliseconds to match their thermal relaxation times[42]. This study would suggest that larger vessels would be unlikely to clear with the 0.45 millisecond pulse duration of 585nm Pulsed Dye Lasers. Currently, the reaction of CM vessels to laser treatment has not been established in vivo.

The amount of competing chromophores within the skin will also affect laser treatment[2]. The skin chromophores are haemoglobin, melanin, water and bilirubin. With the range of wavelengths required to target the selective absorption spectrum of oxyhaemoglobin, water does not compete as a chromophore due to its' very low absorption at these wavelengths. Bilirubin is at a very low concentration in the skin of normal non-jaundiced individuals and again does not practically act as a competing chromophore. Melanin, however has an absorption spectrum similar to oxyhaemoglobin and thus can act as a competing chromophore, especially at higher concentrations in patients with high Fitzpatrick Skin Types. In this situation melanin will absorb some of the incident laser light at the level of the basal cells of the epidermis and cause scattering to light penetrating to the dermis, where the ectatic CM vessels are located[43]. This not only reduces the effectiveness of the laser treatment, but also increases the likelihood of complications from the heating of this layer. To reduce this effect effective epidermal cooling is required[20].

Kimel et al has investigated the effect of laser irradiation on chick chorioallantoic membrane[44]. This tissue contains vessels of corresponding diameters to CM vessels and can allow the effect of laser treatment on these vessels to be imaged in-vivo using real time video. They have found that the effect of laser irradiation is dependent upon whether the vessels being treated are arterioles or venules. They argue that the absorption spectrum of a vessel that is oxygen rich, and therefore contains a higher concentration of oxyhaemoglobin, differs significantly from an oxygen poor vessel, which contains a higher proportion of deoxygenated haemoglobin. They state this may have an effect on the treatment of CMs in humans as these vessels tend to be relatively oxygen poor and would therefore favour the use of longer wavelengths.

Studies by Troilius et al have established that some CMs have abnormal blood perfusion in comparison to normal adjacent skin[45]. They found that prior to laser treatment 15 out of 19 patients had increased blood flow to their CM, whereas following treatment 15 out of 18 had reduced blood flow. Trelles et al has demonstrated that within ectatic capillaries the blood velocity will cause blood to move between 3-4mm during a 200 millisecond pulse duration[46]. Although this study utilises a very long pulse duration in comparison to the lasers used in practice to treat CMs this study does highlight that blood velocity within irradiated vessels may be significant factor affecting laser treatment.

A number of factors related to the laser used to treat a CM can alter its effectiveness. As has already been noted CM vessels tend to have a variety of diameters and thus selecting the most appropriate pulse duration for the laser can affect not only its ability to cause coagulative necrosis but also the possibility of it inducing complications such as pigment change and scarring. Also, as vessels tend to be located at different depths the choice of wavelength of the laser is important. More superficial vessels would tend to respond better to shorter wavelengths, whereas deeper vessels may require a longer wavelength to ensure adequate tissue penetration.

The spot size of the incident laser can also affect its ability to treat CM vessels. Tan et al investigated the effect of treating Guinea Pig skin with a 360 microsecond 577nm Pulsed Dye Laser at energy fluence but with differing spotsizes[47]. They found that although the smaller spotsize had a much greater fluence (j/cm^2) than the larger size the depth of injury on histology was greater for the larger spot size. Keijzer et al investigated this phenomenon theoretically using Monte Carlo Modelling techniques and concluded that the increased depth of penetration was a result of the scattering of the incident laser[31]. Thus for a small spotsize any CM vessel would have less incident photons than by using a larger spotsize due to the effect of scattering of the laser. Although it is now recognised that using larger spotsizes is preferable, this effect has yet to be demonstrated in-vivo.

The use of higher fluences to treat CMs is another factor that can improve response. Kauvar et al studied the effect of long-tem laser treatment of CMs and found that

further improvement could be obtained if the fluence used was increased as response stopped to a maximum fluence when further increments may cause complications[38]. The improvement gained by increasing the fluence may be due to deeper penetration of the laser or because it can cause coagulative necrosis to vessels of non-ideal diameters[39]. Thus, vessels with thermal relaxation times smaller than the incident laser pulse duration may require higher fluences as their vessel wall form a higher proportion of their diameter. This will require more incident energy as to heat the vessel wall and cause coagulative necrosis requires diffusion from the blood within its lumen. Larger vessels, on the other hand, may improve with a higher fluence, as it will allow heat to diffuse through to the blood within the centre of the lumen.

The use of higher fluences to improve the response of CMs is limited by the complications of scarring and pigment change, which are a consequence of the heating of the epidermis. To protect the epidermis from this heating effect requires cooling. Gilchrist et al established that cooling the skin prior to argon laser treatment with topical ice could improve results obtained[20]. Van Gemert et al also found that cooling the skin could improve the results obtained with a dye laser[48]. Recently skin cooling has been achieved with cool air jets (Cryo 5, Zimmer Medical Systems, Irvine, USA) and contact methods (Sapphire Chill Tip, Lumenis, Santa Clara, USA). However these methods are not readily adjustable and the cooling of the skin can be highly variable[49]. To improve this Nelson et al demonstrated the use of timed Cryogen sprays just prior to the laser shot to give controlled predictable cooling of the skin[49]. This technique of Cryogen Spray

Cooling has been incorporated into a number of recent laser systems (Dynamic Cooling Device, Candela Corp, Wayland, USA), and allows much higher fluences to be used in the treatment of patients[50,51].

The current goal of treating CM patients with Pulsed Dye Laser is to be able to assess the characteristics of the lesion that will effect its' response to laser treatment, and then to be able to modulate the parameters used to treat the patients so as to achieve the greatest possible benefit in the smallest number of treatments whilst safeguarding them from complications.

CHAPTER 2

Methods of Assessment of Response To Laser Treatment

The assessment of patients with Capillary Vascular Malformations (CM) has traditionally been carried out by the subjective assessment of the laser practitioner, combined with that of the patient. With the aid of standardised photography it is possible to get an impression as to whether the lesion is continuing to respond to treatment or has become non-responsive[52]. Photography, however, is a potentially unreliable means of recording a CM as it is dependent on any number of factors, such as the angles recorded, the lighting, the film used and even the dress and demeanour of the patient[52]. Even if all these factors are standardised as well as possible, the colour of the CM itself will tend to change with factors such as the time of year, and hence the ambient temperature and amount of melanin deposition within the skin, or whether the patient has exercised or feels stressed. Also, as shown by Currie and Monk, there is significant variability in the assessment of CM stain fading by observers, due both to inter-observer variability and also lack of reproducibility[53].

Due to these factors it is difficult for the laser practitioner and even the patient to be able to tell whether the CM is still responding to laser treatment. This can frequently lead to excessive treatments when little or no improvement is occurring. This is detrimental to the patient as it means they undergo unnecessary treatments, increases the inconvenience to them, requiring them to miss more time from work

or school and also increases their chance of a complication. It is also costly for the healthcare service to carry out unnecessary treatments. For younger children who require general anaesthesia to have the treatment performed it again increases their chance of a complication and is even more expensive[54].

In an effort to standardise the degree of lightening of a CM between treatments and for the purposes of standardising research measures Apfelberg and Gilchrist used a standardised descriptive assessment which characterised an excellent result as over 75% fading, Good between 50% - 75% and so on[20,21](figure 2). Although this allowed some standardisation of outcome measure it was still subject to the error due to inter-observer variability and reproducibility as outlined by Currie and Monk[53]. Attempts were taken to modify and further standardise these measures by Quaba[55] and Koster[56], who used other aspects of a CM as well as colour. Quaba used texture, the ability to apply make up, pigmentary change and the presence of scarring to construct a points score[55]. Koster used shape, size, surface texture and the presence of complications[56]. Both these studies attempted to include other effects of laser treatment to improve the outcome measure in CM therapy, rather than colour alone. However, both these studies relied essentially on subjective methods of assessment and as such are again subject to inter-observer variability and lack of reproducibility.

There is therefore a need to improve the objective measurement of CM response to treatment, and there also exists a need to be able to determine which CMs respond to laser treatment and why, and whether a particular CM be matched to a particular

laser system or set of parameters to achieve the best results. A number of techniques have been advocated to allow both these areas to be assessed.

Figure 2. Subjective Assessment of Fading of Capillary Vascular Malformations Exposed To Laser Treatment

Percentage Degree of Fading	Response
> 75 %	Excellent
50 - 75 %	Good
25 – 50 %	Moderate
<25 %	Poor

Gilchrist et al and Apfelberg et al[20,21]

Colour Analysis

A number of techniques can be used to record the colour of a port wine stain. The simplest techniques involve the use of colour charting to quantify the colour of a Capillary Vascular Malformation (CM). Munsell colour charting, as advocated by Ginsbach[57], allows colour to be rapidly assessed by different observers, is inexpensive and easy to use. For these reasons we have adopted its use for the

colour assessment in these studies. This colour charting system was devised by an American artist and originally published in 1905. It defines a particular colour by variables based on its' hue, lightness and chroma or saturation. The hue scale referring to five cardinal colours: yellow, red, purple, blue and green.

Another colour chart based system, advocated by Malm et al, called the Natural Colour System is based on psychometric testing of normally sighted individuals and describes a colour as an equation based on its elementary attributes[58]. These attributes being: red, green, yellow, blue, white and black. As such each individual colour is described based on how much of these elementary colours it possesses.

Whether either the Munsell or Natural Colour System is adopted, they both allow an aid for the subjective interpretation of colour by different individuals or at different times. To obtain an objective measure of colour, reflectance spectrophotometric evaluation can be performed. This technique is based on the theory of spectral chemical analysis as advocated by Bunsen for the assessment of chemical constituents of compounds and was first applied to normal skin by Sheard et al in 1926[59]. This was then used for the assessment of CM skin by Ohmori et al[60], Tang et al[61], Lanigan et al[62] and Troilius et al[45].

Reflectance spectrophotometry is a technique that employs the use of emitted light of specific wavelengths, which is directed at the skin, and the reflectance and thus the absorption spectrum of the skin noted. To determine the amount of oxyhaemoglobin and melanin within the skin the wavelengths chosen match the

absorption spectra of the compounds, for example 568nm and 655nm, respectively. This technique is used to determine the amount of oxyhaemoglobin in the CM skin and the adjacent normal skin. By doing this an erythema index for the port wine skin can be calculated. Troilius et al found a significant correlation in the erythema index, as calculated by reflectance spectrophotometry and the clinical outcome following treatment as judged by the clinician[45]. Furthermore, they also found that eventual outcome from a number of laser treatments could be predicted from a single laser treatment based on the degree of improvement obtained after one treatment. Also they found no further improvement in the erythema index following five treatments in any of their sixty-six patients.

A recent advance in the colour interpretation for CMs has been the use of computerised colour analysis, either with the use of digital photography or with use of scanned photographs. By using image processing software it is possible to define the colour of a particular pixel of an image using the Commission Internationale de l'Eclairage L*a*b* colour classification system. This is an international standard colour classification and is based on the luminance (L*), change in the balance between green and red (a*) and change in the balance between blue and yellow (b*). Rah et al, using this method found a statistically significant correlation between L*a*b* measurements and subjective clinical evaluation of colour before and after laser treatment[63]. Yong-Gee et al also used image processing software to calculate the exact dimensions of a CM using pixel counting, and showed a reduction in the size of the port wine stain following laser treatment[64].

Blood Flow Analysis

The blood flow within a CM can be calculated using Laser Doppler flowmeter or scanner. This device uses a helium-neon class 3b laser to penetrate the skin to a depth of 0.5-1mm[52,62]. This incident light is then doppler shifted by the blood flowing through ectatic dermal capillaries within a CM and some of it is reflected back to the Laser Doppler device. The Laser Doppler device then calculates blood flow from the doppler shift. The flowmeter records a spot recording and so needs to be repeated in different places within a CM so as to obtain a representative recording of what is a polymorphous condition. The scanning Laser Doppler, in comparison, can record a large area of the CM by scanning the laser over the lesion. In a research setting this device has been used by Lanigan et al to examine changes to blood flow during heating and cooling the skin and its relation to the reflectance spectrophotometric changes seen[62]. They found that although blood flow was increased by temperature of the CM this was not associated with a commensurate increase in the erythema index of the lesion. Troilius et al also found that laser Doppler scanning results did not correlate well with erythema index when using it in combination with reflectance spectrophotometry for the assessment of CMs before and after laser treatment[45]. Apfelberg et al found no correlation between laser Doppler results and outcome after a single Argon laser treatment[65], whereas Troilius et al found that 15 out of 18 patients had increased blood flow prior to treatment and 15 of 18 had reduced flow following treatment[45]. It seems likely, therefore, that although changes in blood flow within a capillary vascular

malformation may influence treatment, it is the presence of ectatic capillaries within the dermis rather than the flow through them that is responsible for the colour seen.

Laser Doppler can be an important tool for examining one characteristic of a CM but does not provide information to predict response to laser treatment, or indeed, to follow response.

Work by Patrice et al using thermography, an indicator of blood flow, also showed no relation between thermographic results and colour of a CM[66]. They did, however find that those CMs with low signal thermograms tended to respond better to laser treatment than high signal ones.

Capillary Vascular Malformation Structural Analysis

Previous theoretical studies by Anderson and Parish[24] and biopsy studies by Hohenleutner[41] and Fiskerstrand[40] have demonstrated the importance of CM structure in determining response to treatment and predicting outcome from treatment.

Previously outlined techniques of colour analysis and blood flow analysis give no information as to the structure of a CM. Work by Troilius et al with high resolution ultrasound has allowed the maximum extent of a CM to be measured in vivo[67]. They found a mean maximum depth of 1 mm with a range of 0.2 –3.7 mm. The

photoacoustic probe as demonstrated by Viator et al relies on a technique of laser-induced ultrasound and can also determine CM depth up to 800 – 900 micrometers[68,69]. This technique can determine the mean and range of depths occupied by the CM vessel plexus but cannot demonstrate individual vessel depth or diameter. Previous studies by Hohenleutner et al have demonstrated that pulsed dye laser of 585 nm wavelength is unlikely to penetrate the skin to a depth of more than 0.65 mm[41]. Modelling studies by Lakmaker et al suggest that capillaries deeper than 0.8 – 0.9mm in the skin (depending upon the melanin content) do not affect the colour of the skin due to the optical properties of light[70]. This study modelled the effect of ectatic dermal capillaries on the colour of skin and found vessels deeper that 0.9mm depth would not contribute to the colour of the skin. It seems likely, therefore that complete resolution of a CM may be a rare occurrence and complete fading of a lesion does not mean that all the vessels in a port wine stain are necessarily removed by the laser, only that those remaining vessels are beyond influencing the perceived colour of the skin. Thus the actual total depth of a CM may not be as important as the microvascular structure of it.

Total port wine depth and depth from the melanin containing epidermal layer to the vascular dermis may allow more accurate use of cryogen spray cooling devices to adequately cool the skin, whilst still allowing sufficient laser energy to penetrate to the ectatic capillaries[69].

A number of techniques have been used to study the depth and diameter of capillaries within a CM. Infrared Tomography (IRT), uses a low energy sub

therapeutic laser shot to heat chromophores within the skin[52]. This then causes the heated chromophores to give off infrared energy. An infrared camera then records this energy. The infrared emission is time resolved, and the results plotted via an equation to give a lateral and longitudinal algorithm for the depth and dimensions of the chromophores. Two peaks in the emission spectrum exist, the first referring to the basal layer melanin pigment and the second to oxyhaemoglobin. This second peak is then used to determine the depth and diameters of capillaries within the CM dermis. This technique is capable of giving depths and diameters for the most superficial capillaries within a port wine stain, as the more superficial ones obscure deeper capillaries. As it is the superficial capillaries which will be heated by a laser treatment this technique allows the properties of these vessels to be ascertained, and therefore may allow the parameters of this laser treatment to be matched to the vessels. Another advantage of this technique is that it allows the reaction of an individuals skin to the low fluence laser pulse to be characterised and therefore give a measure for the highest permissible fluence that can safely be used on the patient[71]. The technique is, however, expensive and so far only applicable to a research setting.

Optical Coherence Tomography (OCT) is a tool that allows the non-invasive determination of CM vessel structure[72,73]. This technique uses a Michelson inferometer to detect the reflection of a broadband light source directed at the skin. This device can then be scanned across the skin surface to create a series of two-dimensional images of the vessel depths within the skin[72,74]. This technique allows

the two dimensional representation of vessels within the skin down to a depth of 1.5 mm.

An advance on the OCT is the development of Colour Doppler Optical Coherence Tomography or Optical Doppler Tomography[72,73]. This technique couples the OCT equipment with a Doppler flow-imaging device. This can then be used, along with image analysis software to give a three-dimensional real time demonstration of the dimensions and blood flow through ectatic CM vessels. The resolution of this device is up to 20 micrometers and therefore may not be accurate enough for the smallest CM vessels[68]. The technique, however, allows the flow through individual capillaries to be imaged and may allow laser parameters to be adjusted so as to achieve cessation of blood flow within ectatic vessels[73].

Videomicroscopy was initially used by Fagrell to investigate normal skin circulation[75]. This technique couples a microscope to a CCD camera and allows vessels within the dermis to be visualised. This technique was applied to CMs by Motley et al[76]. By visualising the dermal capillary patterns within a port wine stain; they hypothesised two types of ectasia. Firstly, a Type 1 pattern which consisted of predominantly blob-like vessels which responded well to laser treatment, and secondly a Type 2 pattern which consisted of predominantly tortuous ring-like vessels which responded poorly to laser treatment. They postulated that the type 1 pattern consisted of superficial ectasia of the papillary dermal plexus vessels running at right angles to the skin and seen end-on, whereas the type 2 pattern

consisted of more deeply placed reticular vessels running parallel with the skin surface.

This technique was applied to the characteristics of CMs on different parts of the body by Eubanks et al[77]. They suggested that areas which are more likely to respond well to laser treatment, such as on the trunk, neck and lateral face, were more likely to have a type1 pattern and areas which more often did badly, such as the extremities and medial face, were more likely to have a type 2 pattern.

Although traditional videomicroscopy has been able to define prognosis based on type of vessel pattern it has been unable to determine actual capillary depths or diameters.

Aim

The aim of this thesis is, firstly, to further develop videomicroscopy as a tool for assessing Capillary Vascular Malformations and, secondly, to investigate the capillary structure of these lesions in-vivo in a non-invasive manner.

CHAPTER 3

Colour Filtering Videomicroscopy

Videomicroscopy allows the dermal capillaries within a CM to be imaged. From this image it is possible to determine the dominant capillary plexus within the CM[76]. Type 1 CMs contain a predominance of superficial papillary vessels and are predicted to respond better to laser treatment than type 2 which contain a predominance of deeper reticular vessels. Videomicroscopy also allows the measurement of vessel diameter to be calculated in-vivo.

One problem, which was addressed early on in the use of videomicroscopy, was that of excessive reflection from the skin surface obscuring the dermal capillaries. By moisturising the skin with oil this reflection is reduced[43]. One disadvantage with this technique is that the oil itself can obscure the skin by picking up sloughed keratinocytes and dirt.

In an effort to reduce the reflection seen with videomicroscopy of the skin we used a green coloured lens (wavelength 530 nm) to filter light incident on the skin. Previous studies using colour filtering have demonstrated that the contrast between shades of red and yellow, as most commonly seen in videomicrographs of skin, can be increased without reducing acuity[78,79].

Aim

The aim of this chapter was to demonstrate whether the use of green light reduces reflection from the skin surface.

Method

It is routine practice to record all patients with capillary vascular malformations attending Canniesburn Laser suite using both photographs and videomicroscopy both prior to treatment and at regular intervals during treatment to monitor response. During the period September 2001 to November 2001 all patients attending for videomicroscopy were invited to enter the study. These patients all had capillary vascular malformations and had received dye laser treatment within Canniesburn Laser Suite. Each patient was examined with a traditional videomicroscope (PW Allen Compact Video Microscope) using a 200x contact lens. The examination was carried out using both a green filtered lens and also no filter. The examinations were carried out in a temperature-controlled room at a temperature of 28 C with the patients sat in the room for 20 minutes to acclimatise. The recordings were then taken using either the green filter or no filter first, selected at random. The images were then captured to film using a Mitsubishi colour video printer.

From these images it was possible to determine the vessel pattern type, either type 1 or type 2. The diameter of the vessels was then calculated using an image taken of a 1 mm stage micrometer graticule (Graticules Ltd, Tunbridge, Kent) using the 200 x videomicroscope lens. Three measurements were taken for vessel diameter per image for both the reticular and papillary vessels for each videomicroscope image and the means recorded.

Results

Twenty-four sites on eighteen patients with a CM were examined using both normal white light and green filter videomicroscopy. Twelve women and six men with a mean age of 33 years (range 9 – 50) were examined. These patients had had between 0 and 28 treatments (mean 12, S.D. 9.7) prior to this study. Figures 3 and 4 shows the results obtained.

There was no statistically significant difference found for the reticular dermal vessels. However, for the papillary dermal capillaries a statistically significant ($P < 0.01$) increase in the measurements taken with the green filtered lens was found using a Wilcoxon Rank Test. This was independent of whether the CM had a type 1 or type 2 pattern.

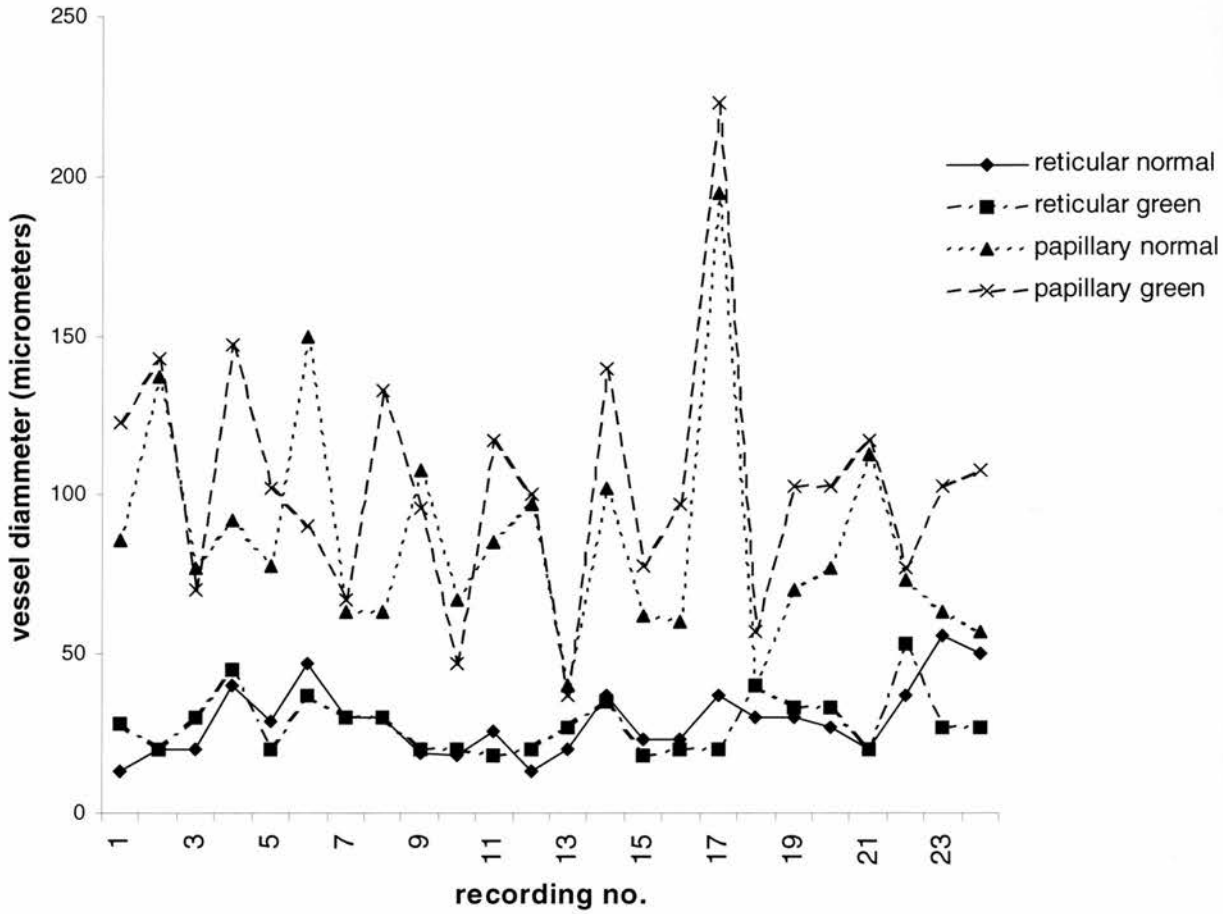
Conclusion

We believe that the difference in diameter measurements of these superficial papillary dermal vessels can be attributed to the reduction in the reflection from the skin surface, which is commonly seen with videomicroscopy, being reduced by the use of green filtering. Figures 5 – 8 demonstrate images taken on two patients using both normal white light and green filter videomicroscopy.

Figure 3. Patient Characteristics and Vessel Patterns

Patient no.	Age	Location	No. Of treatments	Pattern type
1	9	Cheek	10	2
2	10	Leg	2	1
3	12	Cheek	13	1
4	13	Temple	7	2
5	15	Cheek	10	1
6	23	Back	6	1
7	25	Neck	4	1
7	25	Cheek	3	1
8	32	Cheek	16	1
9	35	Leg	10	2
10	35	Leg	28	1
11	36	Nose	26	2
11	36	Cheek	26	2
12	38	Left leg	26	2
13	40	Neck	7	2
14	40	Forehead	12	2
15	42	Cheek	27	2
16	45	Cheek	25	2
17	46	Occiput	0	1
17	46	Neck	19	1
18	50	Cheek	3	1
18	50	Forehead	6	2
18	50	Post auricular	1	1
18	50	Hand	0	1

Figure 4. Vessel diameters in Capillary Vascular Malformations recorded using normal and green filter videomicroscopy.



The above graph shows the vessel diameters recorded for the superficial capillary plexus (papillary) and the deep capillary plexus (reticular) using both the normal videomicroscope lens and the green filtered lens.

Figure 5. Normal filter. Type 1 Pattern

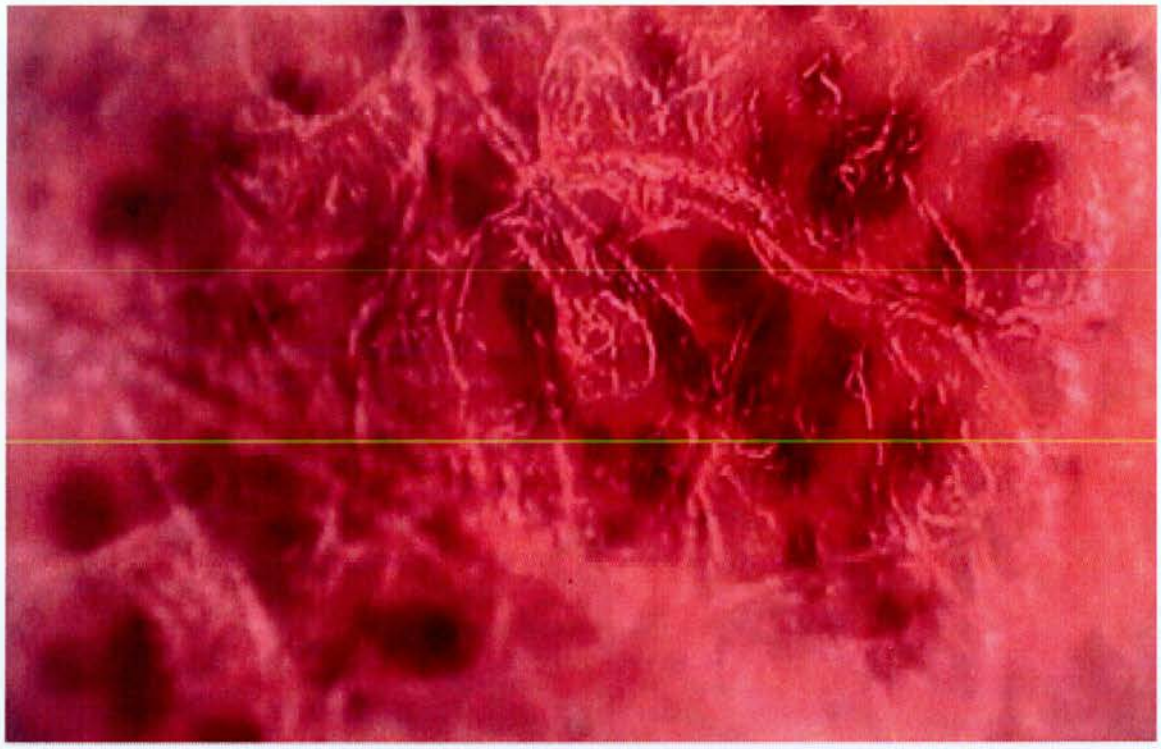


Figure 6. Green Filter. Same Patient as Figure 3.

Figure 7. Normal Filter. Type 2 Pattern

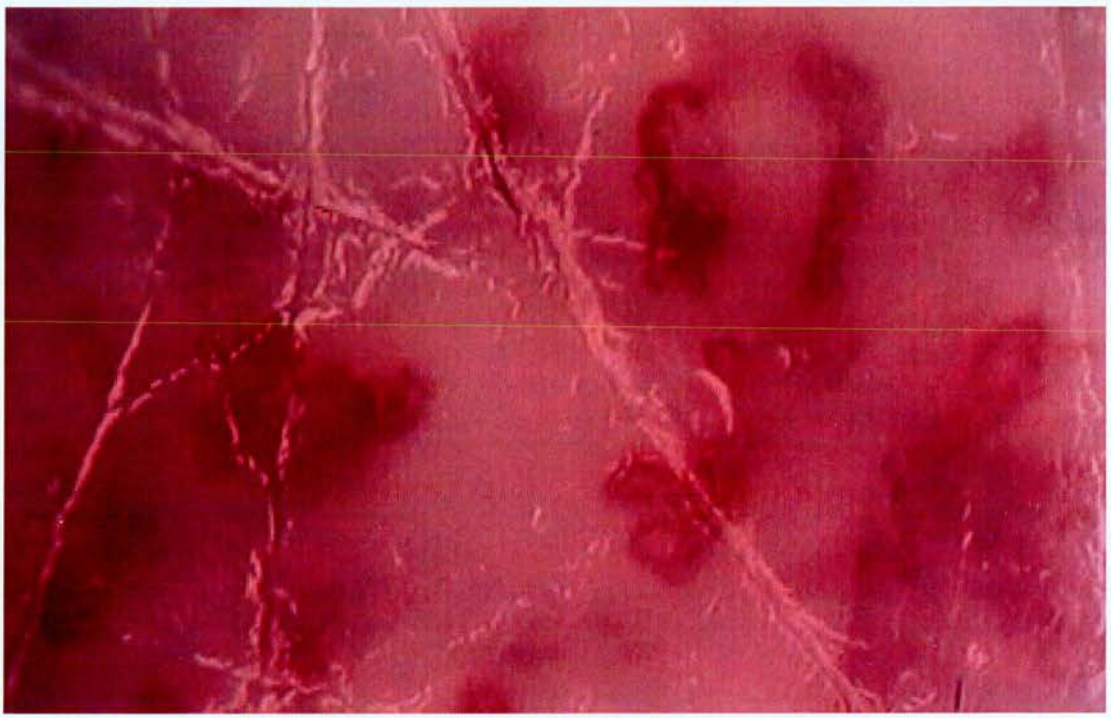


Figure 8. Green Filter. Same Patient as Fig 5

Discussion

The response of CMs to laser treatment is believed to be due to a number of factors:

1. The pattern of capillary ectasia, deep versus superficial (reticular vs. deep)
2. The depth of capillaries
3. The diameter of the capillaries
4. The flow through these capillaries
5. The amount of competing chromophores within the skin.

For clearance of a CM to occur, it is necessary for the laser light to penetrate deep into the dermis with sufficient energy to cause coagulative necrosis in the abnormal capillaries. As these capillaries are of varying diameters it is also necessary for the pulse duration of the incident laser to be long enough to heat the vessel for sufficient time so that damage occurs and the energy is not dissipated. This has led to the development recently of laser systems with longer wavelengths and variable pulse widths.

The results found in this chapter for the diameters of capillaries within a CM correspond well to previously published work by Fiskerstrand et al[40], which was based on biopsy specimens. A number of studies have examined the need for variable pulse duration lasers to enable vessels of varying sizes to be appropriately treated[42,46,80]. By the use of videomicroscopy to record the in-vivo measurement of

vessel diameter it may be possible to tailor pulse duration to that most appropriate for the vessel diameters encountered in a particular CM.

By further developing videomicroscopy with advances such as colour filtering it is hoped that the accuracy of the results can be improved.

CHAPTER 4

Depth Measurement Videomicroscope

Introduction

The principle of Selective Photothermolysis, on which modern laser treatment is based states that a wavelength of laser light should be chosen which will match the thermal absorption spectrum of the compound being targeted – in this case oxyhaemoglobin[24]. Furthermore, if this laser light is pulsed so that each pulse lasts less than the thermal relaxation time of the target, that is the time taken for the target to lose half its' energy, then heat build up and conduction into neighbouring structures will be minimised. It is the conduction of heat away from the target capillaries and into the surrounding skin that leads to the complications of pigment change and scarring.

The goal of recent advances in the development of laser technology has been to attempt to match the pulse duration of the incident laser light to the thermal relaxation time of the target capillaries, this being dependent on their diameter[34,44,81,82]. Also, the depth of the capillaries within the skin has important consequences for laser treatment[83-85]. By increasing the wavelength of the laser used it is possible to increase the depth of penetration of the laser to reach deeper capillaries. Unfortunately, due to the absorption spectrum of oxyhaemoglobin, increasing the wavelength used also requires much more energy (fluence) be used

and thus risks complications. Traditionally the only method to assess the capillary characteristics within a CM has been by biopsy[52].

In an attempt to develop a method of non-invasively determining capillary composition of CMs we have developed the Depth Measurement Videomicroscope (DMV, Figure 9). This consists of a 200x Cy-scope lens attached to a Compact Videomicroscope (PW Allen, Tewkesbury, UK). The advantage of this device over traditional videomicroscope units is that it allows for a higher definition image to be seen and has a focussing scale. This allows individual capillaries to be imaged and their depth and diameter calculated. The lens on the contact tip of this device is made of silicone and is flatter than previous lenses. This greatly reduces the reflection seen when imaging the skin and does not require colour filters. Figure 10 shows the DMV being used on the hand of a volunteer.

Figure 9 The Depth Measurement Videomicroscope – Cyscope lens.

Showing side scale for calculating depth.

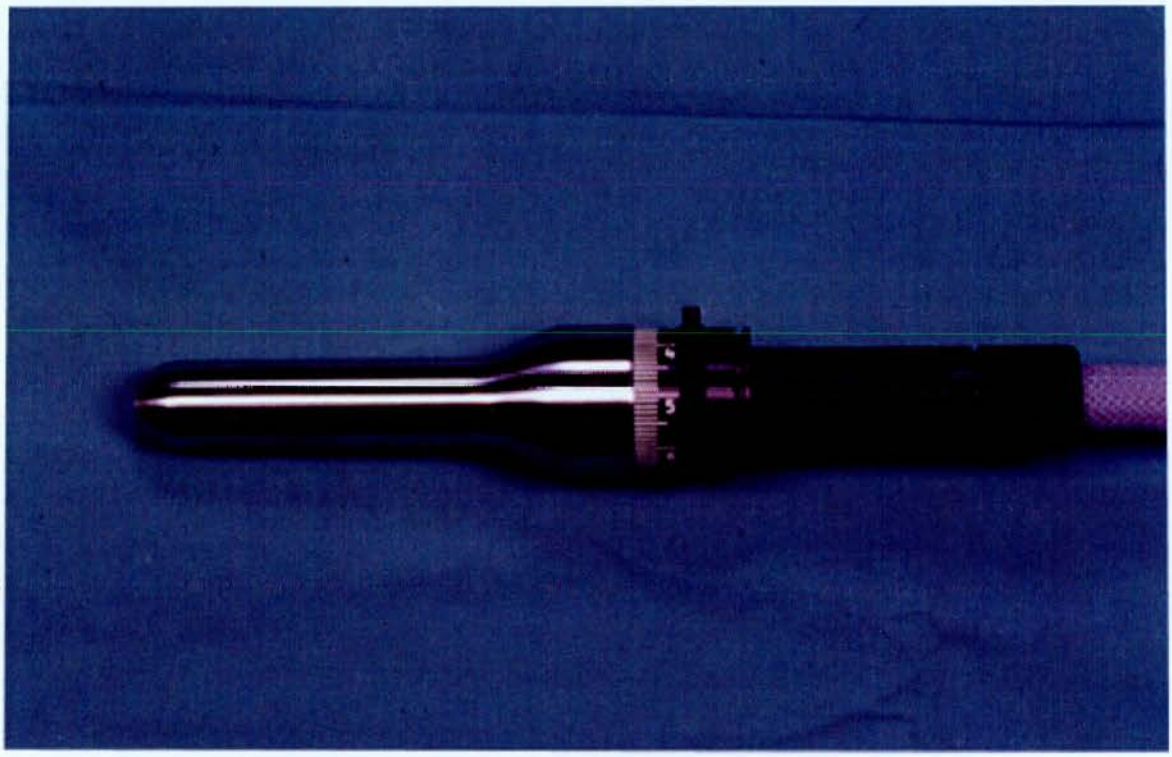
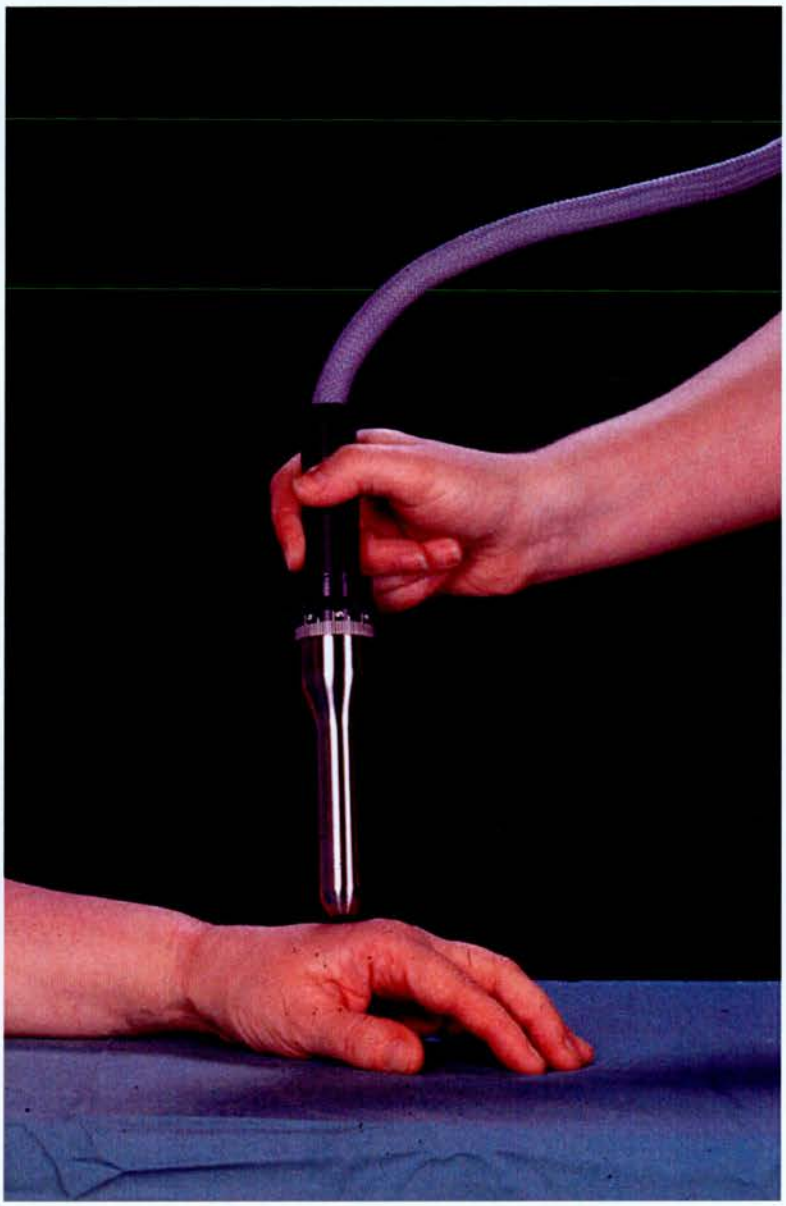


Figure 10. The Depth Measurement Videomicroscope being demonstrated on a volunteer.



Aim

The aim of this chapter was twofold. Firstly, to compare the results obtained in vivo using the DMV with histological measurements obtained through punch biopsies. Secondly, we have attempted to quantify the pressure that can be exerted on the skin surface before measurements are altered. This is a common criticism of many contact methods of assessing CMs.

Method

For the first part of the study ten patients, eight male and two female with a mean age of 47 (range 28 – 85), were recruited. These patients were a mix of either resistant patients, those undergoing treatment or untreated CM patients. Local Ethics Committee approval was gained for this part of the study.

Each patient was allowed to rest for twenty minutes in a temperature-controlled room at 28 ^{0}C. A suitable test area was selected. This consisted of a representative area of CM, which would allow the DMV to be applied to the area without interference, from hair for example. The area was also chosen not to be in an obvious area so that a biopsy would not leave the scar in an unacceptable position.

The DMV consists of a Compact Video Microscope (PW Allan, Tewkesbury, UK) connected to a 200X Cy-scope lens. Images were viewed through a monitor (Sony

Trinitron) and image capture was via a colour printer (Mitsubishi Colour Video Copy Processor).

A DMV examination was carried out on the test area by first adjusting for zero on the skin surface by focusing onto the light reflection from the epidermis (figure 11). To aid zeroing on the skin surface the detail seen on a hair shaft can help (figure 12). Oil was then applied to the skin of the test area to reduce reflection and allow the deeper dermal capillaries to be imaged.

Figure 11 Reflection from the skin surface helps to zero the device.

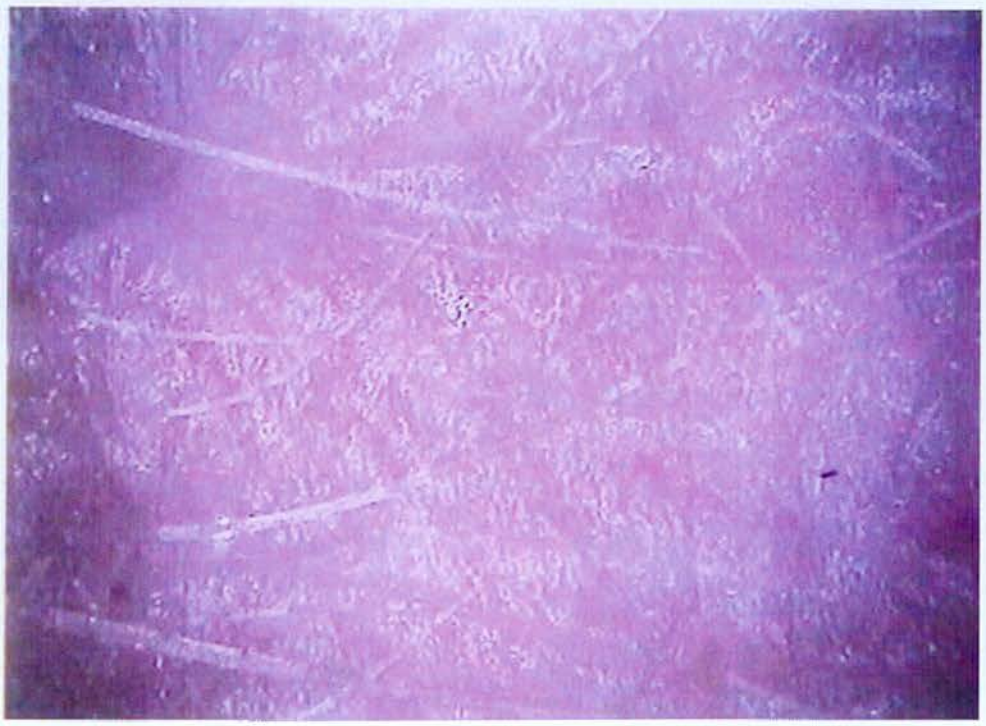
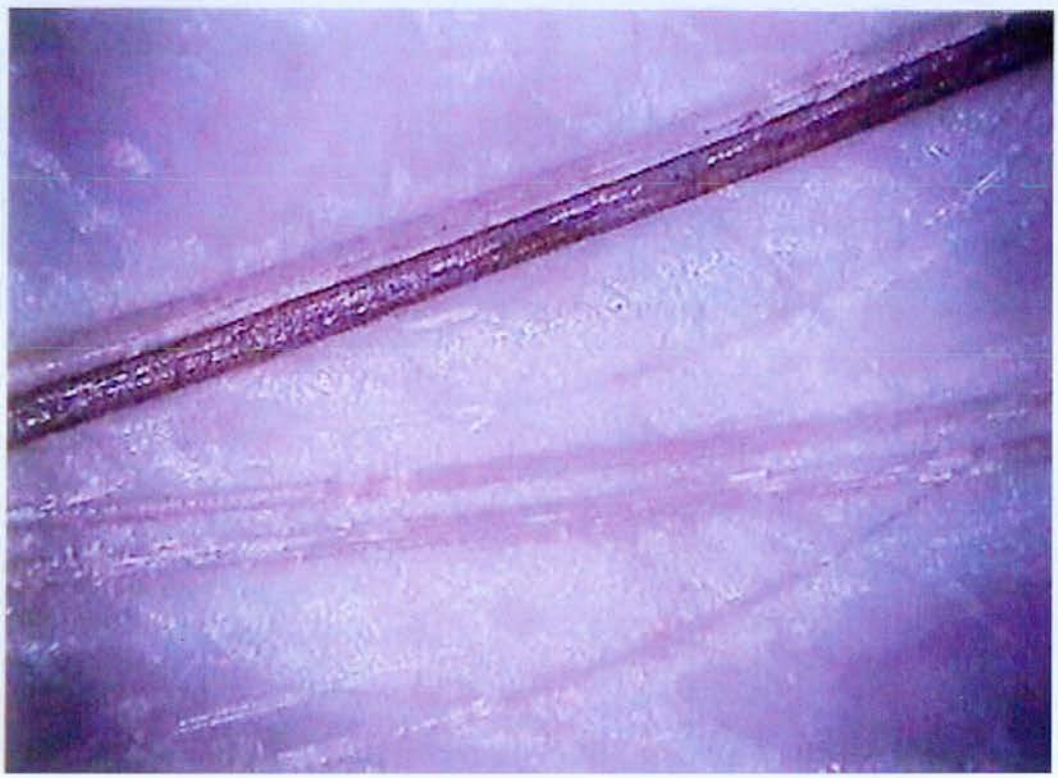


Figure 12 Focussing on a hair shaft can make it easier to zero the device



The handpiece was applied to the skin so that a glass-oil-skin interface was created which did not allow the lens to lift from the skin, thus causing this interface to be lost but also did not apply undue pressure to the skin surface thus causing dermal capillaries to occlude. This was evident from the monitor.

The lens was used to focus down through the skin until the ectatic dermal capillaries came into focus, as judged by the clarity of red blood cells passing

though them. The image was then recorded and a depth measurement obtained. A videomicrograph was then copied to film for later diameter measurement. This was then repeated two further times to increase the accuracy of the recording.

The test area was infiltrated with 2% lignocaine containing no adrenaline. A 3mm punch biopsy was then taken and fixed in formalin. The fixed biopsy specimen was stained with a haematoxylin and eosin stain and examined by a pathologist. The pathologist was blinded as to the values obtained using DMV. The depth and diameter of the capillaries within the specimen was recorded. To improve the accuracy of the histological measurements we analysed the slides using Zeiss Axioscope Microscope and Zeiss KS400 v3 image analysis software. This enabled us to increase the accuracy of our measurements from +/- 100 micrometers to +/- 10 micrometers.

Vessel diameters were measured from the videomicrograph using an image of a 1mm graticule (Graticules Ltd, Tunbridge, UK). Three recordings were taken from each videomicrograph and the mean of these taken.

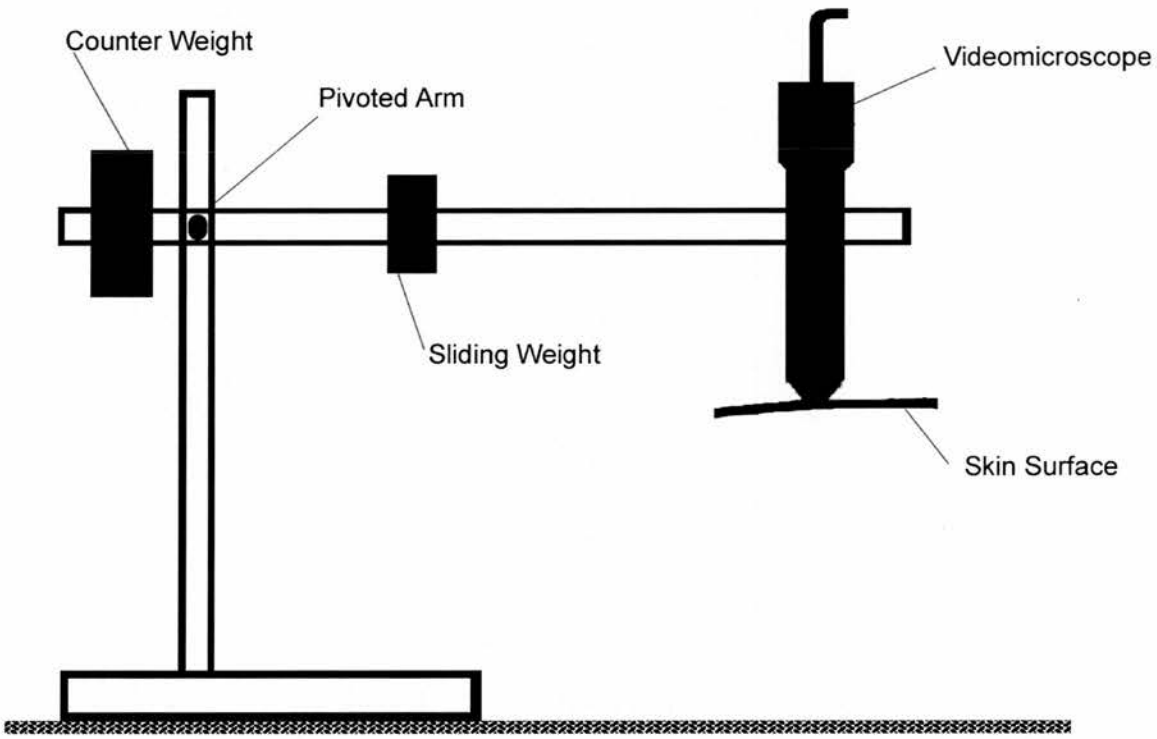
For the second stage of this study to examine the effect of pressure of the DMV tip on the vasculature of a CM we recreated an experiment performed by Schubert and Fagrell to examine the effect of skin pressure on the skin microcirculation in normal skin[86]. Figure 13 shows the device used to measure the effect of pressure on skin microcirculation using a balance with a DMV at one end, which is counterbalance by a weight at the other end. Schubert and Fagrell's original experiment used a

thermister and laser Doppler device to monitor skin microcirculation instead of the DMV we have used here. The device was set up so that the horizontal arm was balanced with the tip of the DMV just in contact with the CM skin surface on the forearm of a volunteer patient. A precision balance was then placed under the balanced DMV and zeroed. The patient again placed their forearm under the DMV and a smaller sliding weight moved along the horizontal arm of the lever to increase the pressure at the tip of the DMV. Once any effect on the CM skin microcirculation was noted (this could be seen as either a reduction in the calibre of the capillaries or an overall reduction in the erythema on the monitor screen, whichever came first), the smaller weight was locked in place. The precision balance was again placed under the DMV and a weight recording taken. This measurement (W), together with the radius of the DMV tip(r), measured using a calliper, and the Density of Mercury Constant (M) was then entered into the following equation to give the pressure on the skin surface:

$$\text{Pressure} = \left(\frac{0.1M\pi r^2}{W} \right)^{-1}$$

This process was repeated five times to reduce error from the recording.

Figure 13 Equipment for assessing skin contact pressure as Modified from Schubert and Fagrell.



Results

Ten patients were recruited into the study and consent gained in accordance with the Local Ethics Committee recommendations. These patients are shown in Figure 14. As can be seen these CM's were mainly untreated patients, with those having had five treatments being resistant. The CMs were mainly facial and where a large lesion was being assessed the temple was chosen for the biopsy, if possible, so as to give a scar in an acceptable position. All the patients entering the study were Fitzpatrick Skin Type 1 or 2.

Figure 15 illustrates an image obtained using the DMV on a Capillary Vascular Malformation. The ectatic capillaries can be clearly seen. The largest diameter of the vessel was taken as the diameter recording. Figures 16 shows a histological section from one patient's biopsy specimen. In both sections abnormally large ectatic vessels can be seen in the upper dermis (as indicated).

Figure 17 illustrates the depth measurements obtained using the DMV and those recorded from the biopsy specimens. Figure 18 shows the diameter measurements obtained with both the DMV and from the biopsy specimens. To compare the two methods of assessment of capillary depth and diameter, namely histological measurement and DMV measurement we have used the Bland and Altman method of assessing the agreement between two methods of clinical measurement[87]. As both the DMV and histological methods of measurement are susceptible to error, for example defining zero with the DMV and fixing and sectioning artefact from the

histological measurements, neither method can be assured to give completely accurate means of measuring in vivo vessel characteristics. In view of this it would be inappropriate to perform a clinical correlation of the two measurements as it is unknown which of the two techniques is the more accurate.

When the depth measurements are examined with the two techniques there is clearly a difference between them, as seen in figure 17. The depth measurements with the DMV appear to be less than with the histology measurements. Figure 19 shows a plot of the difference between the two techniques of measuring capillary depth and the mean of the two methods. As can be seen the depth measurements appear to be less with the DMV than with the biopsy measurements. This bias can be calculated using the Bland and Altman technique[87] and gives a correction factor of 0.102 mm (SD 0.064 mm SE 0.020 mm, 95% confidence interval 0.056 to 0.148). For this to be valid the differences between the two techniques at measuring depth once corrected for bias should be between 2 standard deviations (2sd) of the mean. Figure 20 demonstrates the corrected differences against the mean for the two techniques. This chart also includes the measurements of 2sd. This shows that the measurements obtained with the DMV can be validly compared to biopsy measurements once the bias in the technique is accounted for.

For the diameter measurements (as shown in figure 18) the mean of the differences between the measurement techniques is -0.005 mm (SD 0.051 mm SE 0.016 mm). The 95% confidence interval for this bias is -0.040 mm to 0.030 mm. There is therefore a high concordance between the diameter measurements taken with both

techniques. This can be seen in figure 21 with all the values for the differences between the two techniques clustering around the mean and within 2sd. It would thus be appropriate to compare DMV diameter measurements with histological biopsy measurements without a correction factor.

For the second part of this study, to investigate the pressure required to give anomalous results using the DMV a single patient (two patients failed to attend) was assessed using the modified Schubert equipment seen in figure 13. This gave a mean pressure required to change the DMV recordings of 62 mmHg (Range 61.2 – 64.8, S.D. 2.9).

Figure 14 Characteristics of Biopsy Patients

Patient	Age	No. Of Treatments	Location
1	37	0	Temple
2	85	0	Forehead
3	30	0	Temple
4	41	0	Temple
5	48	0	Temple
6	47	0	Neck
7	28	2	Eyebrow
8	48	5	Forearm
9	55	5	Forearm
10	48	4	Chest

Figure 15. Image obtained using the Depth Measurement Videomicroscope showing ectatic dermal vessels within a Capillary Vascular Malformation.

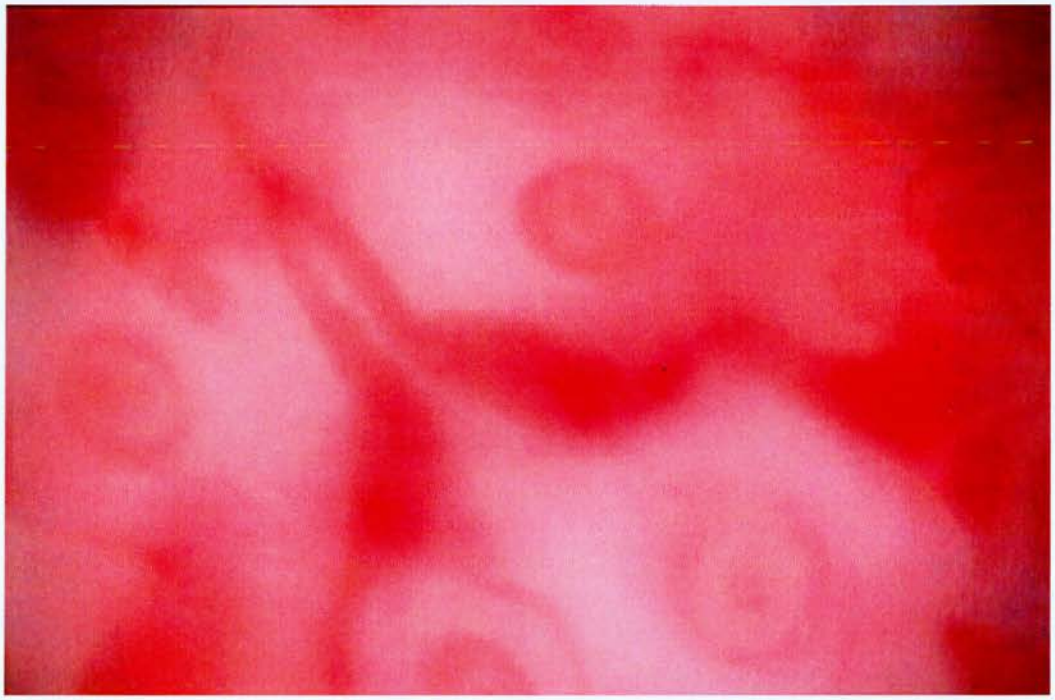


Figure 16. Histological section of a 3mm punch biopsy taken from a Capillary Vascular Malformation illustrating ectatic dermal capillaries (arrowed).

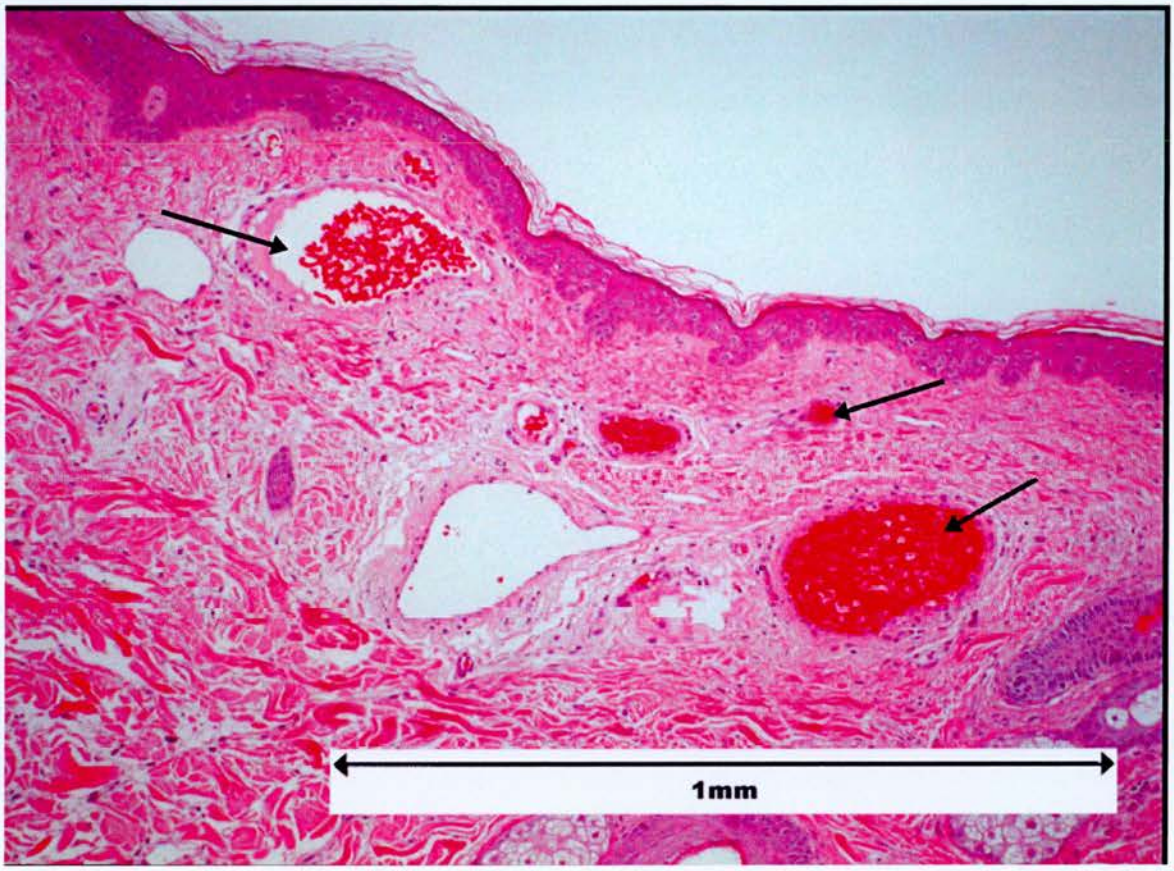


Figure 17 Comparison of Depth Measurement Videomicroscope (DMV) depth and biopsy depth.

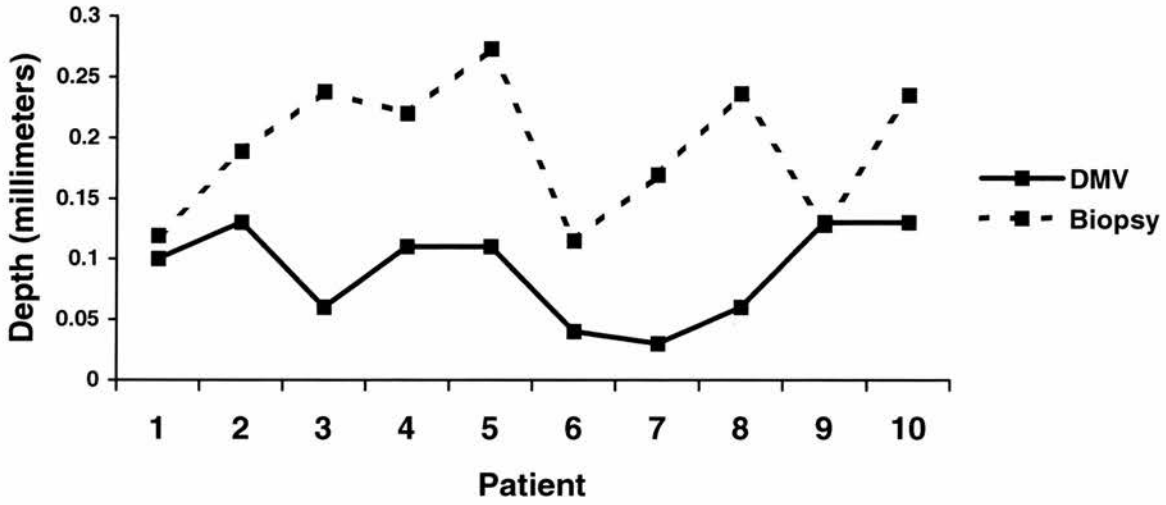


Figure 18. Comparison of Depth Measurement Videomicroscope (DMV) diameter and biopsy diameter.

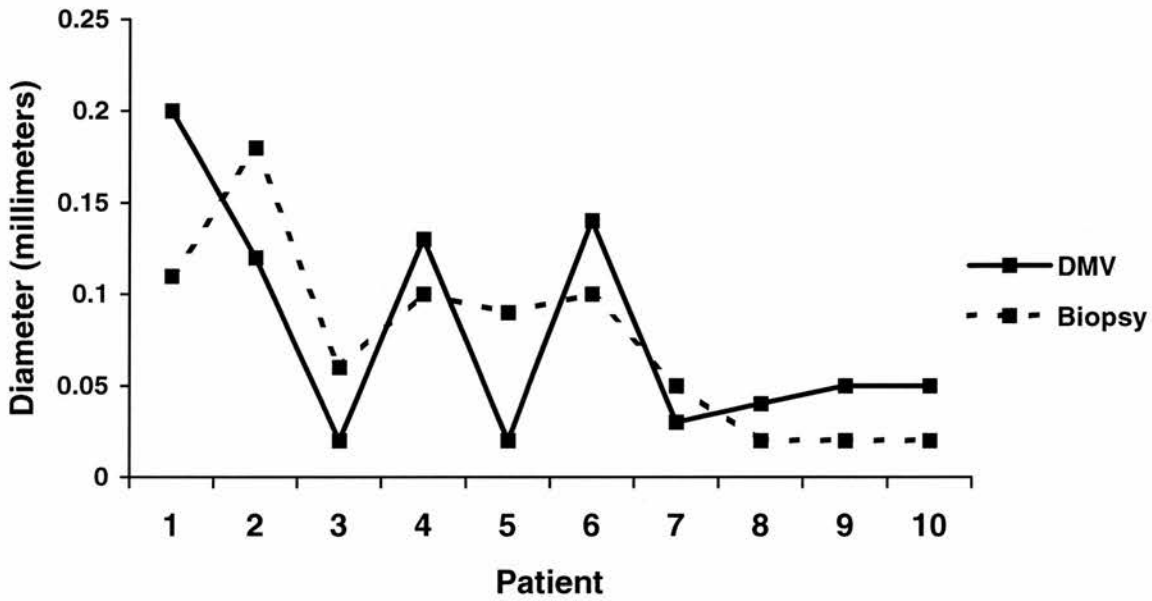


Figure 19. Difference against mean for depth measurement

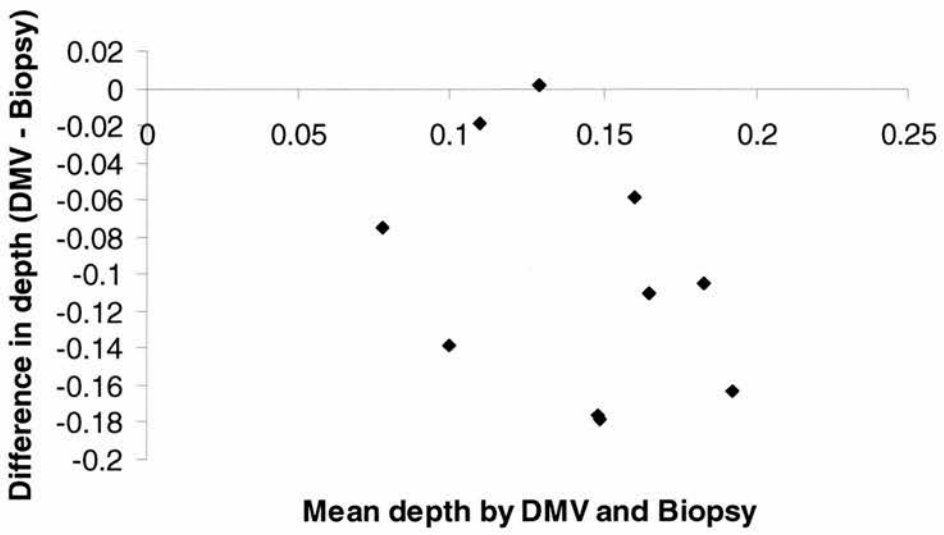
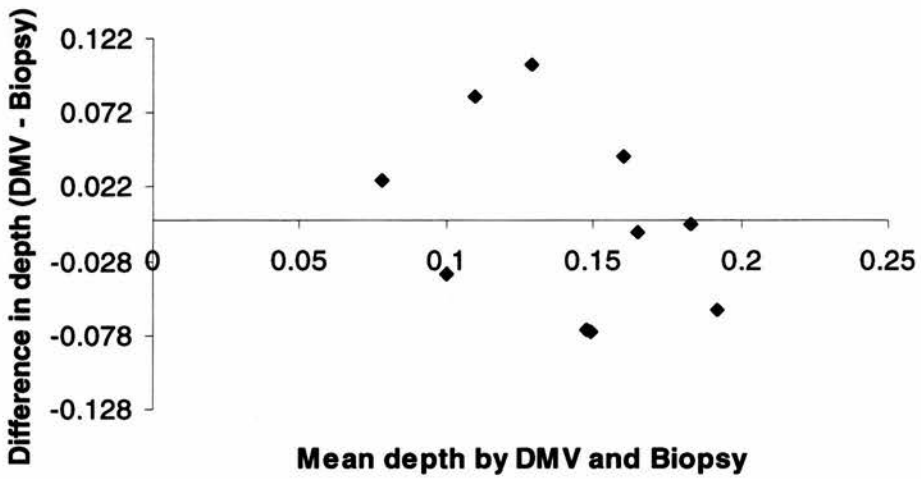


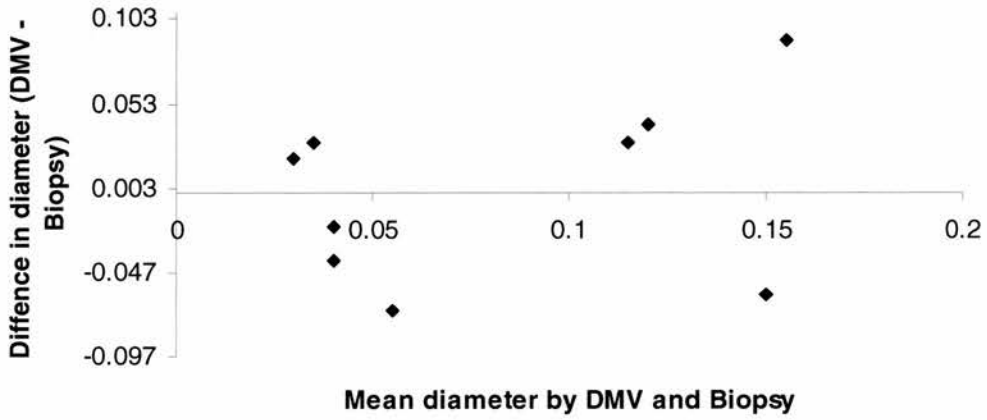
Figure 20. Corrected difference against mean for depth measurements (+ 1.02).



$d + 2sd = 0.128$

$d - 2sd = -0.128$

Figure 21. Difference against mean for diameter measurements.



$d + 2sd = 0.097$

$d - 2sd = -0.107$

Discussion.

There is a need to devise better methods of assessing Capillary Vascular Malformations[45,52,67,72,73,82,88,89]. Currently patients commence a series of treatments with no indication of how long this treatment regime may take. Prognosis following treatment is also hard to quantify with only a minority of patients receiving full clearance of their lesion. Treatments tend to persist until the patient or physician decides that no further improvement is occurring. This inevitably leads to unnecessary treatments and the difficulty of subjectively assessing photographs to look for continued improvement. After ceasing treatment it is recognised that CMs continue to evolve, leading to darkening or re emergence of the lesion[90,91].

In an effort to improve the assessment of CMs a number of objective methods have been devised. Reflectance Spectrophotometry and digital photography aim to provide an objective method of assessing CM colour. These techniques, however, give no information as to the vessel structure and blood flow within a CM. Both these properties have been suggested as important determinants of response to treatment[46,92]. Previously information on vessel structure has been achieved through the characterisation of these malformations through biopsy studies[40,41].

Non-invasive techniques of establishing vessel structure within a CM have been devised. High-resolution ultrasound can determine the maximum depth of a CM[67]. The response of a CM to laser treatment, however, is dependent upon the depth and diameter of the most superficial capillaries within it, as deeper vessels will be

shielded from treatment by the scattering and absorption of the incident laser by the more superficial vessels. The technique of Photoacoustic Probing is able to demonstrate the depth to the superficial vessels within a CM, but not to demonstrate their individual diameters[68]. Colour Doppler Optical Coherence Tomography can allow the three dimensional reconstruction of blood vessels within an animal model, but lacks the resolution required to delineate the smallest CM vessels[72].

The technique of Depth Measurement Videomicroscopy provides a non-invasive in vivo means of determining the depth and diameter of the most superficial vessels within a CM. This study has sought to compare the results obtained with this technique with those obtained histologically. The absolute values obtained with the DMV for vessel depth differ from those obtained from the biopsy specimens. This could be due to a number of factors. The DMV is a non-invasive technique, whereas the biopsy specimens are subject to the trauma of their excision and sectioning and the effect of fixing and staining of the sections. Also, the field of view given by the DMV is approximately 1.5 mm, whereas for this study we have used 3mm punch biopsy. Due to the heterogeneity of these malformations it is likely that the same vessels have not been measured with each technique. Therefore with the depth measurements a difference is found between the DMV recordings and the histological measurements. However, it would be inappropriate to conclude that the DMV was a less accurate method of assessing capillary depth as neither method can be assured to give completely accurate results and thus the use of the DMV is valid only as long as the measurements are compared with measurements taken using the same technique. The Bland and Altman Test used within this study

confirms that the DMV is a valid instrument to measure CM capillaries. This experiment allows one to compare DMV recordings with biopsy values using the correction factor but would not allow comparison with other non-invasive methods of examining CMs, such as optical coherence tomography, unless this these techniques were also compared to histological measurements.

The values obtained for vessel diameter are similar with both the DMV and histological measurements. Therefore the values obtained using the DMV can be accurately compared to histological measurements. To further test the validity of the DMV it would be worth performing measurements on more patients and also repeating those measurements at different intervals to test repeatability.

Although the depth of penetration of a laser can be increased by using longer wavelengths of light this is limited by the reduction in specificity for the absorption spectrum of oxyhaemoglobin. Thus, vessel depth may not be as significant a factor than diameter in determining a CM's response to modern lasers, which allow variation of pulse duration. From the theory of Selective Photothermolysis, the pulse duration of the incident laser should ideally be less than the thermal relaxation time of the target vessels within a CM to reduce the incidence of complications from the treatment[24]. The thermal relaxation time is itself dependent upon the diameter of the vessels to be treated. The DMV provides a non-invasive method of assessing this, which is rapid and relatively inexpensive (approximately £5000) and can be used in a clinic setting.

The second part of this study aimed to evaluate the effect of pressure from the DMV tip on the CM microvasculature. The effect of pressure causing anomalous results is a criticism of many methods of contact assessment of skin lesions, such as reflectance spectrophotometry and ultrasound as well as the DMV. When using the DMV it is essential to rest the tip on the skin so that a Skin-oil-glass interface is created. Too little pressure results in loss of this interface and too much pressure is evident from the monitor. The pressure assessment we have carried out gave a mean pressure of 62 mmHg required to alter the results obtained. Previous studies by Schubert et al found a pressure exceeding 50 mmHg was required to reduce skin microcirculation perfusion in normal skin in the sacral area[86]. Lindan et al found that the normal pressure on pressure points in a sitting position was between 10 - 60 mmHg[93]. The pressure thus required to alter the values recorded using the DMV are significant and much greater than the pressure applied in practice.

We believe the Depth Measurement Videomicroscope provides a useful tool for the assessment of Capillary Vascular Malformations both for research and to practically guide treatment. The ability to assess CVM structure may allow newer Pulsed Dye Lasers to treat CMs more efficiently, as pulse duration and wavelength could be matched to measured vessel diameter and depth, respectively. Also, by determining the depth of CM vessels in relation to the epidermal layer it may be possible to alter the spray timings of Cryogen Spay Cooling Systems, so to improve epidermal protection[68]. By measuring the depth and diameter of vessels within a CM it may be possible to more accurately determine when it becomes resistant and hence prevent patients receiving unnecessary treatments.

CHAPTER 5

The Relationship between Location, Colour and Vessel Structure Within Capillary Vascular Malformations

Introduction

It has been observed that Capillary Vascular Malformations (CMs) in some locations tend to improve more than in others. Nguyen et al[94]divided facial CMs into central forehead lesions, central lesions consisting of the nose, upper lip and medial cheek, and peripheral lesions. They found that central forehead lesions cleared best, then peripheral lesions and central lesions did worst. Renfro et al[95]found also that lesions in the central facial area did less well to laser treatment than more peripheral lesions. They also split patients depending upon the dermatomal distribution of the CMs and found that those involving V2 tended to do less well than those involving V1 and V3.

This chapter uses the technique of Depth Measuring Videomicroscopy (DMV, PW Allen, Tewkesbury, UK) to investigate the relationship between location and vessel structure. Using traditional videomicroscopy has allowed CMs to be characterized into two different types[76]; those involving predominantly ectasia of the superficial papillary plexus termed type1 (figure 22) and those involving ectasia of the deeper reticular plexus as type 2 (figure 23). A minority of CMs also display a mixed pattern (figure 24). It has been found that those CMs displaying a mainly type 1

pattern tend to respond better to laser treatment than those with a type 2 pattern. It has also been suggested that CM Type varies depending on location[77].

This study aims to examine untreated CMs in differing locations with the DMV to investigate whether there is a correlation between site and type of capillary ectasia and depth and diameter of capillaries.

Figure 22. Type 1 Vessel Pattern

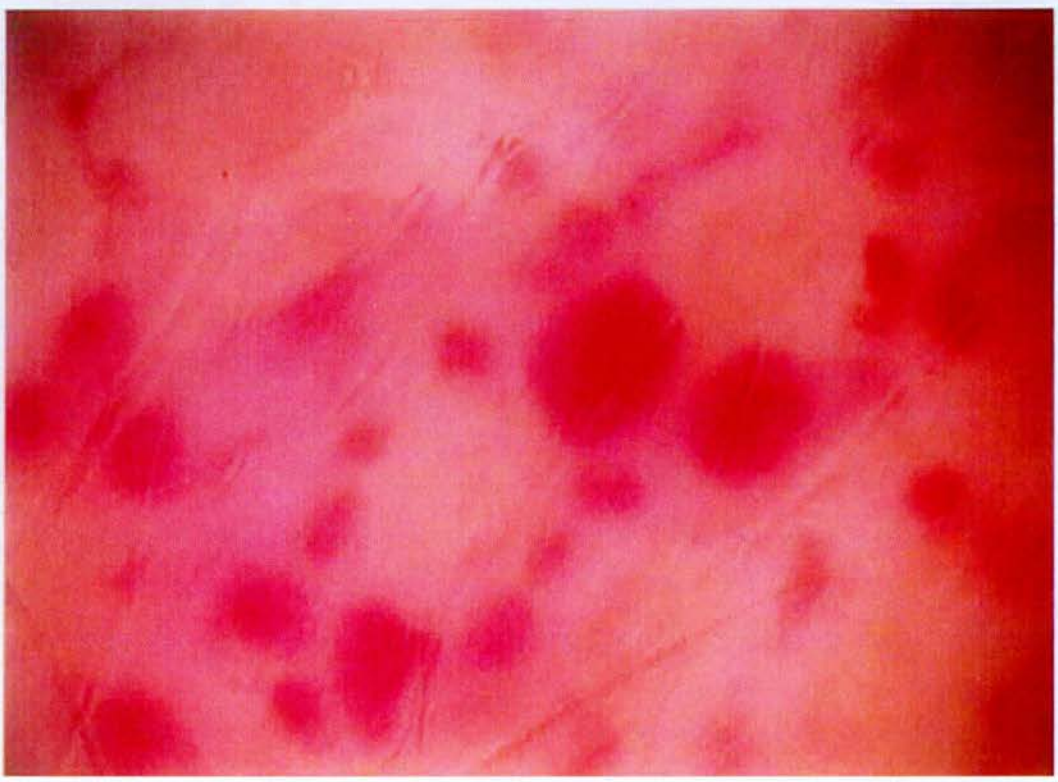


Figure 23. Type 2 Vessel Pattern

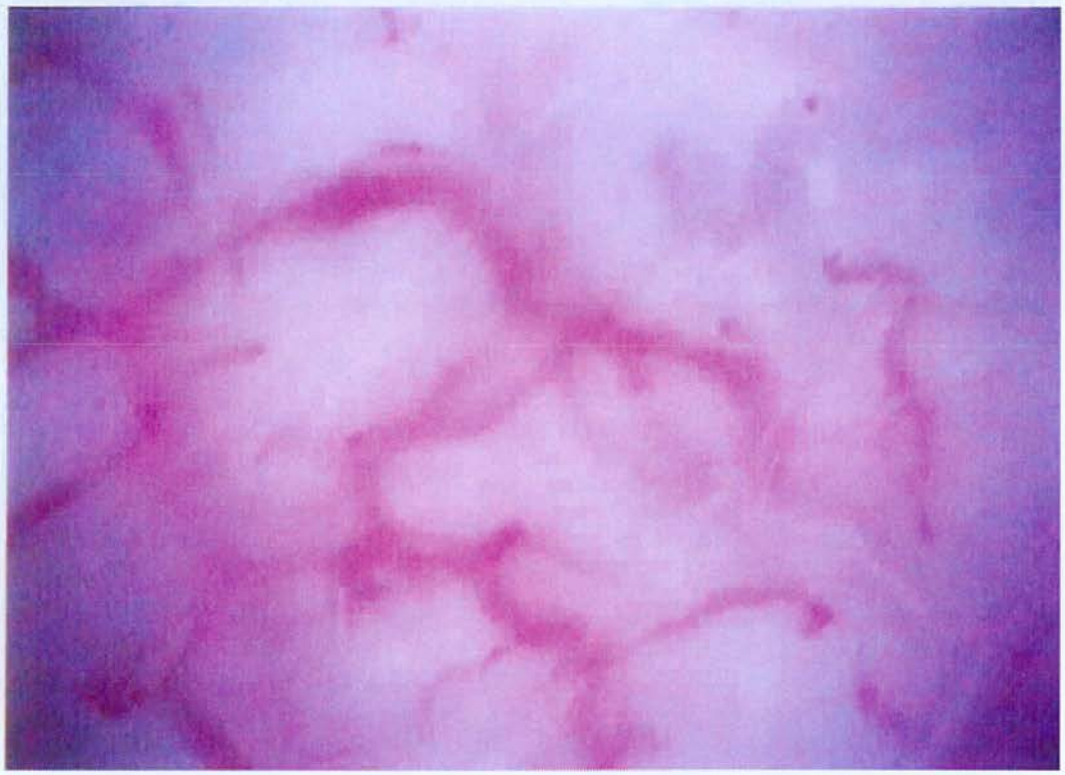


Figure 24. Mixed Vessel Pattern

Method

Fifty sites in forty-four patients were studied. Multiple sites in the same patient were only examined in large CMs covering more than one anatomical unit. Anatomical units were chosen based on existing criteria in previous studies[77,95](Figure 25). None of the patients examined had received previous treatment for their CM.

Patients were rested in a temperature-controlled room at 28 ^{O}C for twenty minutes. This was done to reduce the effect of temperature[62]and exercise on CM capillaries. Colour of the CM was recorded by two observers using a Munsell Colour Chart (GretagMacbeth, New Windsor, NY)[57,95]. This colour chart result was then converted to a scale based on paleness, so as to allow statistical interpretation. Patients were then asked to complete a Combined Skin Type Test (CSTT) questionnaire to determine their skin type.

The Depth Measuring Videomicroscope (DMV) consists of a Compact Video Microscope (PW Allan, Tewkesbury, UK) connected to a 200X Cy-Scope Lens. A DMV recording was taken of the unaffected contralateral side and three recordings taken of the CM to reduce the error from the heterogeneity of the lesion. The DMV recording gave a measure of the depth of the capillaries within the lesion. The DMV measurements were captured to film using a colour video printer (Mitsubishi Colour Video Copy Processor). From the images taken, vessel diameters were recorded based on three vessels in each image using the image of a 1mm graticule (Graticules Ltd, Tunbridge, UK). A mean value was thus attained for CM vessel depth and diameter. Type of capillary ectasia could be visualized on the images obtained.

Results

From the CSTT results, the majority of patients in the study were Fitzpatrick Skin Types 1 – 3. One patient was a Skin Type 4 and no darker skin types were encountered. The number of patients in each anatomic location is shown in figure 25. V2 on the face was further divided into medial and lateral lesions as previous studies have found that medial lesions tend to respond less well to laser treatment than lateral ones. In some groups there was insufficient numbers of patients to allow statistical analysis and groups containing less than three patients were excluded.

The Munsell colour chart scores were adjusted to represent a scale based on paleness of the CM. Figure 26 shows the mean and range of Munsell colour types for each location. As can be seen there is a wide variation in the colours of the CMs in different locations.

There was no correlation found between Munsell colour score and either depth or diameter of the capillary ectasia using a Pearson Correlation calculation. All statistics were carried out using SPSS v10.

When different locations were examined there was a wide variation found in both the depths (Figure 27) and diameters (figure 28) of the CMs. Interestingly, when neighbouring anatomical areas were examined in the same patient there was found to be a change in the type of ectasia seen in some individuals. Figures 29 and 30 show DMV videomicrographs taken on the same patients. The image of the thigh

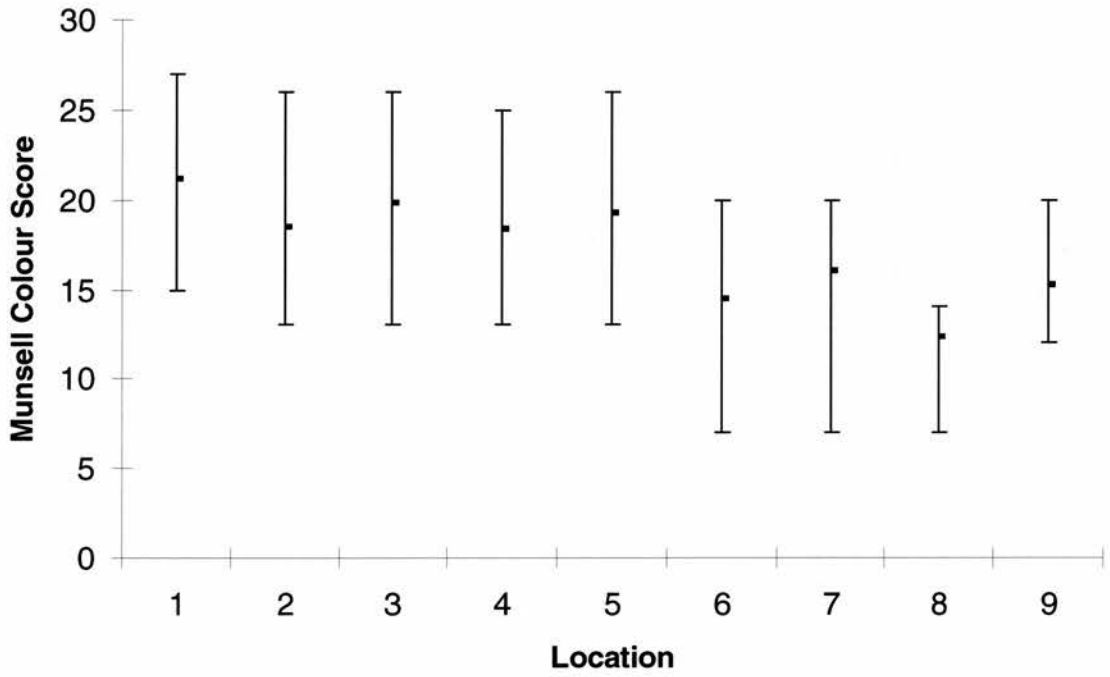
shows a type 1 pattern whereas that of the leg demonstrates a type 2 pattern. Figure 31 shows the distribution of capillary ectasia type for each location. As can be seen no correlation could be found between type of capillary ectasia and those sites known to do badly from laser treatment, namely V2 and distal extremities, overall.

Figure 25. Locations of Capillary Vascular Malformations Studied and Numbers of Patients per Location.

Ref	Location	Number of Patients
1	Lower Limb Distal	7
2	Lower Limb Proximal	4
3	Upper Limb Proximal	5
4	Trunk	5
5	V1	6
6	V2	16
7	V2 medial*	9
8	V2 lateral*	7
9	V3	3
	Other	2
	Upper Limb Distal	1

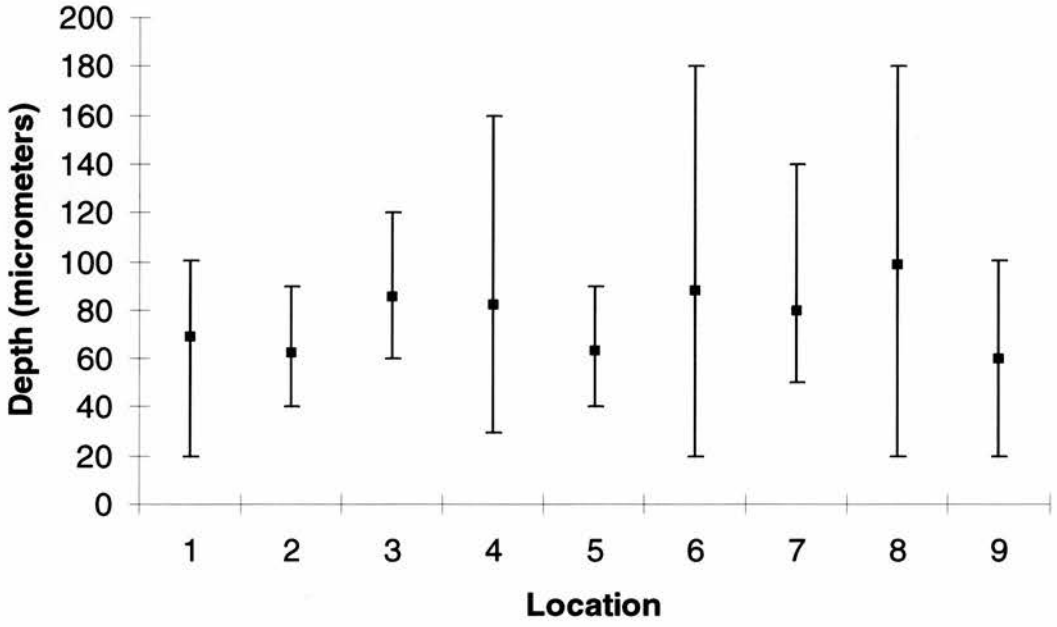
* V2 divided into medial and lateral, depending upon the relationship to outer canthus

Figure 26 Range of Munsell Colour Chart Scores per Location



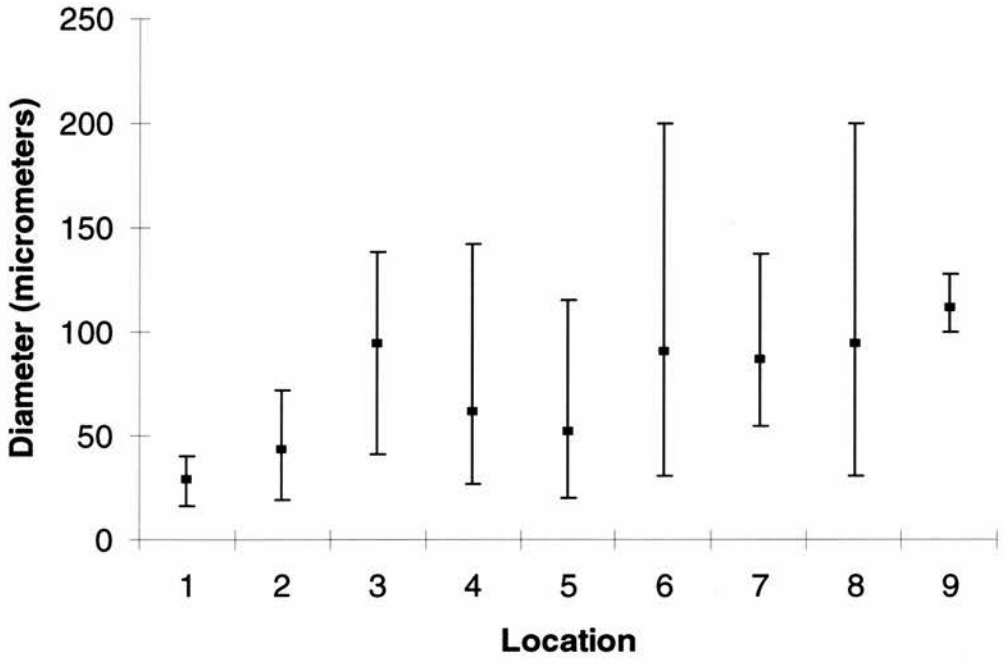
Locations: 1 = Lower limb distal; 2 = Lower limb proximal; 3 = Upper limb proximal; 4 = Trunk; 5 = V1; 6 = V2; 7 = V2 medial; 8 = V2 lateral; 9 = V3.

Figure 27. Range of Depth Measurements per location



Locations: 1 = Lower limb distal; 2 = Lower limb proximal; 3 = Upper limb proximal; 4 = Trunk; 5 = V1; 6 = V2; 7 = V2 medial; 8 = V2 lateral; 9 = V3.

Figure 28. Range of Diameter Measurements per Location



Locations: 1 = Lower limb distal; 2 = Lower limb proximal; 3 = Upper limb proximal; 4 = Trunk; 5 = V1; 6 = V2; 7 = V2 medial; 8 = V2 lateral; 9 = V3

Figure 29. Videomicrograph taken from the thigh showing a type 1 pattern

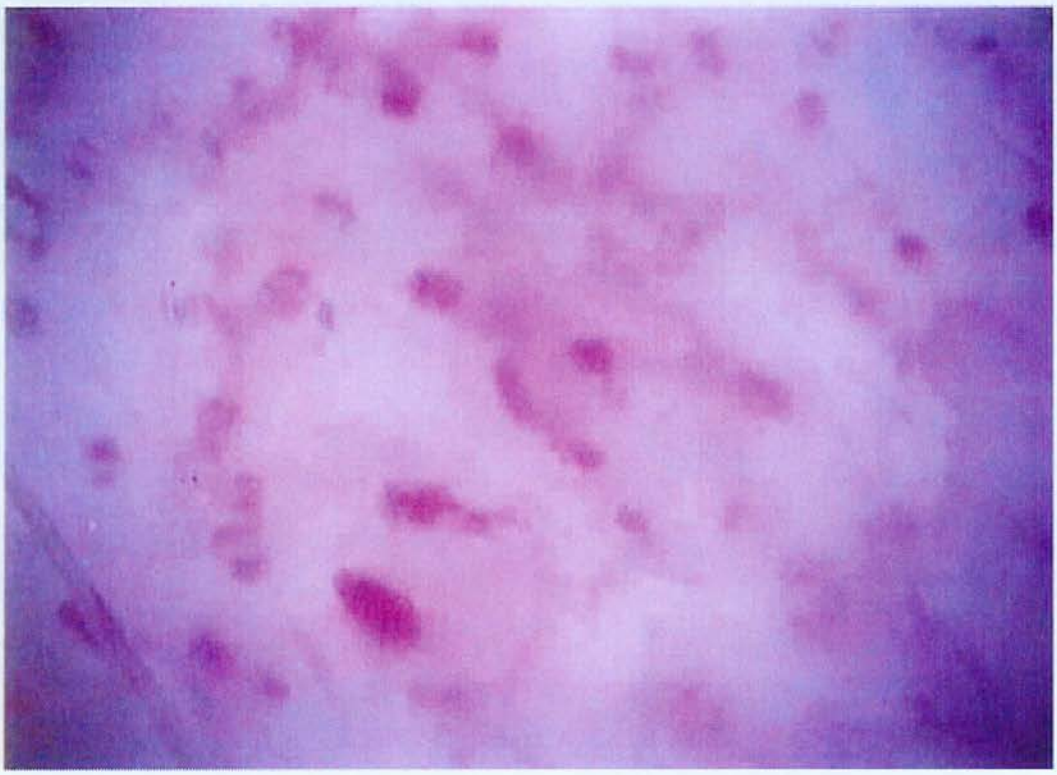


Figure 30. Videomicrograph taken of the leg of the same patient as seen in figure 26 demonstrating a type 2 pattern

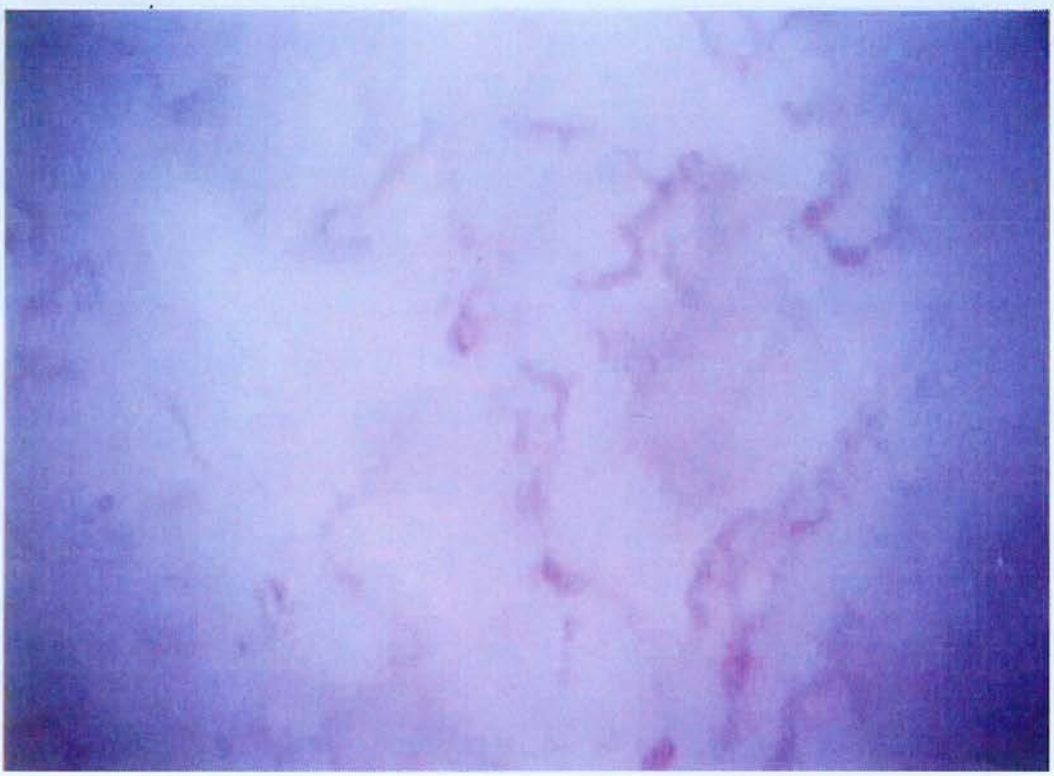
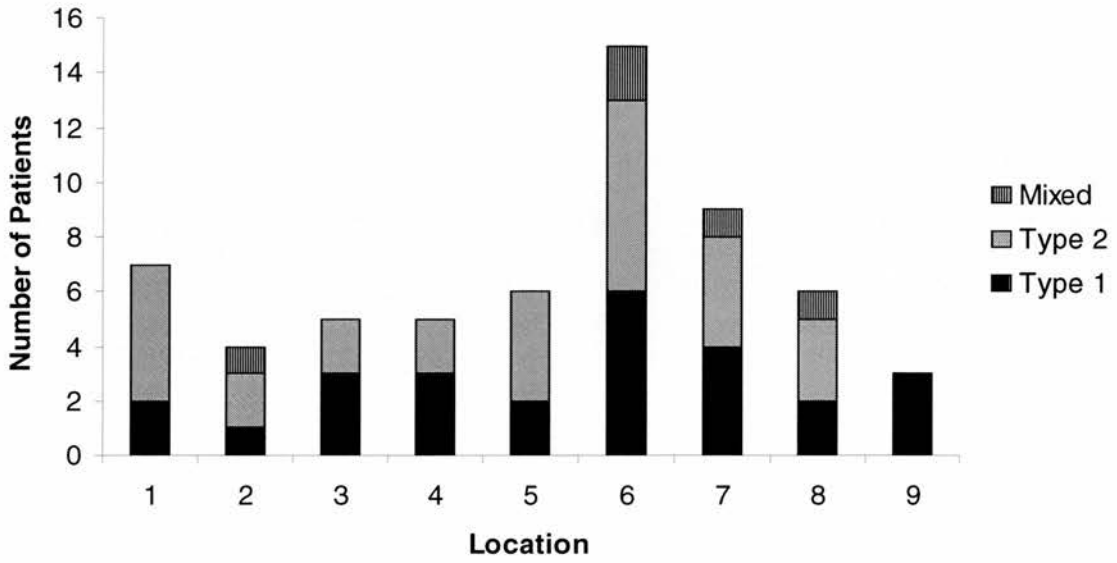


Figure 31. Type of Vessel Ectasia seen per Location



Locations: 1 = Lower limb distal; 2 = Lower limb proximal; 3 = Upper limb proximal; 4 = Trunk; 5 = V1; 6 = V2; 7 = V2 medial; 8 = V2 lateral; 9 = V3

Discussion

It has been widely recognized that certain sites for capillary vascular malformations tend to respond better to laser treatment than others[94]. Lesions on the distal extremities and medial face tend to do less well from laser treatment than lesions on the lateral face or trunk. Eubanks et al attributed this difference to type of vessel ectasia seen using traditional videomicroscopy[77]. Their study, however, looked at only 17 patients and 12 of these had already received laser treatment prior to imaging. Previous unpublished work within our unit examining 111 resistant CM patients (all having had more than ten dye laser treatments) demonstrated that 98 % patients exhibited a type 2 pattern following treatment. In Eubanks' study, 10 of the 12 previously treated patients were found to have a type 2 pattern as opposed to only 1 of the 5 previously untreated patients. Their conclusion that response in different anatomical sites is due to differences in vessel type is, therefore, more likely to be due to whether patients had received prior treatment or not. In this study we have examined only patients who have received no former treatment by any modality.

We have used a Depth Measuring Videomicroscope to examine CMs and determine not only type of ectasia, but also actual vessel diameter and depth. In this study we are measuring the depth to the most superficial of the CM capillaries, rather than mean or maximum depth. With this technique the deeper capillaries are masked by more superficial vessels. The response of an untreated CM to laser treatment will be dependent on the range of depths and diameters of capillaries comprising the lesion.

Biopsy studies by Fiskerstrand[40]and Hohenleutner[41]have demonstrated that CMs with deeply placed and smaller vessels tend to respond less well to laser treatment than those with superficial and large vessels. We have examined the hypothesis that poor response to laser treatment will be related to the depth and diameter of the vessels within the CM.

Colour of a CM has been found to be predictive of outcome following laser treatment, with purple and red lesions tending to respond better than pink ones[96]. This is believed to de due to the theory that pale pink CMs have more deeply located or smaller capillary ectasia. Our results have not demonstrated a relationship between capillary depth or diameter and colour.

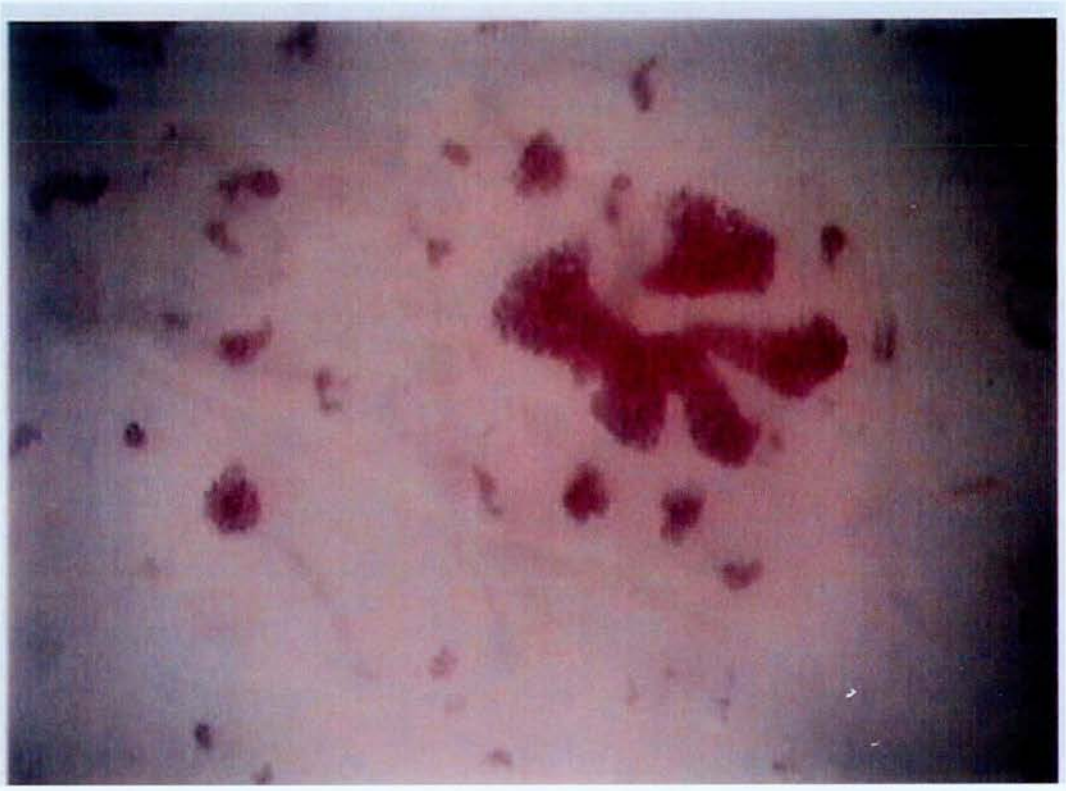
We have found no relationship between the type of capillary ectasia and the site of a CM. It would seem likely therefore that predominance of one type of capillary ectasia over another is not a reliable indicator as to why one anatomical area will respond better to laser treatment than another. Also, it appears that capillary depth and diameter alone cannot be the only factors responsible for the variation of response between different areas.

The technique of Depth Measurement Videomicroscopy has demonstrated multiple clumps of very small vessels in a couple of the treated patients (figure 32). This phenomenon has previously been described by Smithies et al[81] from the computer-aided reconstruction of biopsy specimens, but has not been described in vivo previously. The presence of these clumped aggregated vessels has been proposed as

a possible reason for poor response to laser treatment. However, we have only seen this vessel structure in two patients examined and therefore find it unlikely that this is a major reason for the poor response of some CMs.

This study does not take account of any variation of capillary flow between different locations. Previous studies have demonstrated abnormal flow within CM vessels in comparison to normal skin[45,92]. In these studies, many of the CMs investigated exhibited increased blood flow in comparison to normal skin. Currently no studies have been performed to evaluate the difference in blood flow to CMs in different anatomical locations. It seems likely that factors such as blood flow, and not just the capillary depth and diameter, may be responsible for the differing responses of certain sites to laser treatment.

Figure 32. Demonstrating aggregates of very small vessels.



CHAPTER 6

The Response of Capillary Vascular Malformation Vessel Structure to a Single Pulse Dye Laser Treatment

Introduction

The previous chapters have outlined the use of the Depth Measurement Videomicroscope (DMV) as a new technique to enable the non-invasive assessment of Capillary Vascular Malformation (CM) vessel structure. This chapter focuses on demonstrating any alteration in the capillary structure of CMs following a single therapeutic treatment with a Pulsed Dye Laser.

A number of techniques have been used to provide an accurate means of assessing CM characteristics non-invasively. These include methods for assessing colour, such as reflectance spectrophotometry[45,97], digital[63,64] and traditional photography[52] and methods of assessing flow such as laser Doppler flowmetry[62,65,92]. None of these techniques allows the delineation of actual ectatic capillary structure. A number of studies have established the importance of capillary depth and diameter in determining the results from laser treatment of CMs[30,40-42,80,98].

Recently, new techniques have been devised in an attempt to achieve accurate non-invasive evaluation of CM vessels. Ultrasound and photoacoustic probes may be able to determine maximum CM depth but do not delineate individual vessel

diameter[67,68]. Optical coherence tomography (OCT) uses a light source to scan through the skin and can delineate small vessels from their reflection to down to 10-20 micrometers resolution[73,99]. At present there are no studies examining series of patients with this technique.

To enable the measurement of capillary depth and diameter within a CM we have used the Depth Measurement Videomicroscope. The aim of this study was to examine how capillary characteristics within a CM are affected by dye laser treatment using DMV.

Method

During the period of February to August 2002, all new patients attending Canniesburn laser suite were entered into the trial of the DMV. Thirteen, previously untreated patients were assessed with the DMV prior to and six weeks following a single treatment with a 0.45 ms 585nm Pulsed Dye Laser (SLS Chromos, Wales, UK). For each patient the treatment parameters remained the same: 6.4 J/cm^2 fluence and a 7mm spotsize with topical ice epidermal cooling.

During the DMV examination each patient was allowed to rest for twenty minutes in a temperature-controlled room at 28 ^{0}C. They were also asked to carry out a Combined Skin Type Test (CSTT), which assesses their skin type and recent sun

exposure. The area to be examined was then colour matched using a Munsell Colour Chart (GretagMacbeth, New Windsor, NY) by two observers.

The DMV consists of a Compact Video Microscope (PW Allan, Tewkesbury, UK) connected to a 200X Cy-Scope Lens. Images were viewed through a monitor (Sony Trinitron) and image capture was via a colour printer (Mitsubishi Colour Video Copy Processor).

The normal capillary depth was measured on the contralateral unaffected side of the body prior to the assessment of the CM test area.

A further cohort of eleven resistant patients attending the Laser Suite for final assessment was also examined. These patients had all had over five dye laser treatments (mean 11.6, range 5-29).

Results

Following treatment the majority (12/13) of untreated patients demonstrated some lightening of their CM as assessed using Munsell colour charts. To aid statistical analysis Munsell chart recordings were converted to scores based on paleness (Fig. 30). In this chart the higher scores represent paler lesions. When colour pre and post laser treatment was compared using a Wilcoxon Signed rank test, there was a

significant lightening of the lesions ($p<0.01$). All Statistics were carried out using SPSS V.10.

All the patients in the study were Fitzpatrick Skin Type 1 or 2.There was no statistically significant change in CSTT scores between the two recordings, indicating that sun exposure within the six-week gap between DMV measurements was not sufficient to cause confounding results (Figure 31).

When vessels were examined prior to and six weeks following pulsed dye laser treatment the measured depths were found to be greater following treatment in most cases (Fig. 32), a mean of 68.5 micrometers (range 20-140 micrometers) before and 82.3 micrometers after (range 50-140 micrometers). Figures 33 and 34 show videomicrographs taken of the same previously untreated patient before and following a single laser treatment. This clearly demonstrates the reduction in vessel size seen. Figure 35 illustrates the vessel pattern seen in a resistant CM patient, for comparison.

Fig. 33 Colour change following pulsed dye laser treatment.

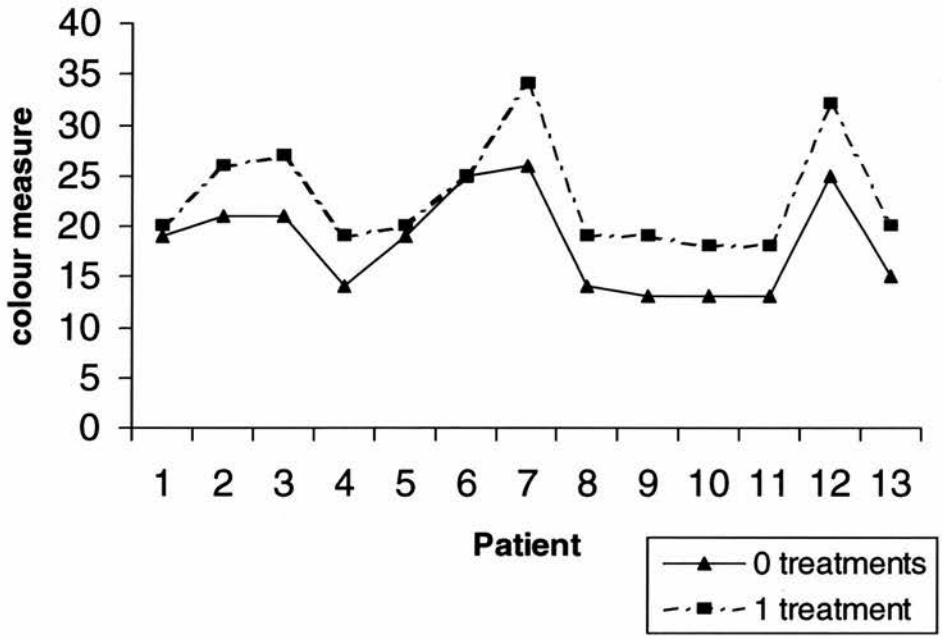


Figure 34. Results for patients receiving first dye laser treatment.

Patient no.	Age	CSTT score[1]		Colour Change[2]	Contralateral Depth		Mean Depth CM		Mean Diameter CM	
		Untreated	Treated		Untreated	Treated	Untreated	Treated	Untreated	Treated
1	11	29	24	1	150	40	140	100	69	24
2	41	13	13	5	30	30	80	90	40	19
3	41	13	13	6	30	30	70	100	34	20
4	37	14	14	5	110	110	60	50	97	86
5	48	26	23	1	100	120	60	100	54	43
6	18	12	12	0	70	70	50	70	24	20
7	14	15	15	6	60	60	120	140	103	20
8	48	22	18	5	80	100	50	70	48	53
9	11	20	20	6	70	70	20	50	31	61
10	19	19	18	13	40	20	40	50	80	34
11	22	16	16	6	110	110	100	120	117	49
12	42	18	18	7	10	10	40	60	29	12
13	43	28	27	5	20	10	60	70	148	57

[1] CSTT – Combined Skin Type Test

[2] Colour change – As Modified Munsell Colour Chart Score

Fig. 35 Capillary depth as assessed by DMV before and after pulsed dye laser treatment.

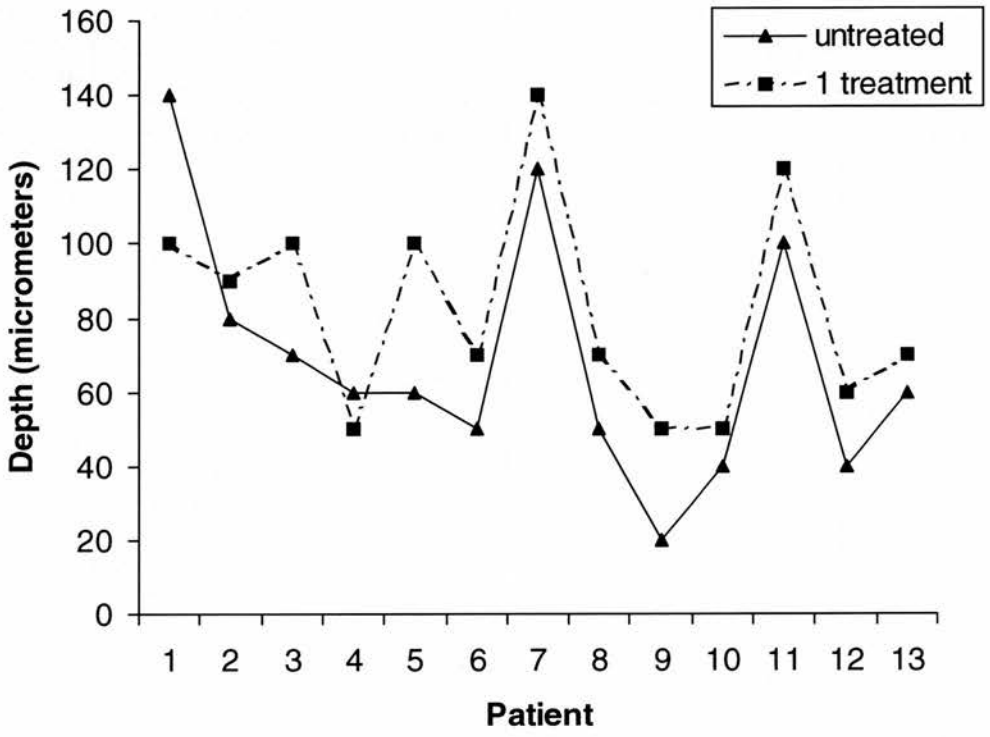
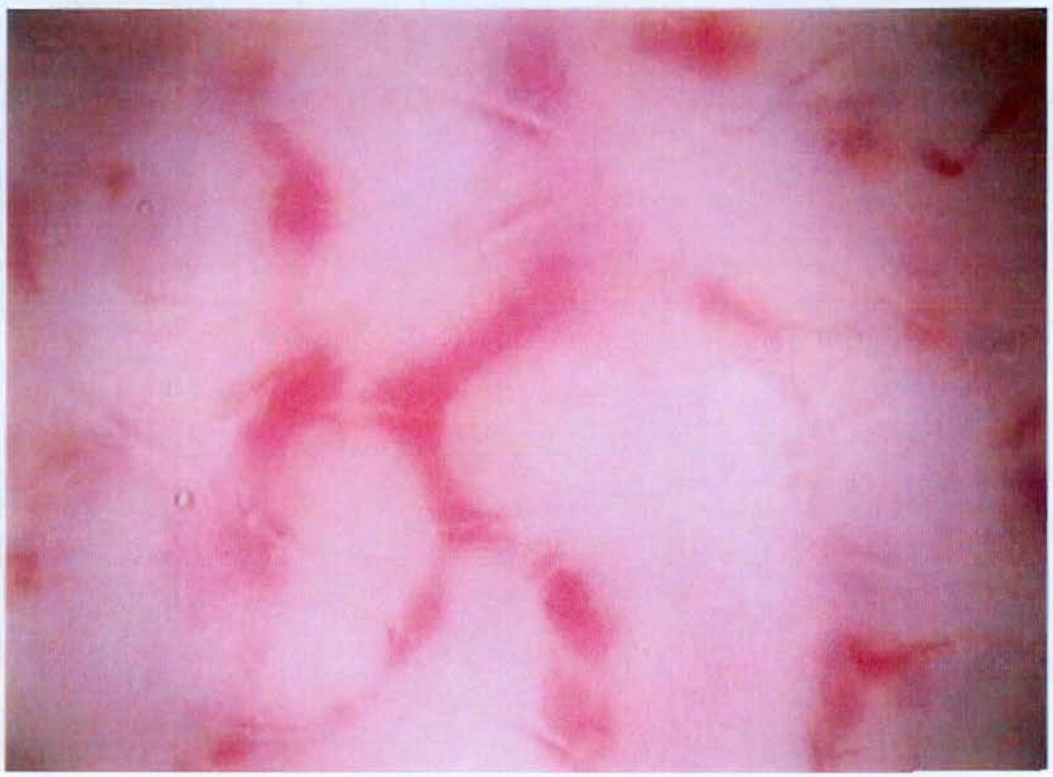


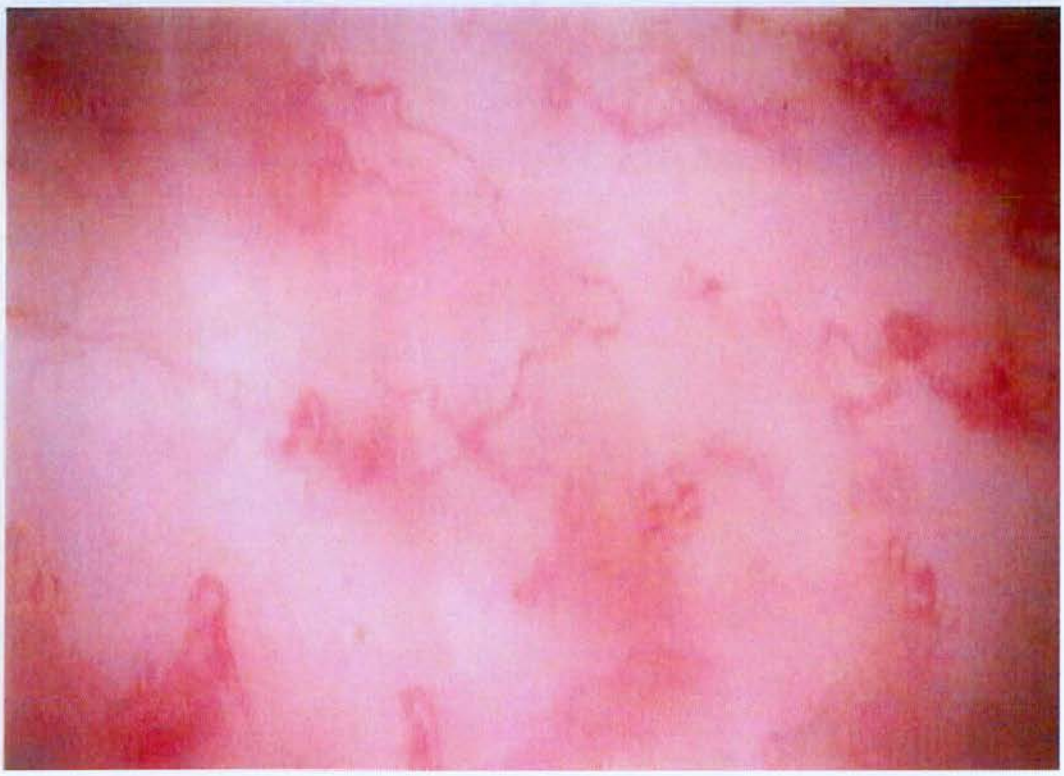
Figure 36. Type 2 Pattern Prior to Laser Treatment.



Mean Depth 80 micrometers.

Mean Diameter 63 micrometers.

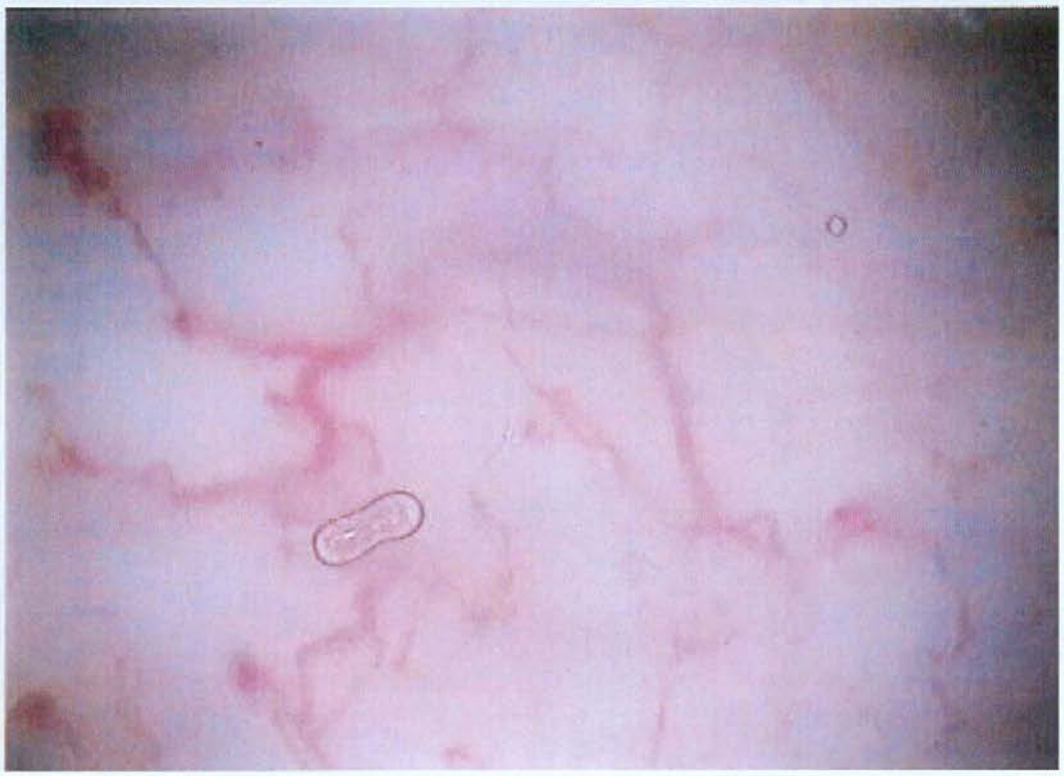
Figure 37. Same patient as figure 33. Again showing a Type 2 pattern but with smaller calibre vessels.



Mean Depth 90 micrometers

Mean Diameter 32 micrometers

Figure 38. A videomicrograph taken of a resistant patient



Mean Depth 130 micrometers

Mean Diameter 21 micrometers

If the depth measurements for the CM vessels are compared to those obtained for the normal contralateral skin the depth of the measured vessels can be seen to increase following treatment (Fig 36). Statistical analysis using a Wilcoxon Signed Rank test showed a significant reduction in the depth measurements following treatment compared to normal capillary depth ($p< 0.01$). This comparison between treated CM and normal contralateral vessel depth was made to test the validity

between the two sets of measurements and reduce any error from zeroing the DMV. As the patient is rested in a temperature-controlled room, fluctuations in capillary filling are minimized. There was no statistically significant difference seen between the normal capillary depth measurements taken pre and post treatment. In addition, as dermal capillary depth varies depending upon location[77], values compared to normal allow comparison between different CM sites.

For vessel diameter, mean measurements were found to be smaller following treatment with a mean of 38 micrometers (Range 12 – 86 micrometers) versus a mean of 67 (Range 24 – 148 micrometers) prior to treatment. This result was statistically significant using Wilcoxon Signed Rank Test ($p<0.02$, Figure 36).

When compared to the pre-treatment group, the cohort of eleven resistant patients was again found to have deeper vessels ($p< 0.01$, Figure 35). Although diameter of vessels was not found to be smaller statistically, a trend is evident from the chart (Figure 36).

Figure 39. Capillary depth compared to normal contralateral capillary depth before and after pulsed dye laser treatment and including a cohort of resistant patients for comparison. Negative values are closer to the skin surface and positive values deeper.

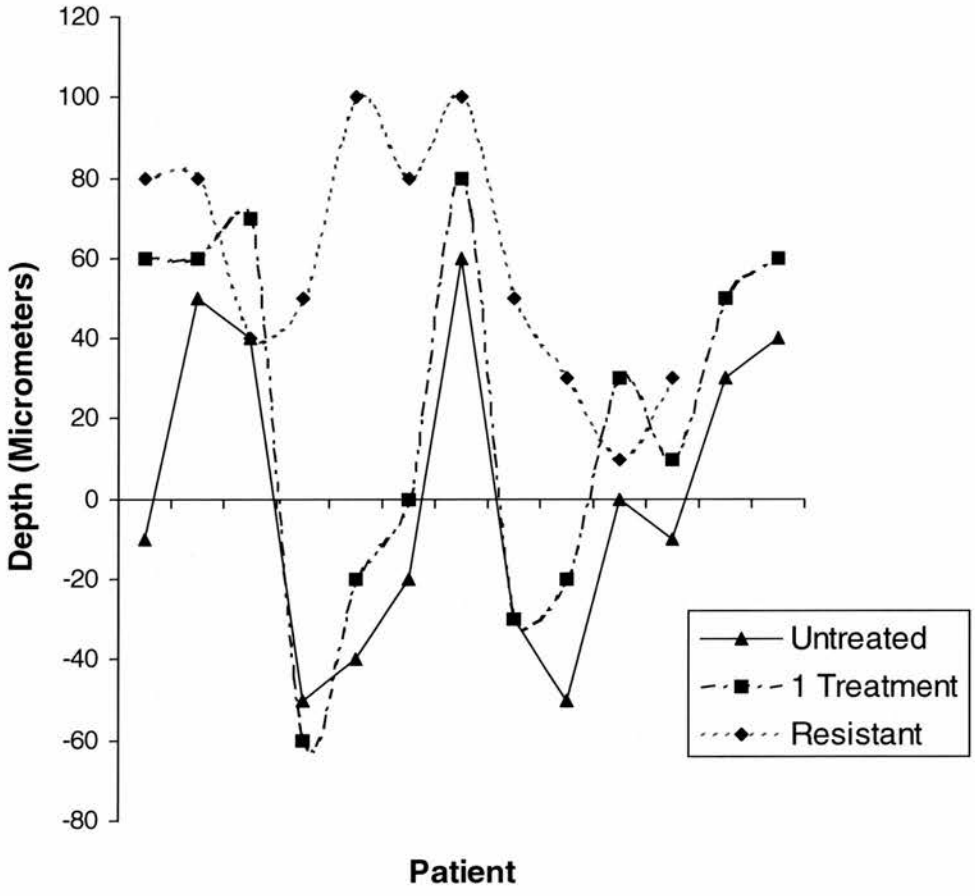
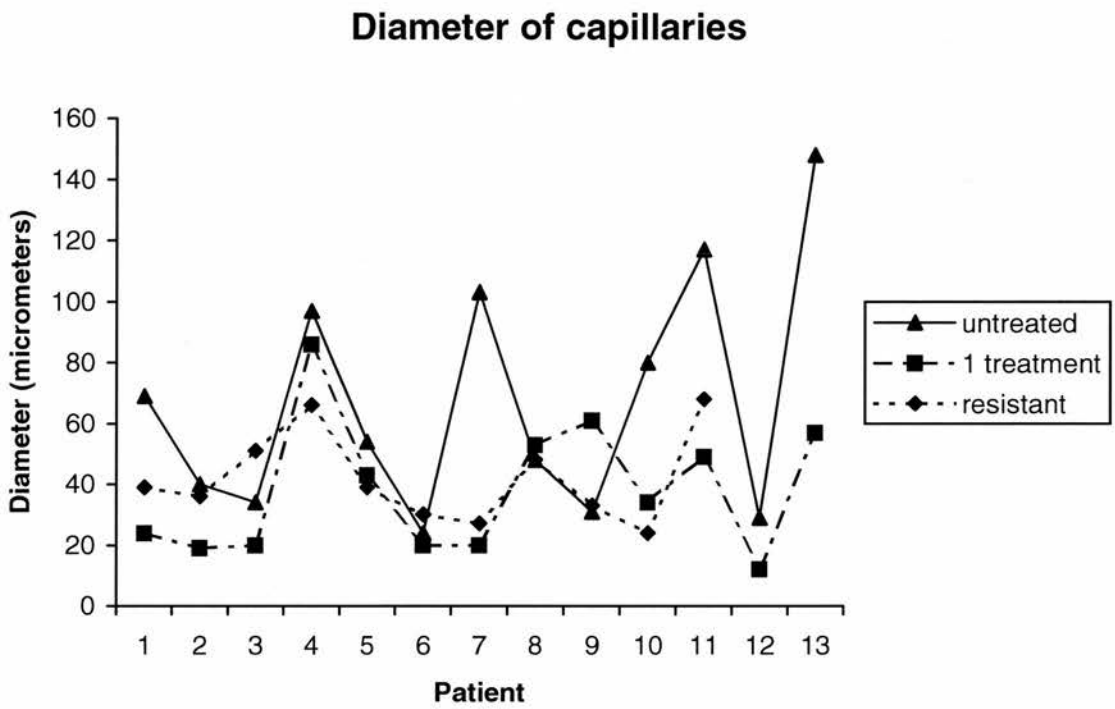


Figure 40. Diameter of CM vessels before and after pulsed dye laser treatment including results for the resistant cohort of patients.



Discussion

The treatment of CMs by pulsed dye laser is based upon the Theory of Selective Photothermolysis proposed by Anderson and Parish[24]. Fundamental to this theory is that vessels will receive sufficient energy to cause photothermolysis. Also, that pulse duration of the incident laser light is less than the thermal relaxation time of the target vessel so as to reduce the risk of complications. In order for incident laser energy to heat a particular vessel to 70 ^{0}C, sufficient to cause photocoagulative necrosis of the vessel wall, implies that sufficient energy reaches that vessel taking into account the processes of reflection, scattering and absorption by other vessels and other chromophores within the skin. This is, as such dependent upon depth of the vessel. Thermal relaxation time for a vessel is a consequence of size of this vessel and therefore dictates the necessary pulse duration of the incident laser light[80]. Pulse duration is therefore dependent upon vessel diameter.

Previous biopsy studies by Fiskerstrand et a[40] demonstrated capillary diameters within the range of 9 – 100 micrometers, with lesions with smaller vessels responding less well to laser treatment than those with larger vessels. These values for vessel diameters respond closely to our in-vivo measurements. In this study the measurements taken for capillary diameter before and after pulsed dye laser treatment reduced suggesting that larger diameter vessels are cleared by the treatment leaving the smaller ones still present.

In terms of vessel depth, Fiskerstrand et al[40] and Hohenleutner et al[41] demonstrated that following dye laser treatment deeper vessels tended to be left uncoagulated within a CM, using biopsy studies. From previous studies using videomicroscopy by Motley and Lanigan[39,76], it is established that there may be abnormality within either the superficial papillary dermal plexus (type 1), the deep reticular dermal plexus (type 2) or a mixed pattern. In this study, the measured capillary depth was found to increase following treatment. This suggests that as vessels that are more superficial are treated the deeper underlying vessels are revealed; these vessels being obscured by more superficial vessels prior to treatment. If the CM is mainly type 1 pattern then the measured depth will be down to the surface of the superficial capillary plexus and if the pattern is type 2 then the measurement will be to the surface of the deep capillary plexus.

In this study, the vessel diameters correspond well to previous biopsy studies. For vessel depth, however, the values obtained appear smaller than previous studies. We believe the reason for this to be two fold; firstly, our measurements have been calculated to the level of the most superficial ectatic dermal capillaries, whether these are part of the superficial type 1 plexus or deeper type 2. We believe this to be a more realistic measurement to follow response of CMs to laser treatment rather than total depth of the CM vessels as these deeper vessels are unlikely to sufficiently improve before the more superficial vessels are cleared. It seems likely that predicting eventual treatment outcome is most likely to depend on the depth of the deeper vessels, and we therefore believe the depth measurements obtained using the DMV are most likely to be useful when taken after the more superficial

capillaries have been cleared by four or five dye laser treatments. Such measurements may be useful in guiding changes to laser treatment parameters, for example using longer wavelengths. Secondly, the measurements we have obtained may not have been taken exactly from the surface of the stratum corneum. Because of this, we measured depth of contralateral normal capillaries and compared them between the two examinations. As there was no statistically significant difference between the normal depth measurements before or after the laser treatment, we believe our depth measurements to be valid.

The ability to assess CM structure may allow newer pulsed dye lasers to treat CMs more efficiently as pulse duration and wavelength could be matched to measured vessel diameter and depth, respectively. In addition, the use of Cryogen Spay Cooling may be used more accurately when depth to CM vessels can be calculated[68]. By measuring depth and diameter of vessels it may be possible to determine more accurately when a CM becomes resistant to further treatment and hence prevent patients receiving unnecessary treatments.

CHAPTER 7

The Assessment of Vessel Characteristics in Capillary Vascular Malformations Exposed to Five Pulsed Dye Laser Treatments.

Introduction

The lack of response of CMs to laser treatments is believed to be due in part to the morphology of the vessels comprising the malformation[30,39-42]. Previous work has established that by adhering to the principles of selective photothermolysis, the treatment of port wine stains can be performed with a low complication rate[24,34]. This theory states that by matching the wavelength of the incident laser light to the absorption spectrum of a particular chromophore within the skin, in this case oxyhaemoglobin, then preferential damage can be caused to this compound. In the case of treating CMs, it is the heating of oxyhaemoglobin that damages the capillaries within the lesion. Also, by choosing longer wavelengths of light deeper penetration of the skin can be achieved, all be it with a commensurate loss of specificity for the chromophore[83,84].

Furthermore, this theory states that by choosing pulse durations less than the thermal relaxation time of the target vessel (the time taken to lose half the incident energy) then diffusion of heat away from the target will be minimized. It is this diffusion of heat into the surrounding structures within the skin, which causes the

complications of skin pigment change, and scaring that marred early laser treatment[36].

The goal of current laser treatment of CMs is to be able to match the wavelength and pulse duration of the incident laser light to the characteristics of the target vessels within the CM, namely vessel diameter and depth[30,68,81,82].

Aim

The aim of this study was firstly to see how the vessel characteristics of untreated port wine stains would alter after five laser treatments, when the majority of any achievable improvement had been achieved[50,94]. By noting the vessel characteristics after treatment it should be possible to elucidate the ideal laser properties to treat these now resistant CMs. Secondly, we wanted to see if eventual outcome could be predicted from the initial capillary characteristics of the untreated PWS.

Method

The delineation of CM vessel characteristics was carried out using the technique of Depth Measurement Videomicroscopy (DMV). Between January 2002 and April 2003 twenty-two previously untreated patients were assessed using this technique

before and six weeks following one laser treatment and six weeks after five full treatments or before if the lesion had totally cleared.

The DMV consisted of a 200x Cy-scope lens on a Compact Videomicroscope (PW Allen, Tewkesbury, UK).

Before the examination was performed patients were rested in a temperature-controlled room at 28^0C for twenty minutes. This was done to reduce the effect of sympathetic stimulation on the CM microvasculature and also maximally vasodilate the vessels at a reproducible temperature. The patients were then asked to carry out a Combined Skin Type Test that evaluated their Fitzpatrick skin type and any recent sun exposure.

Colour was recorded by two individuals using a Munsell Colour Chart (GretagMacbeth, New Windsor, NY)[57], and these values converted to an ordinal scale to aid statistical analysis.

The examination involved using the DMV to record the contralateral normal capillary depth prior to taking three recordings from the CM affected skin. Multiple recordings were taken to reduce the error incurred from evaluating a heterogeneous lesion. These recordings gave values for the depth of the ectatic capillaries from the skin surface and images were than taken and diameter measurements obtained by using the videomicrogram of a 1mm graticule (Graticules Ltd, Tunbridge, UK).

From the DMV examination the type of capillary ectasia was also noted. Previous studies have suggested that type of capillary ectasia (Type 1 being superficial and Type 2 being deep) is an important determinant of response[76,77].

All treatments were carried out using a 0.45 ms 585nm Pulsed Dye Laser (SLS Chromos, Wales, UK). Initial treatment parameters were the same in all patients: 7mm spotsize, 6.4 j/sqcm fluence with topical ice epidermal cooling. Fluences were changed as required during the five treatments to maintain response.

Results

Twenty-two patients entered the trial. Two patients failed to attend for their examination following one treatment, but did attend following five and are thus included in the study. Two patients had their final assessment before five treatments, one at three and another at four treatments because their lesions had fully faded. All statistical analysis was made using SPSS v10.

All but one of the patients was Fitzpatrick Skin Type 1 or 2, the other Skin Type 4. There was no statistically significant difference between CSTT scores between the examinations using a Wilcoxon Rank test. From figure 41, it can be seen that prior to any treatment more of the patients' CMs showed a Type 1 or Mixed Pattern (59%) than following five treatments (18%). This reduction in the proportion of

patients demonstrating a superficial type 1 pattern following five treatments is statistically significant using a Wilcoxon Rank test ($p<0.02$).

Figure 42 shows one patient prior to any laser treatment demonstrating large diameter type1 vessel structure and figure 43 shows the same patient following five laser treatments.

Figure 41. Type of capillary ectasia found in patients exposed to five Pulsed Dye Laser treatments.

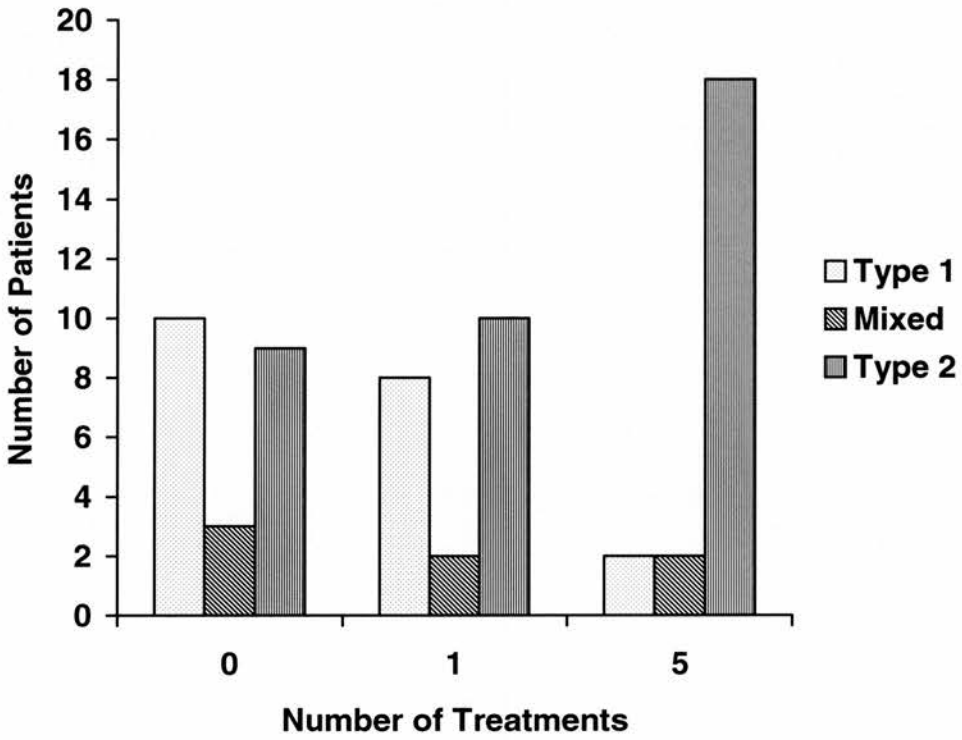
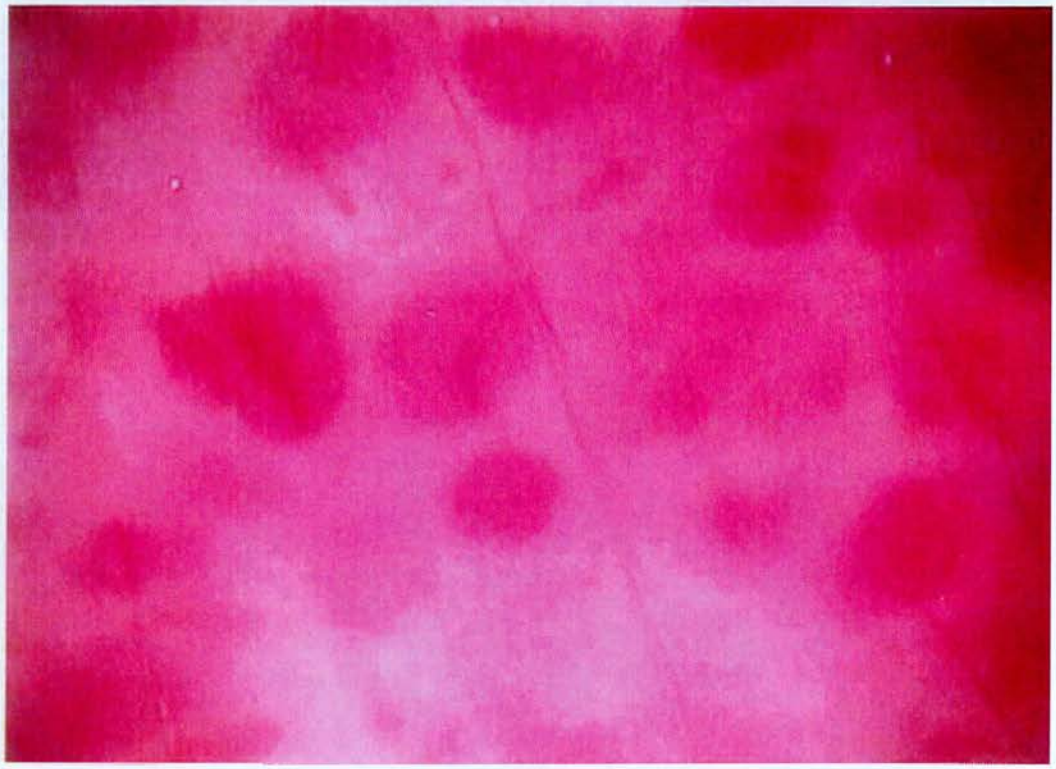


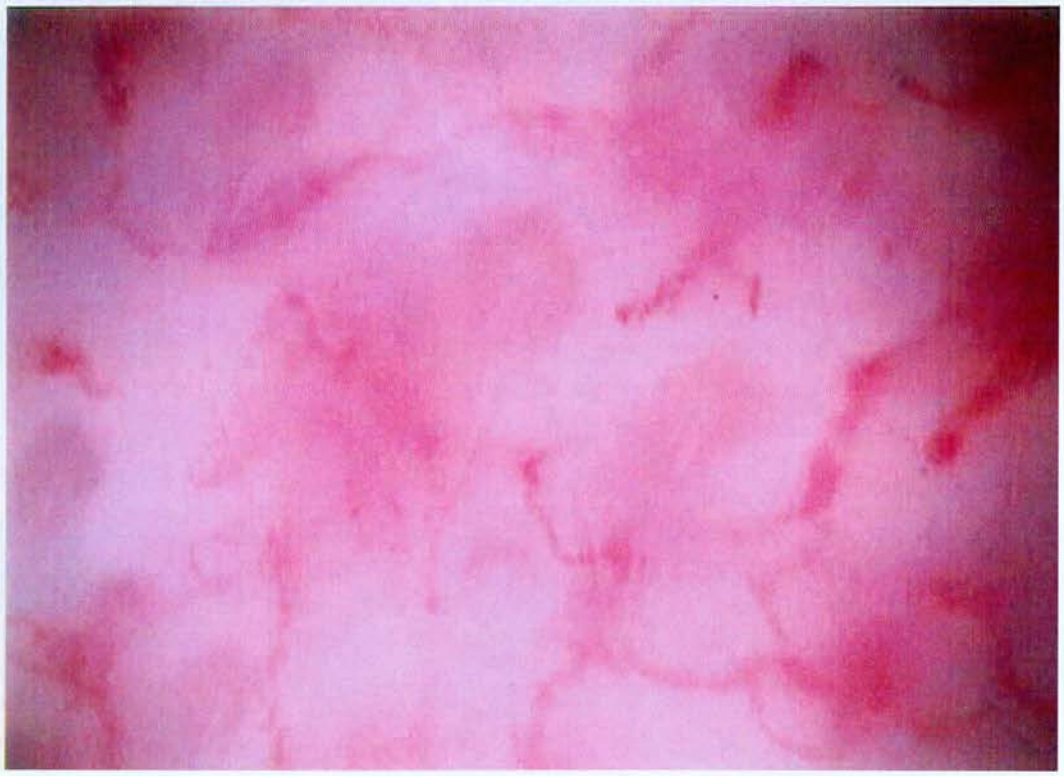
Figure 42. Patient prior to any laser treatment demonstrating a type 1 vessel pattern



Mean Vessel Depth 110 micrometers

Mean Vessel Diameter 200 micrometers

Figure 43. Same patient as figure 38 following five laser treatments.



Mean Depth 220 micrometers

Mean Diameter 87 micrometers

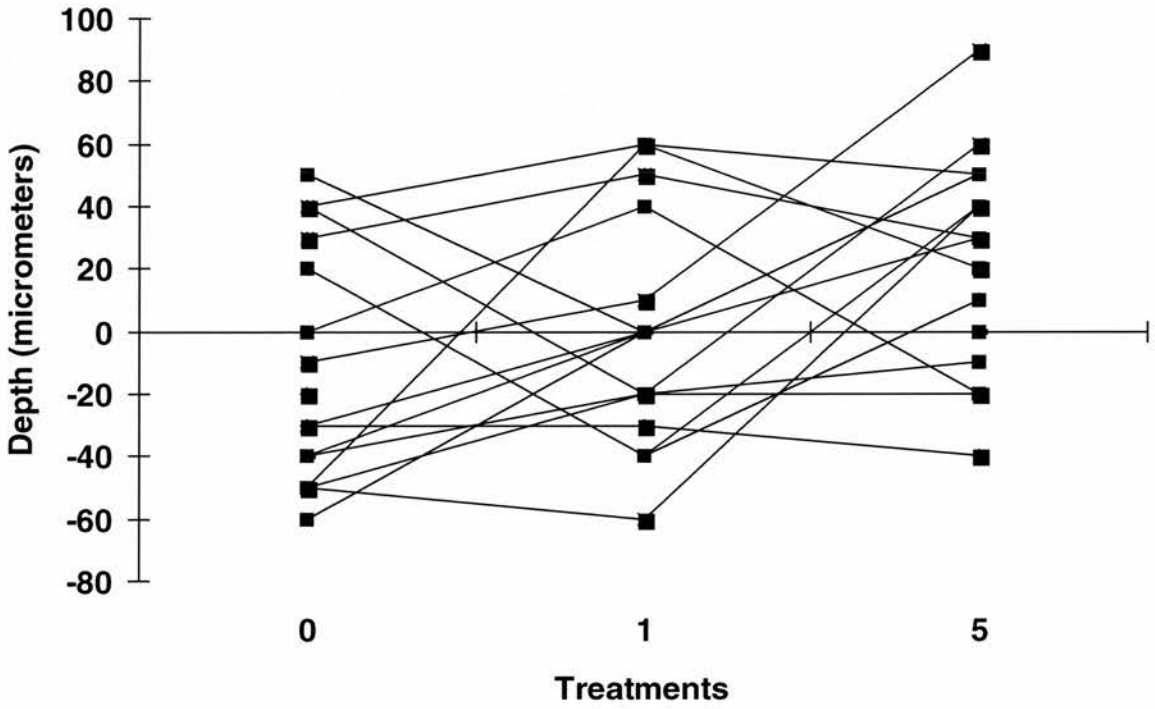
When CM capillary depth is compared to normal capillary depth there is a statistically significant increase in the depth of the recorded CM vessels after five laser treatments ($p<0.02$, figure 44). Capillary Vascular Malformation vessel depth was compared to contralateral capillary depth to reduce error incurred due to the zeroing of the DMV on the skin surface.

When capillary diameter is examined there is a statistically significant reduction in the values using a Wilcoxon Rank Test ($p<0.001$, figure 45). Following treatment the diameters of the capillaries remaining within the CMs were between 10 and 50 micrometers with the larger vessels having been cleared.

For Munsell colour chart assessment there was a statistically significant fading in the lesions after one and after five treatments ($P<0.02$ and $p< 0.001$, respectively, Fig. 46).

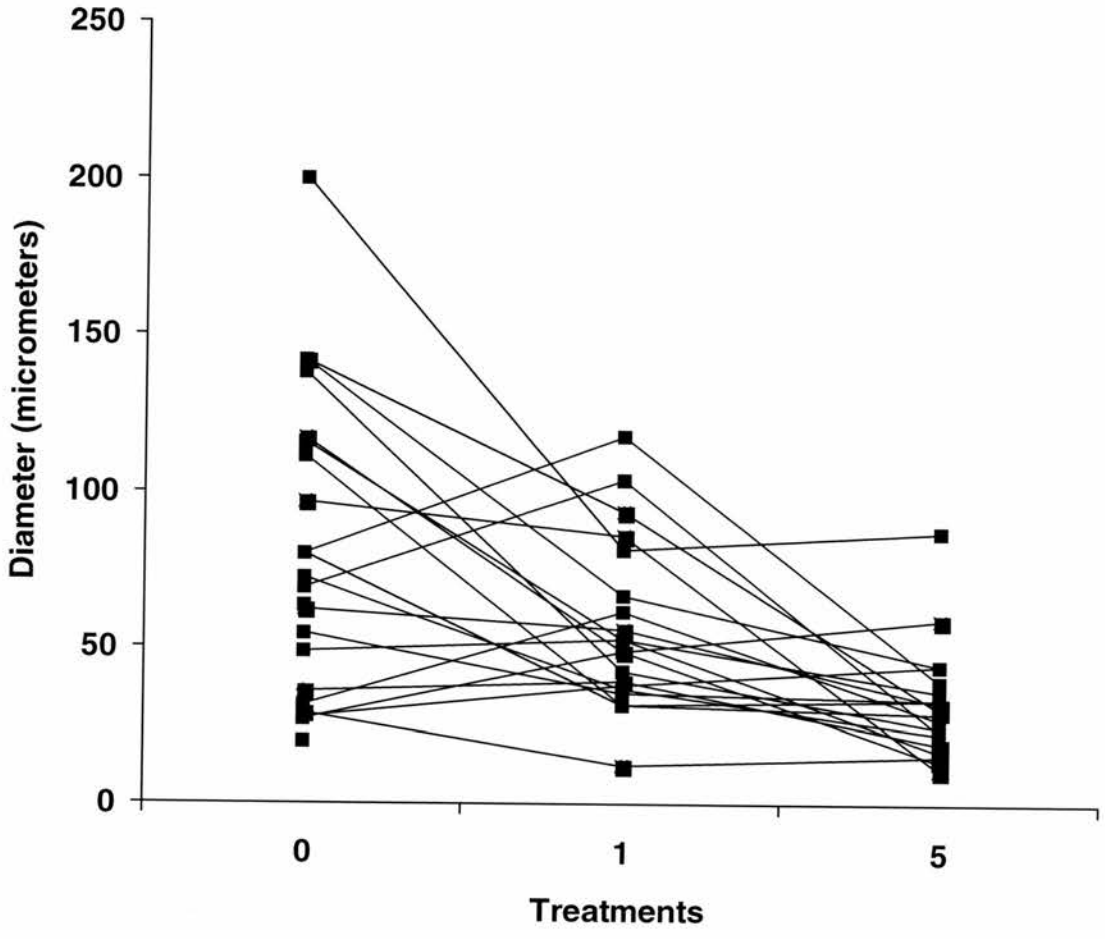
If the eight patients who had the greatest fading of their CM, as evaluated by largest change in Modified Munsell Colour Chart Score, are compared to the eight patients who had the least fading no statistically significant difference can be found between the two groups in either depth or diameter.

Figure 44. The depth of capillaries within Capillary Vascular Malformation compared to normal contralateral skin before and after one treatment and after five treatments.



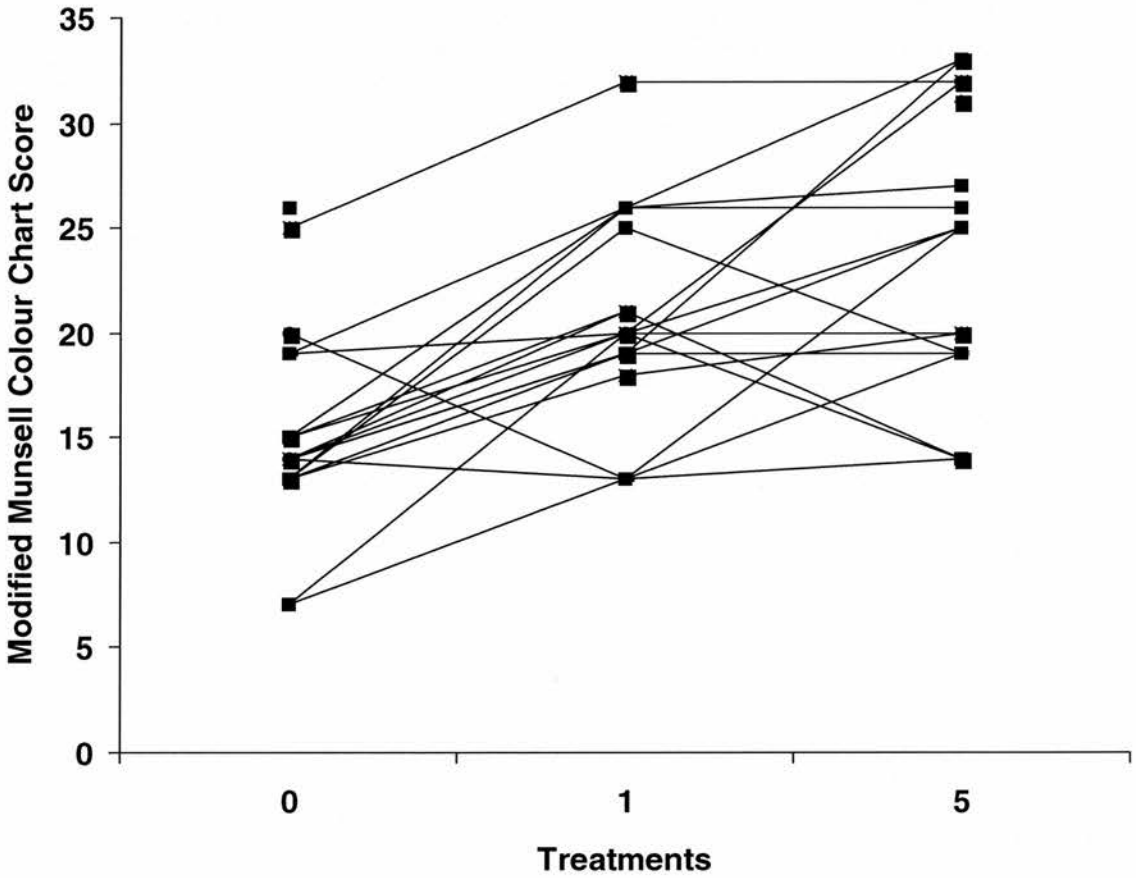
Zero on the X-axis corresponds to normal capillary depth. Positive values are deeper and negative values closer to the skin surface.0= Untreated CM, 1= after one treatment, 5= after five treatments.

Figure 45. The diameter of capillaries within Capillary Vascular Malformations before, after one and after five laser treatments.



0= Untreated CM, 1= after one treatment, 5= after five treatments.

Figure 46. Colour change as evaluated by Munsell Colour Charting before and after one and five laser treatments.



0= Untreated CM, 1= after one treatment, 5= after five treatments. The higher values for the Modified Munsell Colour Chart Score represent paler lesions.

Conclusion

In this study we have attempted to investigate in vivo the change to CM capillary depth and diameter following five Pulsed Dye Laser treatments. In most cases the majority of fading of a CM will occur within the first five treatments, although some longer term treatment regimes may be able to achieve further modest improvement[38].

As previously noted by Fiskerstrand and Hohenleutner through biopsy studies this data supports the hypothesis that it is smaller diameter and deeper capillaries within a CM that remain untreated by Pulsed Dye Laser treatment[40,41]. This study demonstrates this non-invasively and in-vivo by use of the Depth Measurement Videomicroscope.

Recent advances in the development of Pulsed Dye Lasers have allowed longer wavelengths to be generated rather than the traditional 585nm used in this study. It is possible that these longer wavelengths will treat to a deeper depth in a CM despite the less favourable specificity for oxyhaemoglobin[83,84].

This study demonstrates that the vessel diameters that are not being cleared by the laser treatment are between 10 and 50 micrometers. The original work carried out by Anderson and Parrish into the theory of Selective Photothermolysis proposed that to achieve photocoagulation by means of specific thermal damage, and thus limit complications, required a laser with a pulse duration less than the thermal

relaxation time of the target vessels[24]. For cylindrical vessels the theoretical thermal relaxation time of a vessel of known diameter based on a Gaussian temperature distribution is given by the equation[24]:

$$Tr = D^2/16_K$$

Where Tr = Thermal Relaxation time

D = Diameter of CM vessel

$_K$ = Thermal diffusivity of blood ($1.3x10^{-3}$ cm^2 sec^{-1})

Previous work by Dierickx et al using purpura threshold fluence to probe CM vessels found a close correlation between in vivo measurements and those proposed by Anderson and Parrish, based on a Gaussian temperature model[42]. This model predicts maximum heating of the vessel centre following irradiation by a uniform collimated beam and temperature towards the periphery of the vessel falling in a Gaussian curve. Dierickx's study proposes pulse durations of between 1-10 ms for vessels in the range of between 50 and 150 micrometers. In this study we have found that larger vessels of between 50 and 200 micrometers are adequately treated with a 0.45 ms pulse duration laser. It may thus be concluded from this data that longer pulse durations are not required to treat larger diameter CM vessels.

The in vivo determination of thermal relaxation time by Dierickx et al examined CMs using two Pulsed Dye laser pulses, which were both non-purpuric and separated in time[42]. By considering the fluence required of the second pulse at a known time delay required to cause a purpuric reaction and comparing this with the fluence required to produce purpura by a single pulse Dierickx was able to

determine the thermal relaxation of the target vessels. This would suggest an ideal pulse duration of between 1-10 milliseconds to adhere to the principle of Selective Photothermolysis. However, from our study we have found that larger vessels are adequately treated despite using a laser with a relatively short pulse duration. It seems that it is unnecessary to treat these large vessels with a pulse duration equal to their thermal relaxation time and shorter pulse durations would cause sufficient damage to clear the vessel.

A possible theory for the poor treatment of smaller vessels was initially proposed by Anderson and Parrish[24] and has been predicted through Monte Carlo Modelling[30]. This suggests that if the thermal relaxation time of the target vessel is much shorter than the pulse duration of the incident laser then the target may remain untreated due to the greater heat dissipation into the surrounding structures[24]. From our study 13 of the 22 patients examined were found, after five treatments, to have vessels with diameters that (from the above equation), would give a thermal relaxation time less than the 0.45 ms pulse duration of our laser (figure 41).

The use of pulse durations 20 microseconds and less causes destruction to not only the target vessels within a CM, but also causes widespread collateral damage by vaporization and mechanical photodisruption[34,100]. This occurs because the high energy imparted to the chromophore within a short time causes shockwaves to be propagated through the tissue and hence mechanical damage to occur. Therefore to treat smaller diameter capillaries requires a laser with a pulse duration longer than this and less than 450 microseconds as used in this study. Further investigation is

required to examine the effect of using shorter pulse durations once larger vessels have been cleared. Shorter pulse durations in darker Fitzpatrick skin types should be used with caution so as to avoid epidermal damage[101].

Previous work using traditional non-depth measuring videomicroscopy carried out by Motley et al[76] suggested that type of capillary ectasia, either Type 1 superficial or Type 2 deep, found in a particular CM could give prognostic information as to how it would respond to laser treatment. In our study we have found a correlation between type of vessel ectasia and colour of a CM, suggesting that Type 2 lesions were generally paler in colour than Type 1 lesions. This would fit with the previous belief that paler lesions tended to be located deeper in the skin than darker lesions. We also found that, following treatment, initially the majority of CMs had a superficial Type 1 plexus of capillaries whereas by the fifth treatment the majority of lesions now consisted of Type 2 deeper plexus. We found no statistically significant difference in either final colour or degree of colour change between the initially Type 1 CMs and the initially Type 2, using a Wilcoxon Rank test. Therefore, from this study data we found no evidence that initial type of capillary ectasia gives prognostic information as to how a port wine stain will respond to laser treatment.

This study finds no statistically significant correlation between response and either vessel diameter or depth. The initial capillary characteristics of an untreated CM are thus not predictive of eventual outcome. A reason for this may be that other factors play a role. This study gives no information as to flow in the CM vessels. Previous

studies have demonstrated that some CMs have greater flow than the surrounding unaffected skin[45,46]. The combined effect of flow and capillary composition requires further study.

The other aim of this study was to evaluate how the vessel characteristics of CMs altered following Pulsed Dye Laser treatment. This study suggests that the vessels remaining following treatment tend to be located deeper and be smaller in diameter. The use of longer wavelengths may allow deeper vessels to be treated and this effect requires further investigation. The lack of response from smaller vessels would tend to suggest that shorter pulse durations would produce further improvement. The latest Pulse Dye Lasers generate their pulse using a series of very short micropulses, rather than the smooth output of the Pulse Dye Laser we have used[44]. The effect of this change in beam characteristics on smaller diameter capillaries is unknown.

CHAPTER 8

The Effect of Varying Pulse Duration, Wavelength, Spot Size and Fluence on the Response to Pulsed Dye Laser Treatment of Previously Treated Capillary Vascular Malformations.

Introduction

Capillary Vascular Malformations are heterogeneous lesions consisting of abnormal dermal capillaries of differing diameters and depths[6]. The pulse duration of many 585nm pulsed dye lasers is fixed at 0.45 ms. Previous studies have suggested that this pulse duration is not adequate to treat many of the vessels within the lesion[34,42,44,82].

More recently, third generation pulsed dye lasers have become available. Two of these, the Scleroplus (Candela Corp. Wayland, MA, USA) and V-Beam Lasers (Candela Corp. Wayland MA, USA) have the ability to alter the wavelength (Scleroplus) and pulse duration (V-Beam), they produce. Both Lasers also use a Cryogen Spray Cooling (Dynamic Cooling Device, DCD, Candela Corp. Wayland, MA, USA) device to cool the skin and allow increased fluences to be used whilst providing epidermal protection[50]. The Scleroplus laser allows the wavelength of the laser to be altered from 585nm to 600nm in 5nm increments. This laser produces higher fluences than the previous 585 nm 0.45 ms Pulsed Dye Lasers to compensate for the reduction in specificity for oxyhaemoglobin at these higher wavelengths.

The pulse duration of the Scleroplus laser is fixed at 1.5 ms. The V-Beam laser, however, allows the pulse duration of the laser to be altered from 1.5 ms to 40 ms with a fixed wavelength of 595 nm.

It has been suggested that these lasers would allow greater fading of CMs that have become resistant to 585nm Pulsed Dye Laser[50,83,84,102].

Aim

The aim of this study was to treat previously resistant patients with these newer lasers and to investigate the influence of changing the spotsize, pulse duration, wavelength and fluence on the capillary characteristics and color of these lesions. Also, by examining the capillary dimensions of resistant CMs, it was hoped that predictive information could be established as to whether a lesion would respond to further laser treatment and what would be the most appropriate settings to use.

Method

Twenty-five patients were randomly selected from a database of 250 resistant CMs, previously treated with a 585nm 0.45 ms Pulse Dye laser (SLS Chromos, Wales UK). All patients on this database had received a minimum of five pulsed dye laser treatments. They had been assessed with the use of standardized photography and

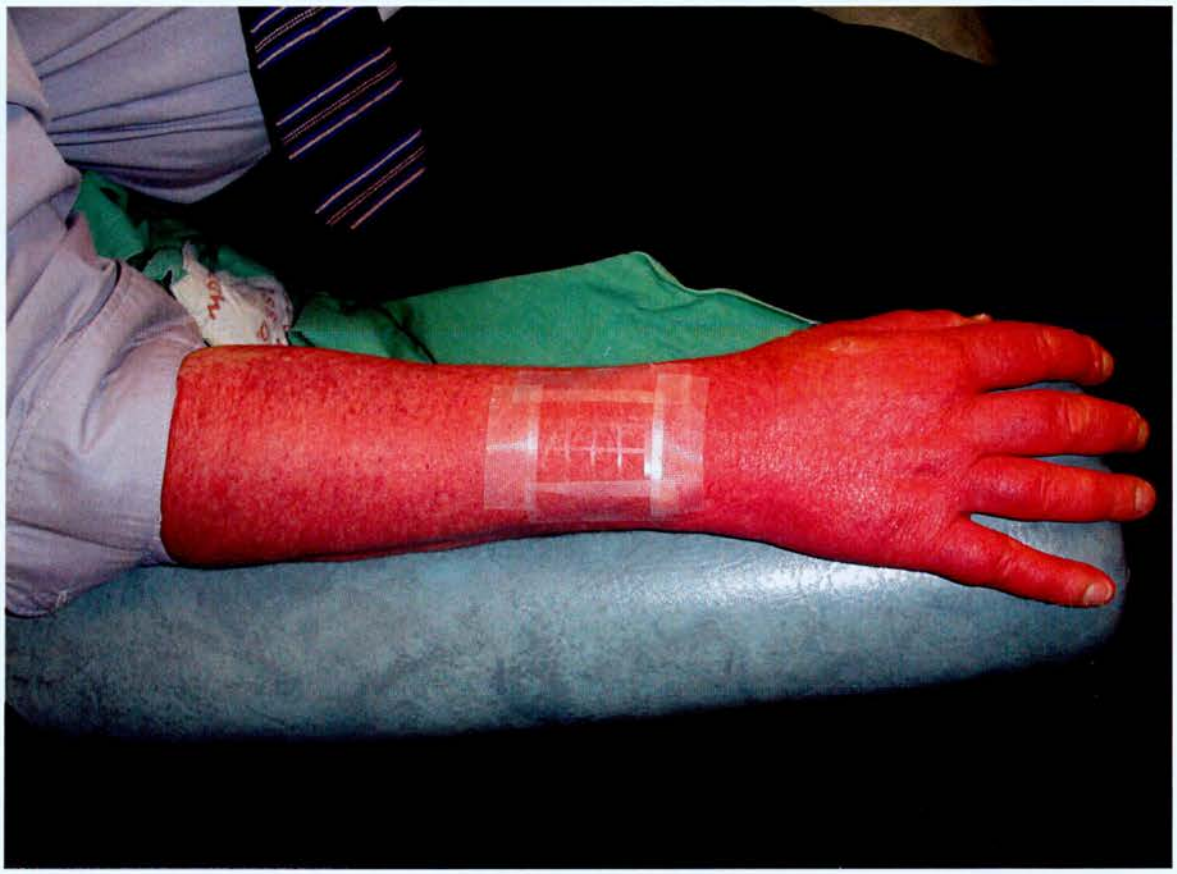
determined to be no longer achieving improvement. The only selection criterion was that their CM was large enough to allow a 3 X 4 cm grid to be placed on an area of uniformly coloured malformation.

Each patient was rested within a temperature-controlled room at 28^0C for twenty minutes to reduce error from sympathetic stimulation and maximally vasodilate the capillaries. A location was selected on the patients CM that would allow a 3 x 4 cm grid to be taped onto the skin (figure 47).

To record the vessel characteristics of diameter and depth we used the technique of Depth Measurement Videomicroscopy[103]. This technique uses Cy-scope lens (PWAllen, Tewkesbury, UK), to measure depth to the superficial vessels within a CM and their diameter.

Three recordings were taken from the normal contralateral side of the patient and then five recordings from the area to be covered with the grid. The grid was then taped on to the patient and a digital photograph of the area taken. This photograph was taken to aid the replacement of the grid in the same place when the patient returned for assessment.

Figure 47 Illustrating the laser test patch template in situ.



The treatment was carried out by test patching the grid with the parameters outlined in Table 48. These parameters were chosen so that only one variable (spotsize, wavelength, fluence or pulse duration) was altered between two test patches. For the Scleroplus laser, three of the test patches with the 7mm spot were carried out with the same fluence (12 j/sq cm) and pulse duration (1.5 ms) and only wavelength altered (585 nm, 590 nm, 595 nm). These were undertaken at 12 j/sqcm, as a higher fluence at a 585 nm wavelength was deemed potentially unsafe. The test patches at 585 and 590 were then carried out with a 5mm spot, but with the same fluence (12 j/sq cm) so to give a comparison between a 5mm and 7mm spotsize. For 595 nm and 600 nm with the 5mm spot we used the maximum fluence achievable with the Scleroplus Laser (14 j/sq cm and 20 j/sq cm respectively), as longer wavelengths will require higher fluences to be effective due to the lack of specificity for oxyhaemoglobin. Lastly, a test patch was performed with the ScleroPlus at 595nm, 14 j/sqcm and a 7mm spot so as to give a comparison with the V-Beam laser using exactly the same settings, but with a different output profile[44]. The V-Beam laser relies on a series of micropulses rather than the smooth output seen with the Scleroplus and earlier laser systems.

For the V-Beam treatments the parameters of fluence and spotsize were kept constant and only pulse duration changed. The maximum pulse duration chosen was 10 ms as from previous studies a longer pulse duration was unlikely to be suitable[42].

With both laser systems the DCD cryogen spray cooling systems were kept at 30 ms spray duration and 30 ms delay for all the treatments[104,105].

The patients returned for assessment 3 months later and were again rested in a temperature-controlled room at 28^0C and the grid replaced with the aid of the digital photograph. A blinded impartial observer using a standardized outcome scale then assessed the percentage improvement in color in each of the test patch sites. The assessment scale rated the improvement in color of the CM subjectively on a scale of: > 75% as excellent, 50-75% as good, 25-50% as moderate, < and 25% as poor and 0 as no improvement. A DMV examination was then performed at each site to determine any changes to the vessel structure following treatment.

Table 48. Parameters used for Test Patches

Laser	Wavelength (nm)	Fluence (j/sqcm)	Spotsize (mm)	Pulse Duration (ms)
ScleroPlus	600	20	5	1.5
	595	14	5	1.5
	590	12	5	1.5
	585	12	5	1.5
	585	12	7	1.5
	590	12	7	1.5
	595	12	7	1.5
	595	14	7	1.5
V-Beam	595	14	7	1.5
	595	14	7	3
	595	14	7	6
	595	14	7	10

Data analysis

Two analyses of variance (ANOVAs) were carried out, of depth measurements and of the natural logarithm of diameter measurements, respectively[106]. A logarithmic transform of capillary diameter was used in order to achieve approximate homogeneity of variance. Patients were treated as a "blocking factor" in the analyses, and the treatment factor had 13 levels, which included a level for "pre-treatment measurement" (Figures 49 and 50). Each ANOVA table yielded two F-tests: first, a test of the overall effect of laser treatment averaged over all 12 treatments, and secondly, a test of the null hypothesis of no difference between the effects of these 12 treatments. Regardless of the outcome of the second of these hypothesis tests, various follow-up tests were also carried out, comprising two F-tests of the effects of differing pulse durations for the V-beam laser, and the effects of differing wavelengths for the Scleroplus laser (at fluence 12 j/sq cm and spotsize 5mm) respectively, and various *t*-tests of comparisons of interest. Note that all follow-up tests were formulated in advance of data collection. For F-tests, the relevant sums of squares were obtained from the full ANOVA sum of squares for treatments, partitioned appropriately, and for t-tests, the residual mean square from the ANOVA was used as an estimate of error variance.

Differences between the 12 treatments in the percentage improvement in color were tested for statistical significance using Friedman's test[106].

Figure 49. Capillary depth measurements following test patches for all patients and three groups based on initial capillary depth.

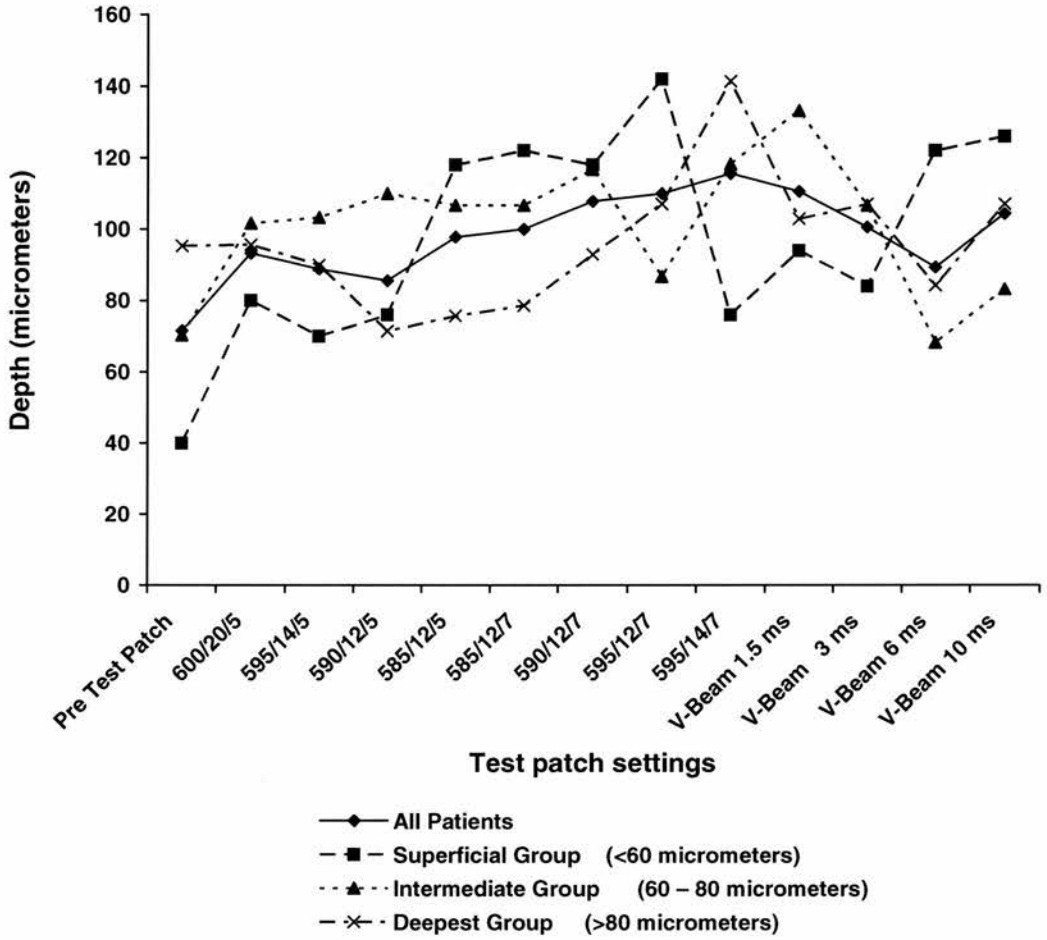
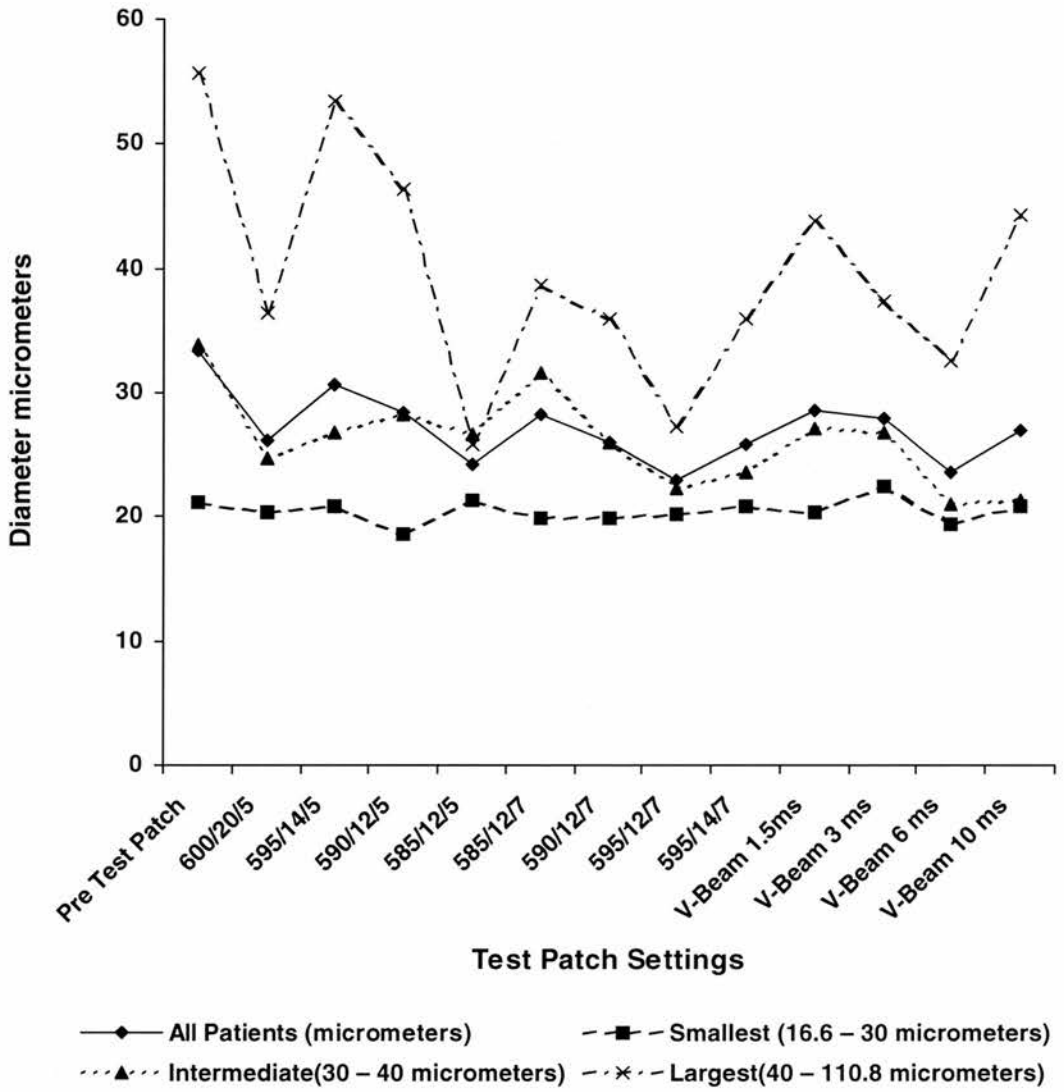


Figure 50. Capillary diameter measurements following test patches for all patients and three groups based on initial capillary diameter.



Results

Five patients failed to attend for their review, one patient's CM was insufficiently large to allow the placement of the grid on a uniform area of malformation and one patient had received previous treatment with a Copper Vapor laser and so are excluded from the study. The remaining 18 patients attended for review. These patients had received a mean of 18.8 previous laser treatments with a 585nm 0.45ms Pulsed Dye Laser (range 5 – 30). The mean time since last treatment was 22 months (range 4-54 months, sd 13.9). All patients were being seen on a yearly review basis with photographic assessment.

The Effect of Treatment on Color of the Capillary Vascular Malformation

When the effect of treatment on the color of the CMs was analyzed there was found to be a great variation in the response. Only 8% of the test patches undertaken showed more than 75% improvement in the color of the CM, and only 17% demonstrated a greater than 50% improvement, whereas 61% showed either no improvement or less than 25%. None of the test patch settings managed a median improvement of greater than 50% improvement. Using Friedman's Test, there was found to be no statistical difference in the subjective assessment of improvement between the different Test Patch settings overall. However, eight patients (44%) had greater than 75% clearance of their malformation in at least one of the test patches, and 10 (55%) had more than 50% improvement. Therefore, although no one setting was superior to the others statistically in lightening the color of the CMs, more than

half the patients received a significant amount of lightening in at least one test patch area. There was little difference in the ability of the Scleroplus or V-Beam lasers in eliciting a significant improvement in a test patch site (8/18 and 9/18 patients receiving over 50% clearance, respectively).

The initial capillary depths, diameters and the best response achieved by both laser systems are shown in Table 51. As can be seen, those CMs that did respond to laser treatment, tended to respond to both laser systems (Spearman's rank correlation coefficient 0.614, $0.002 < P < 0.01$). No correlation can be found between initial capillary measurements and eventual outcome in terms of color change, although CMs with small, deeply located vessels tended to improve least following treatment.

The Effect of Treatment on Capillary Depth and Diameter

When depth of the capillaries within the CM was examined using the DMV and analyzed using an Analysis of Variance (ANOVA), there was found to be a statistically significant increase in the vessel depths following treatment ($P= 0.005$, see Figure 49). The effect of laser treatment, averaged over the chosen parameter settings and the two laser systems, was to increase mean capillary depth from 71.6 μm to 100.3 μm. However, when the test patch settings were considered individually there was no statistically significant difference between them (F test $P= 0.477$).

For vessel diameter, there was a statistically significant effect of treatment overall (Figure 50, F-test P= 0.008). Geometric mean capillary diameter decreased from 33.3 μm to 26.5 μm. However, again there was no statistically significant difference found between the different test patch settings (F test P = 0.37).

The Effect of Altering Pulse Duration

Changing the pulse duration of the V-Beam laser demonstrated that the 1.5 ms pulse duration tended to have the most effect on vessel depth (see Figure 49). However, an F-test of differences between the four mean depths was not statistically significant (P= 0.485).

The effect of changing the pulse duration had no statistically significant effect on the capillary diameters overall (F-Test P= 0.36).

The Effect of Altering Spot Size

When the effect of increasing the spotsize from 5mm to 7mm is compared across all the patients (fluence 14 j/sq cm, wavelength 595 nm), the 7mm spotsize tended to have more of an effect on vessel depth than the 5mm spotsize and this approaches statistical significance (*t*-test P= 0.056).

There was no statistically significant effect found from altering the spot size on the vessel diameter (t-test P= 0.15).

The Effect of Altering Wavelength

For the Scleroplus laser, the effect of changing the wavelength from 585 through 590 to 595 nm (fluence = 12 j/sq cm, spotsize 7mm) on the depth of the capillaries was not significant, with no particular wavelength statistically better than any of the others overall (F-test P = 0.75, t-test for trend P = 0.47).

For the Scleroplus laser, there was no evidence that the effect upon capillary depth of varying the wavelength from 585 nm to 590 nm depended on whether the spotsize was 5 mm or 7 mm (recall that the fluence setting for the four patches concerned was 12 j/sq cm). That is, in statistical terminology, there was no evidence of interaction between wavelength and spotsize. In view of this result, *t*-tests were carried out of the effect of wavelength averaged over the two spotsizes, and of spotsize averaged over the two wavelengths. In both these tests, neither the effect of wavelength, nor of spotsize, was found to be statistically significant.

Changing the wavelength from 585 through 590 to 595 nm (fluence = 12 j/sq cm, spotsize 7mm) appeared to reduce the diameters of the capillaries, and the trend, though strictly not statistically significant, was suggestive of a possible effect (t-test for trend P = 0.076).

No significant interaction was found between wavelength (585 and 590 nm) and spotsize (5 and 7 mm) in respect of their effects upon diameter, at a fluence setting of 12 j/sq cm (t-test P = 0.14). Further, the effects upon diameter of wavelength

averaged over the two spotsizes, and of spotsize averaged over the two wavelengths, were not significant (t-test P = 0.64, 0.66 respectively).

The Effect of Increasing Fluence

For increasing the fluence of the Scleroplus laser from 12 to 14 j/sq cm (wavelength 595 nm, spotsize 7 mm) there was no statistically significant effect seen, although the mean depths increased slightly following treatment with higher fluences (figure 49).

There was no statistically significant effect found of increasing the fluence upon the vessel diameters (t-test P=0.30).

Comparison of Two Laser Systems.

When the two lasers were used on the same settings (595nm, 14 j/sqcm, spotsize 7 mm, 1.5 ms pulse duration) there was no statistically significant difference found between the results obtained either on vessel depth (t-test P= 0.72), or on vessel diameter (t-test P= 0.41).

The Effect of Vessel Morphology on Outcome

A. Vessel Depth

The second part of this study was to examine if the ideal laser parameters for a particular CM could be selected from the vessel characteristics measured using the DMV. For the analysis of capillary depth the patients were grouped into three similar sized groups on the basis of initial capillary depth (Figure 49). Thus, the three groups consisted of a "superficial" group of five patients with vessels less than 60 micrometers from the skin surface, an "intermediate" group of six patients with vessels between 60 – 80 micrometers and a "deep" group of seven patients with vessels deeper than 80 micrometers.

Capillary Vascular Malformations with Superficial Vessels

From Figure 49, it can be seen that for the superficial group all the test patch sites showed an increase in vessel depth following treatment and this is statistically significant (F-test P=0.005). However, differences between treated patches were not statistically significant (F-test P = 0.162). For these superficial vessels there was no statistically significant difference found between using a 7 mm spotsize and a 5mm spotsize, with the Scleroplus laser (wavelength 595 nm, fluence 14 j/sq cm). For the V-Beam laser the longer pulse durations tended to increase capillary depth more than the shorter pulse durations for this group of patients, though this was not statistically significant (P = 0.13). Increasing the fluence from 12 j/sqcm to 14

j/sqcm, at a wavelength 595 nm and a spotsize of 7 mm, had a statistically significant effect on the results achieved in this group (P = 0.025).

Capillary Vascular Malformations with Intermediate Depth Vessels

In the intermediate depth group there was again a significant increase in vessel depth with treatment (F-test P=0.033). However, as for the superficial group, an F-test showed no significant differences between treated test patches. This result is consistent with figure 49, in which there appears to be little difference between the results obtained for any of the Scleroplus test patch settings, although 595nm 14 j/sqcm 7mm spot had the greatest effect on vessel depth. Again, for this wavelength and fluence, there was no statistically significant difference found between the 5mm and 7mm spot sizes. When the V-Beam test patches are examined, the shorter pulse durations tended to produce more of an effect than the longer pulse durations and this is a statistically significant trend (*t*-test for trend, P=0.013).

Capillary Vascular Malformations with Deep Vessels

For the "deep" group of patients, whose vessel depths ranged from 80 – 123.3 micrometers, there was found to be no statistically significant effect of treatment overall (F-test P = 0.960). However, an F-test of differences between treated patches approached the conventional level of significance (P = 0.084). At fluence 12j/sq cm, and wavelengths of 585 and 590 nm, the 7mm spotsize treatments appeared to increase vessel depth more than the 5mm spotsize, but t-tests showed no significant effects. However, comparison between spotsizes 5 and 7 mm for

wavelength 595 nm and fluence 14 j/sq cm, gave a statistically significant result (t-test, P=0.014), and it is notable that of all the settings, 595nm 14 j/sqcm 7mm appeared to have the most effect on vessel depth. Varying the pulse duration with the V-Beam laser appeared to have little effect on these deeper vessels.

B. Vessel Diameter

Patients were also grouped according to their capillary diameters, as measured using DMV. Three groups were defined: a small diameter group of seven patients with diameters in the range 16.6 – 30 micrometers, an intermediate group of five patients with diameters between 30 – 40 micrometers and a large diameter group of six patients with diameters in the range 40 – 110.8 micrometers. Figure 50 shows the effect on initial mean capillary diameter of the different test patch settings for the three groups.

Capillary Vascular Malformations with Large Diameter Vessels

For the large diameter vessel group there was a statistically significant effect of treatment over all the test patches (F-test P= 0.05). However there were no statistically significant differences between the means for each of the different test patch settings (F-test P = 0.32).

Capillary Vascular Malformations with Intermediate Diameter Vessels

When the intermediate group is examined, the effect of treatment approaches significance at the conventional level of 5% (F-test P= 0.07), but again, differences between test patches are not significant (F-test P = 0.80). When figure 50 is examined it can be seen that for this intermediate group, there is little difference between the results obtained with the Scleroplus Laser test patch settings. The longer pulse durations of the V-Beam Laser tended to be most successful at reducing vessel diameter, but a test for trend was not statistically significant (*t*-test P = 0.18).

Capillary Vascular Malformations with Small Diameter Vessels

When the group with the smallest diameters is examined, there is no longer any statistically significant effect of treatment (F-test P=0.62), and there is no evidence that any of the test patch settings reduced capillary diameter more than the others (F-test P = 0.96). There appeared to be no statistically significant difference in the results obtained by changing the pulse duration (t-test P= 0.60), the wavelength (t-test P= 0.35), the spot size (t-test P= 0.98), or the fluence (t-test P= 0.77).

Table 51. Initial Capillary Measurements and Best Response to Each Laser System.

Patient	Initial Depth (micrometers)	Initial Diameter (micrometers)	Best Response	
			ScleroPlus	V-Beam
1	73.3	15.6	<25	<25
2	42	19.3	25-50	0
3	66	19.3	>75	50-75
4	66	19.3	>75	>75
5	123.3	23.3	0	0
6	50	25.3	<25	0
7	92	29.3	25-50	25-50
8	53.3	30	25-50	25-50
9	96.7	34.4	50-75	50-75
10	16	35	>75	<25
11	38	35.3	>75	50-75
12	80	35.3	>75	>75
13	80	41.1	>75	50-75
14	74	45.3	>75	>75
15	86	48.7	<25	>75
16	66	49.3	<25	0
17	76.7	60	25-50	50-75
18	110	110.8	25-50	25-50
Mean (SD) / Median	71.63 (26.09)	37.59 (21.93)	25-50	Between 25-50 and 50-75

Discussion

The recent advent of Pulsed Dye Lasers with variable pulse durations and wavelengths has provided hope that further fading of 585nm 0.45ms PDL resistant Capillary Vascular Malformations may be possible. Previous studies have demonstrated that the use of longer wavelength lasers may allow deeper ectatic capillaries to be treated[83,84]. The use of longer wavelengths, however, necessitates the use of higher fluences as the selectivity of the laser to the absorption spectrum of oxyhaemoglobin is reduced. This increased fluence requires adequate epidermal protection through more efficient methods of cooling the skin. The lasers used in this study both use Cryogen Spray Cooling devices to provide epidermal cooling. Previous studies by Chang et al and Edstrom et al have established the role of longer wavelength Pulsed Dye Lasers in the treatment of some patients[83,84]. Both these studies, however find that for the majority of patients undergoing early treatment of their CM, 585 nm is the wavelength of choice. Previous biopsy studies[40,41] and non-invasive assessment within our unit[103] have established that following laser treatment the deeper and smaller capillaries within a CM tend to be left untreated. Kimel et al's study examining the effect of varying wavelengths produced by a Scleroplus laser on chick chorioallantoic membrane has found that smaller calibre vessels tend to be better treated by shorter wavelengths[85]. However, in previously treated CMs it is likely that the longer wavelengths may be required to allow for greater tissue penetration.

In this study we have sought to investigate the effect of a variable wavelength and variable pulse duration laser on previously treated CMs. In terms of response to these laser systems, both have been found to be effective in the treatment of these resistant patients. All these patients had previously been treated with a 585nm 0.45 ms PDL (SLS CHROMOS, Wales, UK), up to fluences of 7.7 j/sqcm with a 7mm spotsize and topical ice epidermal cooling. These newer laser systems allow much higher fluences to be used as their Cryogen Spray Cooling systems are much more efficient at ensuring epidermal protection[107]. The majority of patients within this study have responded favourably to some test patches. It is possible that the increase in fluence alone could account for this. This may also explain why little difference in efficacy was seen between the two lasers.

It was hoped that by examining initial capillary characteristics of depth and diameter using the DMV that proposed ideal treatment parameters might have been indicated. From our results it can be seen that patients whose CMs contain superficial large diameter capillaries are likely to receive the greatest further fading of their lesions with further treatment. Only patients with deep and small diameter vessels are unlikely to receive further fading. However, it is not possible from this data to predict the optimum treatment parameters for an individual patient. This may be for a number of reasons. This study has a relatively small sample size and relies on test patch information only. It is possible that prolonged treatment of a larger group of patients may have provided more predictive information. Also, other factors may play a role in selecting ideal treatment parameters. All these patients were Fitzpatrick Skin Type 1 or 2, so it is unlikely that melanin content is a

confounding variable within the study. This study takes no account of capillary perfusion and previous studies have suggested that this may be an important consideration when choosing treatment parameters[45,46].

This study found no statistically significant difference in the results obtained by both laser systems at the same parameters (595nm 14 j/sqcm 1.5 ms). This comparison was carried out to investigate whether there was any demonstrable difference in the results obtained as both these lasers use differing methods of producing their laser pulse[44]. For the Scleroplus (and the SLS Chromos 585nm laser used previously) a smooth energy output is achieved during the pulse, whereas the V-Beam uses a series of micropulses. No practical difference was found in the results obtained, however.

This study used the DCD cryogen spray cooling devices on a uniform setting, 30ms spray duration and 30ms delay, across all the testpatches. Recent studies have suggested that these settings should be altered to match the capillary characteristics of the CM to be treated[68,104,108]. Therefore, for deeper vessels where a higher fluence and possibly a longer wavelength is to be used it would be appropriate to increase the cooling system to provide more cooling to the epidermis without risking cooling the abnormal vessels excessively. The role of epidermal cooling and vessel depth requires more clinical study.

Our previous studies have indicated that as treatment progresses the capillaries within a CM tend towards being small and deeply located as the larger and more

superficial vessels are cleared[103]. This study also demonstrates this, as patients with more superficial and larger vessels tended to have more of a change in these characteristics following treatment than patients with deeper and smaller vessels. It is noticeable in this study that there was a wide variation in the initial capillary depths and diameters measured. This may be due to a number of factors. It is likely that response to treatment is a multifactorial phenomenon, with capillary depth, capillary diameter, capillary flow rates and epidermal melanin concentration all interacting to influence non-response to treatment. Also, a number of these patients had not been treated for over three years. As it is well established that CM vessels continue to evolve with age, and even completely treated lesions may recur, it is possible that more superficial and larger vessels had developed in these malformations since their last treatment[90,91].

A number of other studies have also examined the effect of varying laser wavelength and pulse duration on the response to treatment of CMs. Greve et al examined 15 patients previously untreated patients and varied both wavelength and pulse duration[109]. They found 585 nm to be the ideal wavelength to treat previously untreated patients. Scherer et al examined the response of 62 previously untreated patients and also found 585 nm to be the most effective wavelength[110]. Both these studies examine the treatment of new patients. Our previous work[111] demonstrated that the depth of vessels within CMs undergoing treatment tends to increase and their diameter reduce. It seems likely that 585 nm with its higher specificity for oxyhaemoglobin is an effective wavelength for the initial treatment of CMs but as the ectatic capillaries become deeper and smaller longer wavelengths may become

more important. Laube et al examined the treatment of 15 resistant CMs using the V-Beam laser and found further lightening in 67% of patients, and similar percentage to this study (55%)[89]. Interestingly, they found the settings 595nm 14j/sgcm 1.5ms pulse duration to be the most effective when treating resistant CMs, as was also the case within this study. Bjerring et al examined the treatment of resistant CMs with an Intense Pulsed Light (IPL) system and demonstrated improvement in just under half the patients[112]. However, this study gives little information as to the nature of the previous pulsed dye laser treatment the patients had undergone, nor how resistance to laser treatment was defined.

In general our results suggest that patients whose CM vessels are not too deep and not too small, as assessed by DMV, can expect a good response from treatment with these newer laser systems. This study demonstrates trends that conform to the commonly accepted belief that increasing the fluence, spotsize and using longer wavelengths improves the results when treating resistant CMs. For example 595 nm wavelength, 7mm spotsize and 14 j/sqcm fluence appeared to be the most effective parameters when treating the malformations with the deepest vessels. It must be borne in mind though that the majority of test patches within this study produced less than 25% improvement in the color of the CM. A previous study by Woo et al showed only 5 out of 22 patients with resistant CMs improved following treatment with a V-Beam laser[113]. Their study, however, used only two different treatment settings and it is possible that these settings were not well matched to the majority of the CMs. This illustrates the need for a technique, such as Depth Measurement Videomicroscopy, that would allow those patients most likely to respond to

treatment (i.e. those with large and superficial vessels) to be identified and thus reduce the likelihood of excessive unnecessary treatments. This study emphasizes that patients who's CM may have become resistant to PDL may derive further improvement with laser treatment a few years later.

CHAPTER 9

Summary/Further Developments

The aim of this thesis was to further develop videomicroscopy as a tool for assessing capillary vascular malformations. The assessment of Capillary Vascular Malformations (CMs) is an evolving area as different techniques are developed in the hope of guiding laser treatment. The use of standardised digital photography and image analysis has allowed the colour of CMs to be recorded rapidly and should allow assessment within Laser Clinics. This is an advantage in comparison with regular photography where subjective interpretation is still required. Although not as accurate as reflectance spectrophotometry, digital photography has the advantage that it can be performed on all patients and not just those within a trial. Colour recording, however, gives no information regarding the factors peculiar to a CM that define its response to laser treatment. These factors of capillary structure, blood flow and melanin content demand other methods of assessment.

Throughout this thesis the standardised questionnaire, the Combined Skin Type Test, has been used to both determine Fitzpatrick Skin type and thus melanin content, and also to give information regarding recent sun exposure. This is important as recent sun exposure can change the subjective impression of Fitzpatrick skin type and may increase the likelihood of complications from treatment. The assessment of blood flow within these lesions has not been carried

out and little is known about the contribution of perfusion to the response of CMs to laser treatment.

Videomicroscopy has been used within this thesis as a non-invasive, rapid and relatively low cost method of assessing CMs. The advantage of this technique over other methods of assessing CM vessel structure is that it can be used relatively easily within a clinic setting. Tools such as ultrasound, thermography and photoacoustic probing can give a measurement of depth from the skin surface down to the superficial vessels within a CM but do not give information regarding individual vessel diameters. In this regard they may be of use in deciding on a typical wavelength to use for treatment or for setting Cryogen Spray Cooling durations but they do not give any information as to the ideal pulse durations required. The Depth Measurement Videomicroscope, however, provides information regarding both depth to the superficial ectatic vessels and also diameters of these vessels. Optical coherence tomography and Optical Coherence Doppler Tomography also allow the delineation of capillary depth and diameter within a CM, but rely on expensive equipment and are restricted to use within clinical trials. There may also be some doubt as to whether the resolution of this device is small enough to measure the smallest CM vessels.

With the development of the Depth Measurement Videomicroscope (DMV) we found that the improved sapphire glass lens at the tip of the handpiece, that was much flatter than a traditional videomicroscope lens, resulted in much less

reflection from the target tissue. Thus, we no longer required the use of colour filtering to improve the images we were seeing.

The DMV has been compared to histological measurements and the results obtained found to be comparable. These are entirely different measurement techniques and some discrepancy is expected. None of the other methods of non-invasive determination of vessel structure have been compared to biopsy measurement so comparisons are not possible.

The DMV has been used, within this thesis to probe capillary structure within CMs in different locations. Our results challenge the evaluation by Eubanks et al that differences in CM response to treatment in different locations may be due to vessel pattern. We found no correlation between vessel structure and site.

When the reaction of CM vessels to laser treatment was assessed using the DMV there was an increase found in the depth of vessels within the lesion. Also following laser treatment the larger vessels were cleared leaving smaller calibre vessels untreated. This was also true after five laser treatments and in comparison with a cohort of resistant patients within chapter 6. It appears that following laser treatment deeper and smaller vessels are being left untreated. This may be because the pulse duration of the laser we have used is not sufficient short to target these vessels or it may be that deeper within CM's the vessels tend to be less ectatic. From the biopsy studies carried out in chapter 4 it can be seen that large diameter ectatic capillaries are present throughout the dermis. Thus it seems likely that it is

the smaller vessels that are not being cleared. Another possible hypothesis to explain why smaller vessels remain following laser treatment is that the treatment itself causes new vessel formation due to the release of angiogenic factors following coagulative necrosis. This seems unlikely, though, as a minority of CMs do fade completely following laser treatment. The phenomenon of larger vessels being cleared and smaller ones remaining has not been demonstrated non-invasively before although it has been suggested from serial biopsy studies.

The DMV provides a tool to give information regarding the characteristics of the superficial vessels within a CM. Therefore it cannot be used as a tool to give prognostic information to previously untreated patients as the eventual degree of lightening they can expect cannot be predicted. It can, however, give some clues as to whether a CM is likely to respond to continued treatment. For example a patient who has had a number of laser treatments and, on DMV assessment, is shown to have relatively deep vessels less than 50 micrometers in diameter, is unlikely to respond to further treatment.

As larger diameter vessels are cleared by laser treatment, the suggestion in some previous studies that longer pulse durations are required to clear resistant large vessels appears incorrect. It may be possible to achieve further improvement with shorter pulse durations. No lasers available at present have a pulse duration shorter than 0.45 milliseconds. In chapter... the cohort of previously treated patients demonstrated a variety of vessel diameters. This is most likely due to further ectasia developing in CM that had not been treated for a number of years. When the cohort

with the smallest vessel diameters is examined in isolation it is found that they have vessel patterns similar to the patients studied in chapter 7 following their five laser treatments. These patients had little improvement in their CMs from treatment at longer pulse durations.

The lack of correlation between vessel characteristics and improvement following laser treatment throughout this thesis demonstrates that vessel characteristics alone cannot account for the response of a CM to treatment. It seems likely that the flow through the capillaries is another factor that contributes to response. From observations carried out whilst using the DMV is can be seen that the passage of red blood cells through small vessels is much quicker than through the larger calibre vessels. The larger vessels appear to suffer from clumping of red blood cells. This may partly explain why larger vessels tend to respond better as there is less convection of heat away from the target vessel due to blood flow. It seems likely that the reaction of a CM to laser treatment is due to the interaction of vessel characteristics and blood flow. Another important factor may be the density of vessels within the malformation. Malformations with more densely packed vessels may respond more poorly to laser treatment as there is more scattering of the incident laser.

The DMV may be further improved through computerisation. Currently the measurement of diameter requires that a videomicrograph of the screen image be printed via a colour video copy processor. Diameter measurements are then obtained by overlaying a scale and recording the values by hand. The input of the

signal through a video card into a computer allows the image to be frozen and then measurements obtained by placing two points on the screen. So far there has been an unacceptable loss in definition once the image has been seen on a computer screen, although this should improve with further development. Also by using image analysis software it should be possible to record the area on the screen occupied by the capillaries and thus give a value for the density of vessels within the CM.

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Chapter 11

Appendix - Publications

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DEVELOPMENTS IN THE VIDEOMICROSCOPIC ASSESSMENT OF CAPILLARY VASCULAR MALFORMATIONS.

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ABSTRACT

The use of pulsed dye laser for the treatment of Capillary Vascular Malformations (CVMs) only leads to complete clearing of the abnormality in a minority of cases. In order to examine the characteristics of a CVM, which determine its response to laser treatment, we have used videomicroscopy. By using a videomicroscope to determine the vessels diameters within a CVM in-vivo it is hoped that treatment parameters can be chosen which would be most appropriate to achieve the best response. To increase the clarity of our images and reduce reflection we carried out a videomicrographic examination using both normal white light and green filtered light on 24 sites on 18 patients. For the deeply placed reticular vessels we found no difference between the results obtained with the two techniques. However, for the superficial papillary vessels we found a statistically significant ($p < 0.01$) increase in the recordings taken with the green filtered lens. We attribute this difference to the reduction in reflection seen with the green filter. We have also developed a depth measuring videomicroscope, which allows the calculation of vessel depth as well as vessel diameter.

Key words: capillary vascular malformations, videomicroscopy, videomicrographic examination,papillary vessels

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1. INTRODUCTION

Capillary vascular malformations (CVMs) or port wine stains are congenital vascular malformations, which tend to be present at birth and grow commensurately with the child. They consist of abnormally sized dermal capillaries[1] and have an incidence of 1 – 3 per thousand births[2]. They cause considerable psychological distress[3] and can be treated with pulsed dye laser treatment[4,5].

The pulsed dye laser was designed on the basis of the theory of Selective Photothermolysis[6], matching wavelength to the selective absorption spectrum of oxyhaemoglobin (the chromophore). Early studies showed that, although the CVM's were improved in terms of fading of colour, the response in most cases was not complete[7]. After a number of treatments with the pulsed dye laser the patients would stop improving and become resistant. This leads to uncertainty for the patient about to embark on treatment and frequently leads to excessive treatments in order to achieve a degree of lightening which is not possible.

A number of techniques have been used to study the response of CVMs to laser treatment and to try and predict this response at the outset of treatment. These include photography, laser Doppler imaging, colorimetry and infrared tomography[8].

Videomicroscopy allows the dermal capillaries within a CVM to be imaged. From this image it is possible to determine the dominant capillary plexus within the CVM[9]. Type 1 CVMs contain a predominance of superficial papillary vessels and are predicted to respond better to laser treatment than type 2 which contain a predominance of deeper reticular vessels. Videomicroscopy also allows the measurement of vessel diameter to be calculated in-vivo.

One problem, which was addressed early on in the use of videomicroscopy, was that of excessive reflection from the skin surface obscuring the dermal capillaries. By moisturising the skin with oil this reflection is reduced. One disadvantage with this technique is that the oil itself can obscure the skin by picking up sloughed keratinocytes and dirt.

Proceedings of SPIE Vol. 5287 Laser Florence 2002: A Window on the Laser Medicine World, edited by Leonardo Longo, Alfons G. Hofstetter, Mihail-Lucien Pascu, Wilhelm R. A. Waidelich (SPIE, Bellingham, WA, 2003) · 1605-7422/03/$15.00

In an effort to reduce the reflection seen with videomicroscopy of the skin we used a green coloured lens (wavelength 530 nm) to filter light incident on the skin. Previous studies using colour filtering have demonstrated that the contrast between shades of red and yellow, as most commonly seen in videomicrographs of skin, can be increased without reducing acuity[10,11].

AIMS: To demonstrate whether the use of green light reduces reflection from the skin surface.

2. METHODS

It is routine practise to record all patients with capillary vascular malformations attending Canniesburn Laser suite using both photographs and videomicroscopy both prior to treatment and at regular intervals during treatment to monitor response. During the period September 2001 to November 2001 all patients attending for videomicroscopy were invited to enter the study. These patients all had capillary vascular malformations and had received dye laser treatment within Canniesburn Laser Suite. Each patient was examined with a videomicroscope (PW Allen Compact Video Microscope) using a 200x contact lens. The examination was carried out both using a green filtered lens and also no filter. The examinations were carried out in a temperature-controlled room at a temperature of 27 C with the patients sat in the room for 20 minutes to acclimatise. The recordings were then taken using either the green filter or no filter first, selected at random. The images were then captured to film using a Mitsubishi colour video printer.

From these images it was possible to determine the vessel pattern type, either type 1 or type 2. The diameter of the vessels was then calculated using an image taken of a 1 mm stage micrometer graticule (Graticules Ltd, Tunbridge, Kent) using the 200 x videomicroscope lens. Three measurements were taken for vessel diameter per image for both the reticular and papillary vessels for each videomicroscope image and the means recorded.

3. RESULTS

Twenty-four sites on eighteen patients with a CVM were examined using both normal white light and green filter videomicroscopy. Twelve women and six men with a mean age of 33 years (range 9 – 50) were examined. These patients had had between 0 and 28 treatments (mean 12, S.D. 9.7). Figures 1 and 2 shows the results obtained.

There was no statistically significant difference found for the reticular dermal vessels. However, for the papillary dermal capillaries a statistically significant ($P < 0.01$) increase in the measurements taken with the green filtered lens was found using a Wilcoxon Rank Test. This was independent of whether the CVM had a type 1 or type 2 pattern.

4. CONCLUSION

We believe that the difference in diameter measurements of these superficial papillary dermal vessels can be attributed to the reduction in the reflection from the skin surface, which is commonly seen with videomicroscopy, being reduced by the use of green filtering. Figures 3 – 6 demonstrate images taken on two patients using both normal white light and green filter videomicroscopy.

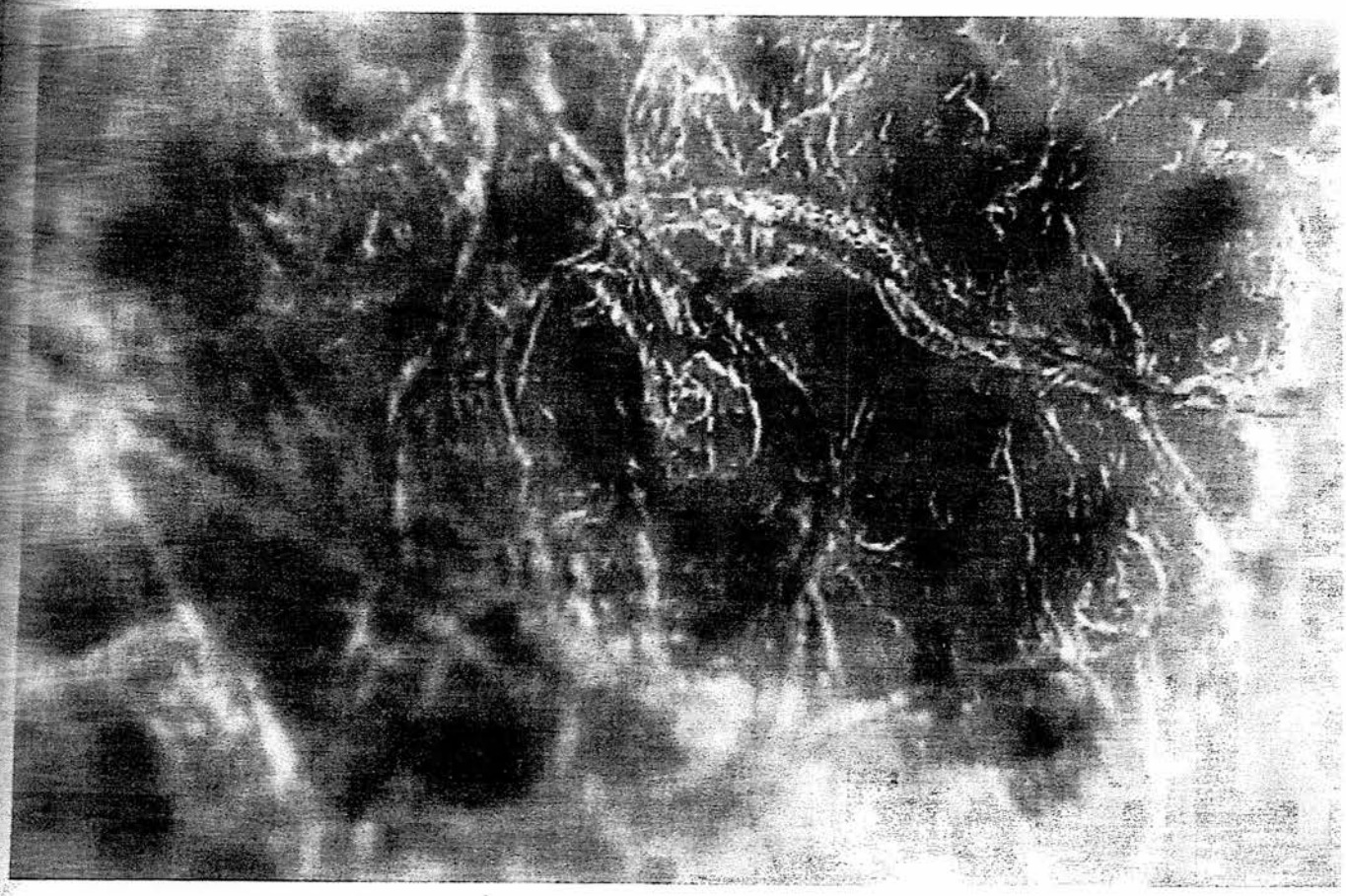

Figure 3 Normal

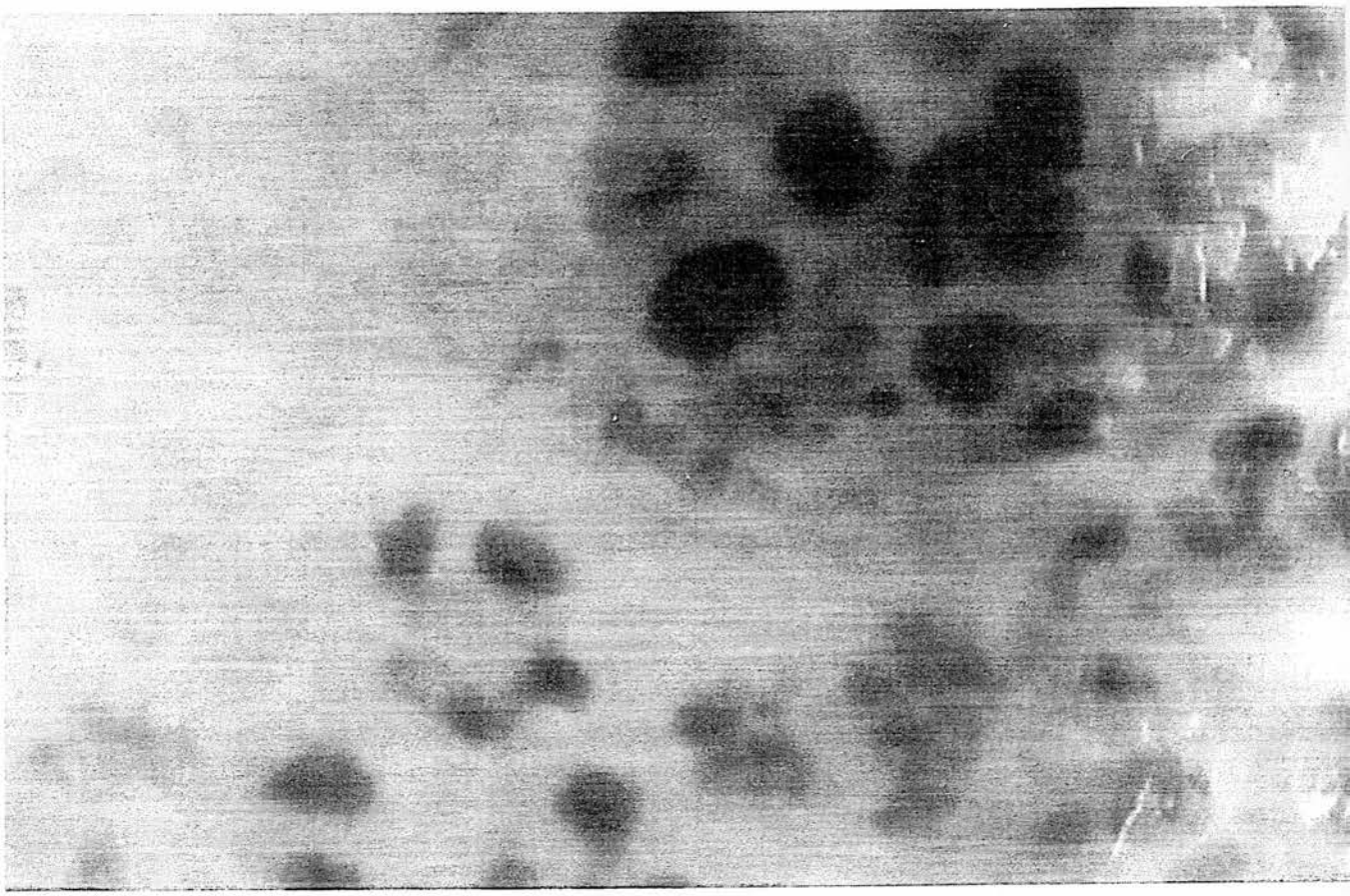

Figure 4 Green Filter

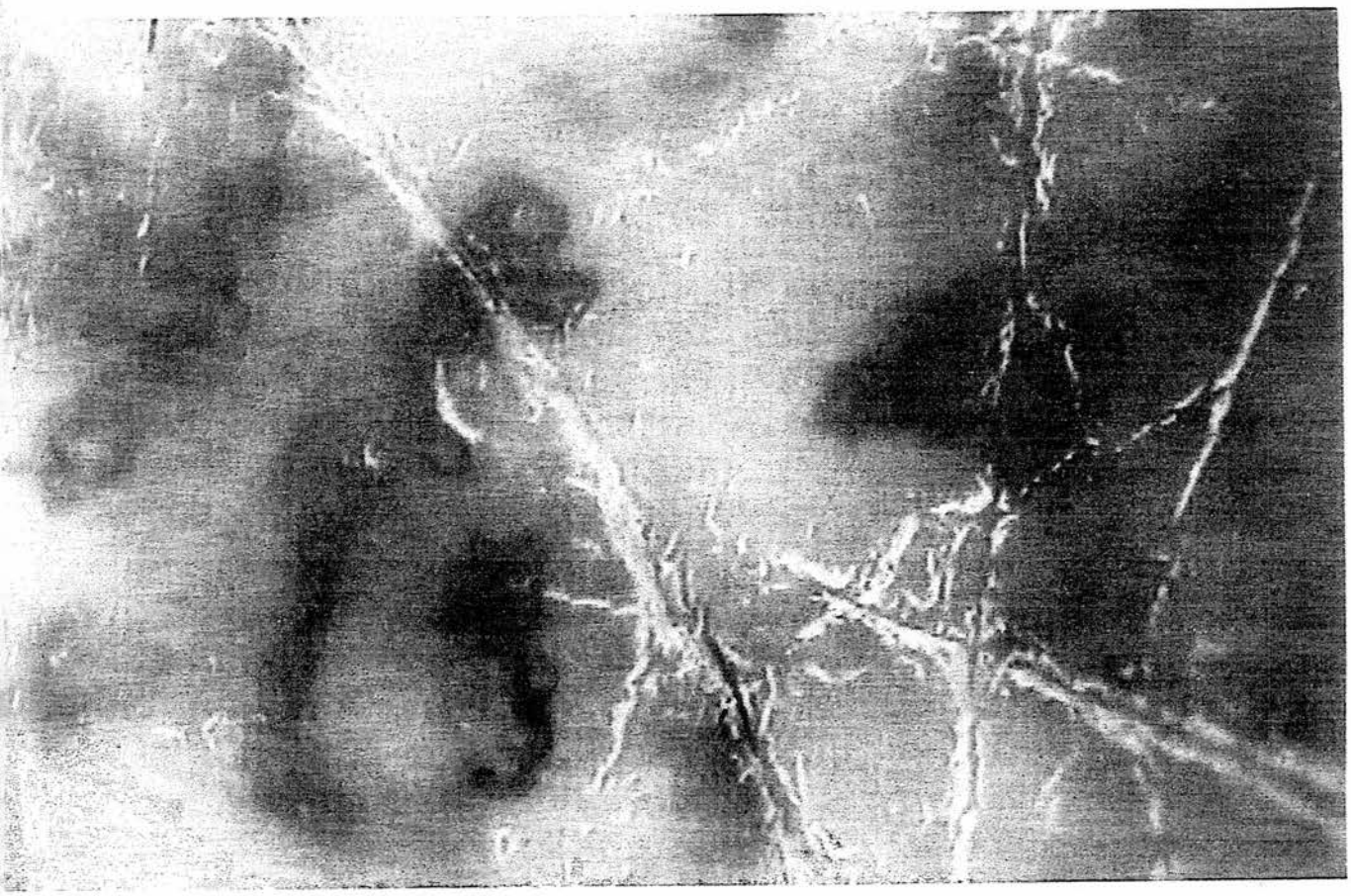

Figure 5 Normal

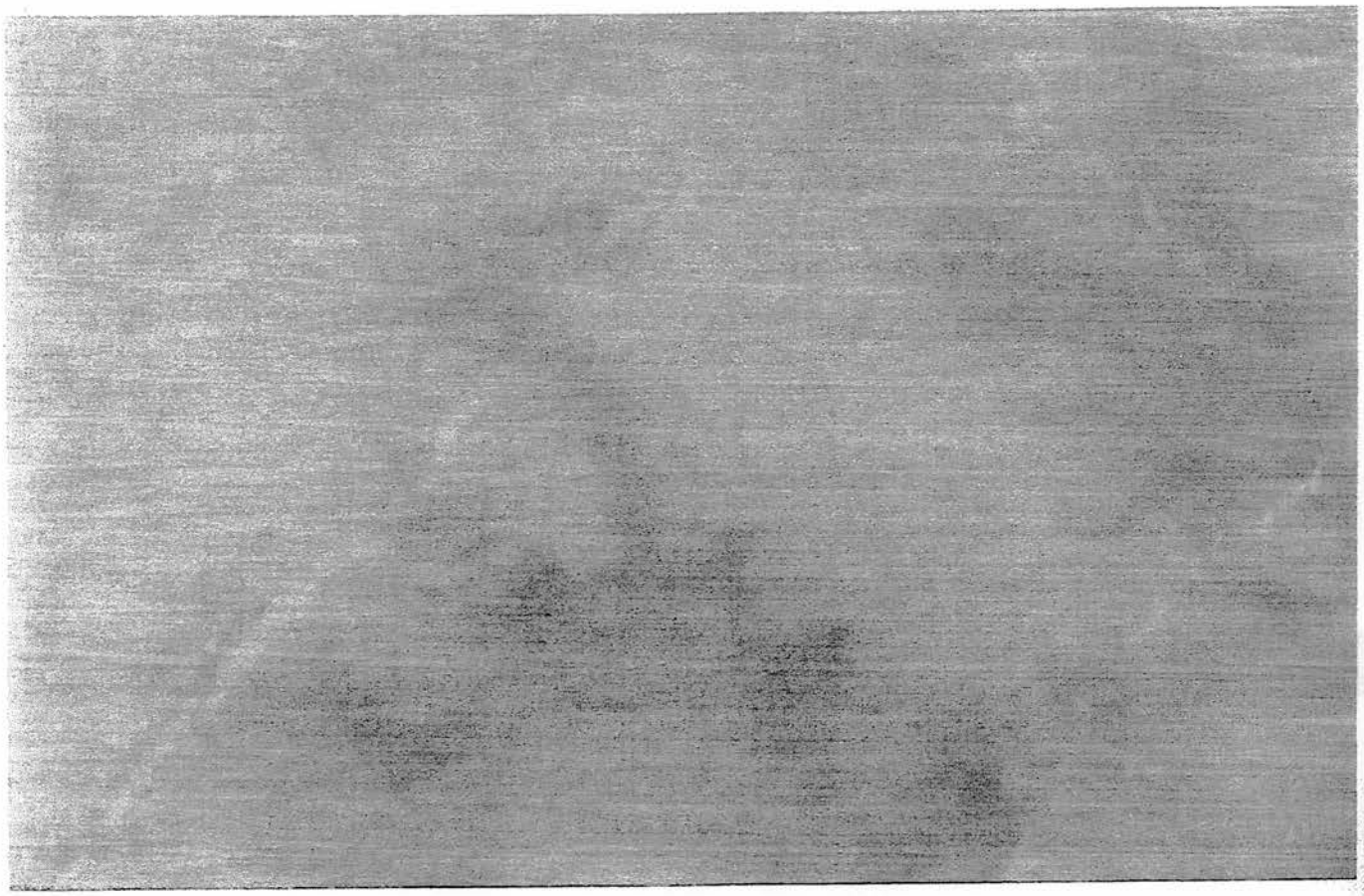

Figure 6 Green Filter

5. DISCUSSION

The response of CVM's to laser treatment is believed to be due to a number of factors:

1. The pattern of capillary ectasia, deep versus superficial
2. The depth of capillaries
3. The diameter of the capillaries
4. The flow through these capillaries
5. The amount of competing chromophores within the skin.

For clearance of a CVM to occur, it is necessary for the laser light to penetrate deep into the dermis with sufficient energy to cause coagulative necrosis in the abnormal capillaries. As these capillaries are of varying diameters it is also necessary for the pulse duration of the incident laser to be long enough to heat the vessel for sufficient time so that damage occurs and the energy is not dissipated. This has led to the development recently of laser systems with longer wavelengths and variable pulse widths.
The results found in this study for the diameters of capillaries within a CVM correspond well to previously published work by Fiskerstrand et al[12], which was based on biopsy specimens. A number or studies have examined the need for variable pulse duration lasers to enable vessels of varying sizes to be appropriately treated[13--15]. By the use of videomicroscopy to record *the in-vivo measurement of vessel diameter it may be possible to tailor pulse duration to that most appropriate for the* vessel diameters encountered in a particular CVM.

By further developing videomicroscopy with advances such as colour filtering it is hoped that the accuracy of the results can be improved. Another advance is the use of a newly developed depth measuring videomicroscope; which makes it possible to calculate the depth of capillary plexi within a CVM as well as their diameter. Figures 7 and 8 show images taken with the depth measuring videomicroscope. This 200 x contact lens has a flat quartz crystal window, which increases the clarity of the image and greatly reduces reflection from the target. By measuring the depth of capillaries within a CVM it may also be possible to tailor the wavelength of a laser system to the characteristics of the lesion.

Figure 7 Depth Measurement 120 micrometers
Type 1 Pattern

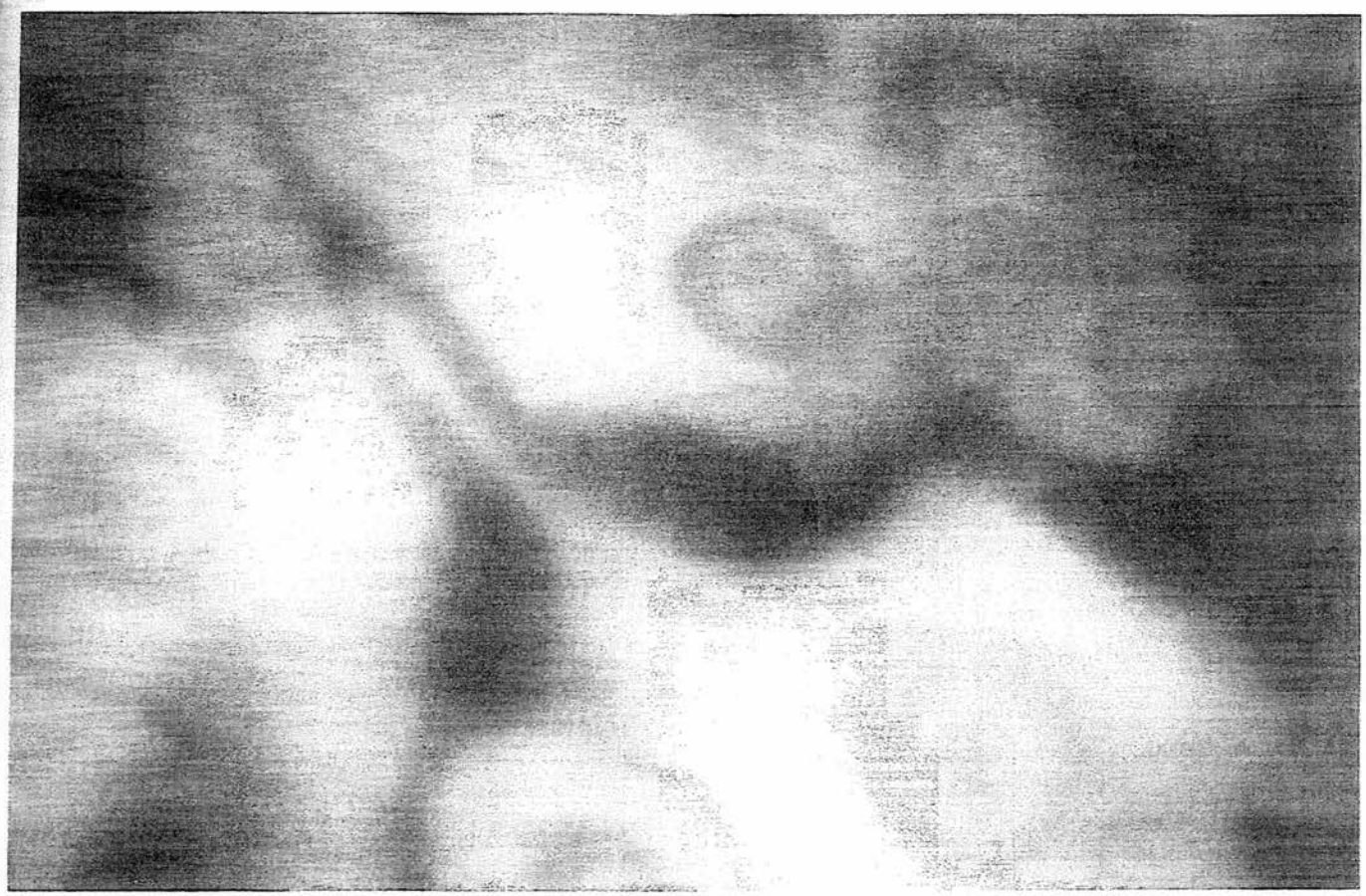

Figure 8 Depth Measurement 240 micrometers
Type 2 Pattern

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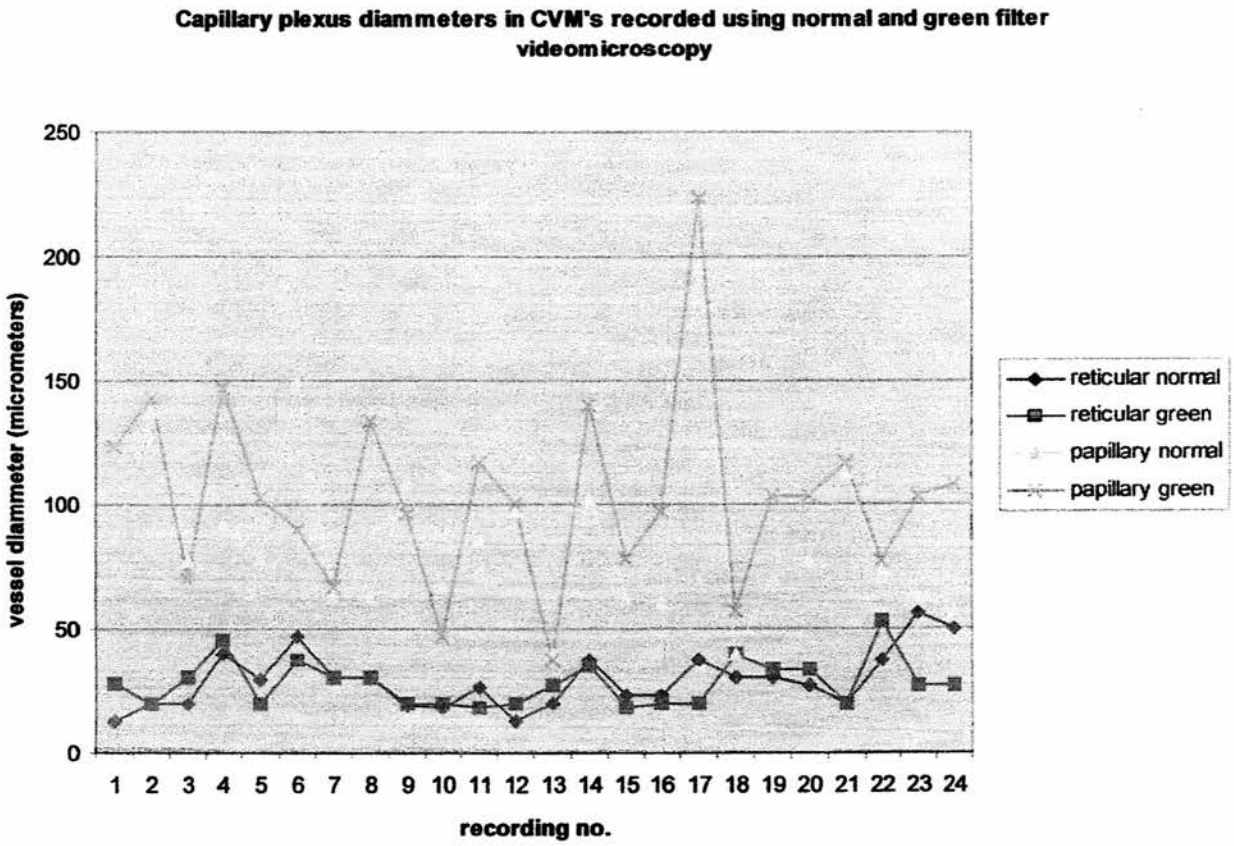
Figure 1 **Patient Characteristics and Vessel Patterns**

Patient no.	Age	Location	No. of treatments	Pattern type
1	9	Cheek	10	2
2	10	Leg	2	1
3	12	Cheek	13	1
4	13	Temple	7	2
5	15	Cheek	10	1
6	23	Back	6	1
7	25	Neck	4	1
7	25	Cheek	3	1
8	32	Cheek	16	1
9	35	Leg	10	2
10	35	Leg	28	1
11	36	Nose	26	2
11	36	Cheek	26	2
12	38	Left leg	26	2
13	40	Neck	7	2
14	40	Forehead	12	2
15	42	Cheek	27	2
16	45	Cheek	25	2
17	46	Occiput	0	1
17	46	Neck	19	1
18	50	Cheek	3	1
18	50	Forehead	6	2
18	50	Postauricular	1	1
18	50	Hand	0	1

Figure 2


Capillary plexus diammeters in CVM's recorded using normal and green filter videomicroscopy
vessel diammeter (micrometers)
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
recording no.
reticular normal
reticular green
papillary normal
papillary green

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The validation of the Depth Measurement Videomicroscope (DMV) as a noninvasive tool for the assessment of capillary vascular malformations☆

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KEYWORDS
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Laser;
Biopsy;
Pressure

Summary The assessment of capillary vascular malformation (CM) morphology can be performed using videomicroscopy. Previously only the type of capillary pattern could be demonstrated. The Depth Measurement Videomicroscope (DMV) allows both depth and diameter of CM vessels to be measured. The aim of this study was to examine how videomicroscope recordings correlated with biopsy recordings and to investigate pressure-related changes in recordings when using the device.

For the first part of the study, 10 patients with CMs resting in a temperature-controlled room were assessed with the DMV. Following this a 3 mm punch biopsy of the area was taken. The depth and diameter measurements taken with the DMV were compared to those obtained histologically. For the second part of the study, pressure measurement was used to determine the amount of pressure required on the tip of the DMV to alter the results obtained. Five recordings were taken on the forearm of one volunteer.

When the DMV and biopsy measurements are compared using a Bland and Altman Test to determine their relationship there is a close agreement with the diameter measurements and a correction factor of −0.100 mm for the depth measurements.

The pressure required to alter the skin microcirculation when placing the DMV on the skin surface was found to be 62 mmHg. This corresponds closely with other

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studies of pressure effects on the skin microcirculation and exceeds the pressure used when using the DMV. The DMV thus provides a useful tool for assessing CM capillary structure.

Capillary Vascular Malformations (CM) or Port Wine Stains are congenital vascular malformations, which consist of ectatic dermal capillaries within the skin.[1] As defined by Mulliken and Glowacki, they have a normal rate of endothelial cell turnover and grow commensurately with the child.[1] This is distinct from haemangiomas, where the rate of cell turnover is increased in the proliferative phase and reduced in the involuting phase. The incidence of these vascular abnormalities is between 1 and 3 per thousand live births[2] and they may cause considerable psychological upset for both the patient and parents.[3–6]

The treatment of CMs by laser has been attempted for a number of years, initially with the Argon Laser[7] and more recently with the Pulse Dye Laser.[8–11] The results of treatment are highly variable, with only a minority of patients receiving full fading of the lesion.[12] This lack of response is believed to be due to the capillary composition of the lesions,[13–15] the flow through the capillaries[16,17] and the amount of melanin in the skin.[18] The principle of selective photothermolysis, on which modern laser treatment is based, states that a wavelength of laser light should be chosen which will match the thermal absorption spectrum of the compound being targeted – in this case oxyhaemoglobin.[18] Furthermore, if this laser light is pulsed so that each pulse lasts less than the thermal relaxation time of the target, that is the time taken for the target to lose half its' energy, then heat build up and conduction into neighbouring structures will be minimised. It is the conduction of heat away from the target capillaries and into the surrounding skin that leads to the complications of pigment change and scarring.

The goal of recent advances in the development of laser technology has been to attempt to match the pulse duration of the incident laser light to the thermal relaxation time of the target capillaries, this being dependent on their diameter.[15,19–21] Also, the depth of the capillaries within the skin has important implications for laser treatment.[22–24] By increasing the wavelength of the laser light used it is possible to increase the depth of penetration of the laser to reach deeper capillaries. Unfortunately, due to the absorption spectrum of oxyhaemoglobin, increasing the wavelength used also requires much more energy (fluence) be used and thus risks complications. Traditionally the only method to assess the capillary characteristics within a CM has been by biopsy.[25]

In an attempt to develop a method of non-invasively determining capillary composition of CMs we have developed the Depth Measurement Videomicroscope (DMV). This consists of a 200× Cy-scope lens attached to a Compact Videomicroscope (PW Allen, Tewkesbury, UK). The advantage of this device over traditional videomicroscope units is that it allows for a higher definition image to be seen and has a focussing scale. This allows individual capillaries to be imaged and their depth and diameter to be calculated.

The aim of this study was twofold. Firstly, to compare the results obtained in vivo using the DMV with histological measurements obtained through punch biopsies. Secondly, we have attempted to quantify the pressure that can be exerted on the skin surface before measurements are altered. This is a common criticism of many contact methods of assessing CMs.

Method

For the first part of the study 10 patients, eight males and two females, with a mean age of 47 (range 28–85), were recruited. These patients were a mix of either resistant patients, those undergoing treatment, or untreated CM patients. Local Ethics Committee approval was gained for this part of the study.

Each patient was allowed to rest for 20 min in a temperature-controlled room at 28 °C. A suitable test area was selected. This consisted of a representative area of CM, which would allow the DMV to be applied to the area without interference, from hair for example. The area was also chosen not to be in an obvious area so that a biopsy would not leave the scar in an unacceptable position.

The DMV consists of a Compact Videomicroscope connected to a 200× Cy-scope lens. Images were viewed through a monitor (Sony Trinitron) and image capture was via a colour printer (Mitsubishi Colour Video Copy Processor).

A DMV examination was carried out on the test area by first adjusting for zero on the skin surface by focusing onto the light reflection from the epidermis. Oil was then applied to the skin of the test area to reduce reflection and allow the deeper dermal capillaries to be imaged.

The handpiece was applied to the skin so that a glass–oil–skin interface was created which did not allow the lens to lift from the skin, thus causing this interface to be lost but also did not apply undue pressure to the skin surface thus causing dermal capillaries to occlude. This was evident from the monitor.

The lens was used to pan down through the skin until the ectatic dermal capillaries came into focus, as judged by the clarity of red blood cells passing though them. The image was then recorded and a depth measurement obtained. A videomicrograph was then copied to film for later diameter measurement. This was then repeated two further times to increase the accuracy of the recording.

The test area was infiltrated with 2% lignocaine containing no adrenaline. A 3 mm punch biopsy was then taken and fixed in formalin. The fixed biopsy specimen was stained with a haematoxylin and eosin stain and examined by a pathologist. The pathologist was blinded to the values obtained using DMV. The depth and diameter of the capillaries within the specimen were recorded. To improve the accuracy of the histological measurements we analysed the slides using a Zeiss Axioscope Microscope and Zeiss KS400 v3 image analysis software.

Vessel diameters were measured from the videomicrograph using an image of a 1 mm graticule (Graticules Ltd, Tunbridge, UK). Three recordings were taken from each videomicrograph and the mean of these taken.

The second stage of this study examined the effect of the pressure of the DMV tip on the vasculature of CM skin. To do this we recreated an experiment performed by Schubert and Fagrell to examine the effect of skin pressure on the skin microcirculation in normal skin.[26] Fig. 1 shows the device used to measure the effect of pressure on skin microcirculation using a balance with a DMV at one end, which is counterbalanced by a weight at the other end. Schubert and Fagrell's original experiment used a thermister and laser Doppler device to monitor skin microcirculation instead of the DMV that we have used here. The device was set up so that the horizontal arm was balanced with the tip of the DMV just in contact with the CM skin surface on the forearm of a volunteer patient. The patient's arm was then replaced by a precision balance, which was then zeroed. The patient again placed their forearm under the DMV and a smaller sliding weight moved along the horizontal arm of the lever to increase the pressure at the tip of the DMV. Once any effect on the CM skin microcirculation was noted (this could be seen as either a reduction in the calibre of the capillaries or an overall reduction in the erythema on the monitor screen, whichever came first), the smaller weight was locked in place. The precision balance was again placed under the DMV and a weight recording taken. This measurement (W), together with the radius of the DMV tip (r), measured using a calliper, and the Density of Mercury Constant (M) was then entered into the following equation to give the pressure on the skin surface:

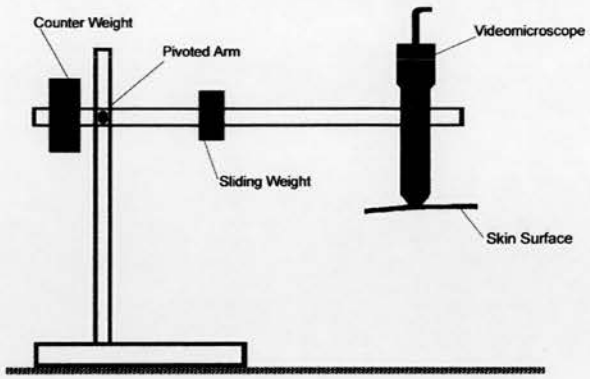

Figure 1 Equipment for assessing skin contact pressure as modified from Schubert and Fagrell.

$$\text{Pressure} = \frac{(0.1M\pi r^2)^{-1}}{W}$$

This process was repeated five times to reduce error from the recording.

Results

Ten patients were recruited into the study and consent gained in accordance with the Local Ethics Committee recommendations. These patients are shown in Fig. 2. As can be seen these CMs were mainly untreated patients, with those having had five treatments being resistant. The CMs were mainly facial and where a large lesion was being assessed the temple was chosen for the biopsy, if possible, so as to give a scar in an acceptable position. All the patients entering the study had Fitzpatrick Skin Type 1 or 2.

Fig. 3 illustrates an image obtained using the DMV on a CM. The ectatic capillaries can be clearly

Patient	Age	No. Of Treatments	Location
1	37	0	Temple
2	85	0	Forehead
3	30	0	Temple
4	41	0	Temple
5	48	0	Temple
6	47	0	Neck
7	28	2	Eyebrow
8	48	5	Forearm
9	55	5	Forearm
10	40	4	Chest

Figure 2 Characteristics of biopsy patients.

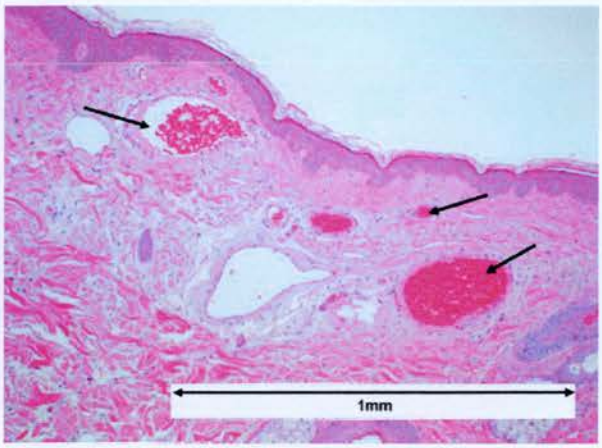

Figure 4 Histological section of a 3 mm punch biopsy taken from a CM illustrating ectatic dermal capillaries (arrowed).

seen. The largest diameter of the vessel was taken as the diameter recording. Fig. 4 shows a histological section from one patient's biopsy specimen. In both sections abnormally large ectatic vessels can be seen in the upper dermis (as indicated).

Fig. 5 illustrates the depth measurements obtained using the DMV and those recorded from the biopsy specimens. Fig. 6 shows the diameter measurements obtained with both the DMV and the biopsy specimens. To compare the two methods of assessment of capillary depth and diameter, namely histological measurement and DMV measurement we have used the Bland and Altman method of assessing the agreement between two methods of clinical measurement.[27] As both the DMV and histological methods of measurement are susceptible to error (for example defining zero with the DMV and fixing and sectioning artefact from the histological measurements) neither method can be assured to give completely accurate means of measuring in vivo vessel characteristics. In view of this it would be inappropriate to perform a correlation of the two measurements as it is unknown which of the two techniques is the more accurate. Correlation coefficients used to test the agreement between two measuring devices are susceptible to a number of errors, for instance the two sets of measurements may differ by a factor of 10 for example but may still give a high correlation as absolute values are not taken into account.[27] The Bland and Altman technique of assessing the agreement between two methods of clinical measurement is thus a more accurate test of agreement.

When the depth measurements are examined with the two techniques there is clearly a difference between them, as seen in Fig. 5. The depth measurements with the DMV appear to be less than with the histology measurements. Fig. 7 shows a plot of the difference between the two techniques of measuring capillary depth and the mean of the two methods. As can be seen the depth measurements appear to be less with the DMV than with the biopsy measurements.

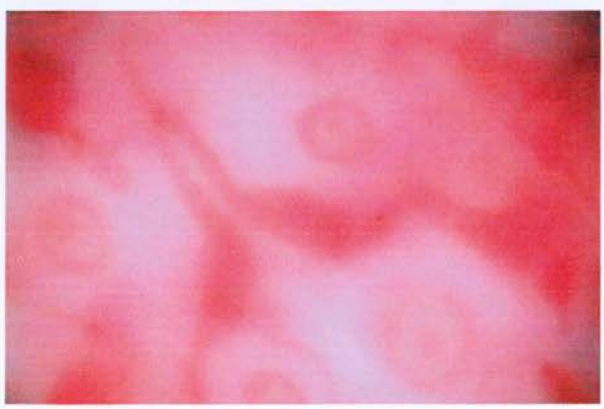

Figure 3 Image obtained using the DMV showing ectatic dermal vessels within a CM.

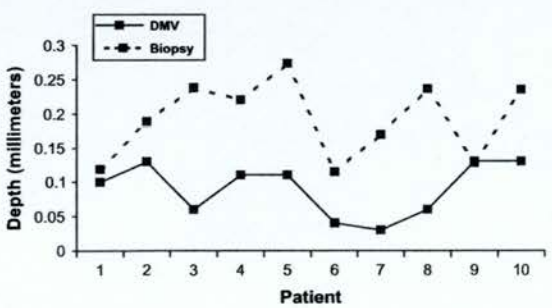

Figure 5 Comparison of DMV depth and biopsy depth.

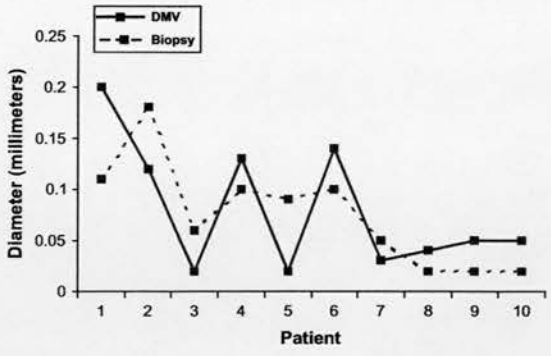

Figure 6 Comparison of DMV diameter and biopsy diameter.

This bias can be calculated using the Bland and Altman technique[27] and gives a correction factor of 0.102 mm (SD, 0.064 mm; SE, 0.020 mm; 95% confidence interval, 0.056–0.148). For this to be valid the differences between the two techniques at measuring depth once corrected for bias should be between 2 standard deviations (2 SD) of the mean. Fig. 8 demonstrates the corrected differences against the mean for the two techniques. This chart also includes the measurements of 2 SD. This shows that the measurements obtained with the DMV can be validly compared to biopsy measurements once the bias in the technique is accounted for.

For the diameter measurements the mean of the differences between the measurement techniques is −0.005 mm (SD, 0.051 mm; SE, 0.016 mm). The 95% confidence interval for this bias is −0.040 to 0.030 mm. There is therefore a high concordance between the diameter measurements taken with both the techniques. This can be seen in Fig. 9 with all the values for the differences between the two techniques clustering around the mean and within 2 SD. It would thus be appropriate to compare DMV diameter measurements with histological biopsy measurements without a correction factor.

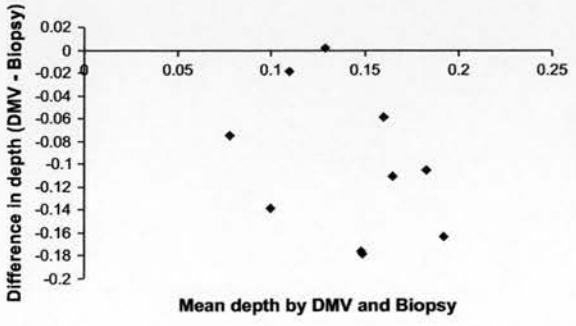

Figure 7 Difference against mean for depth measurement.

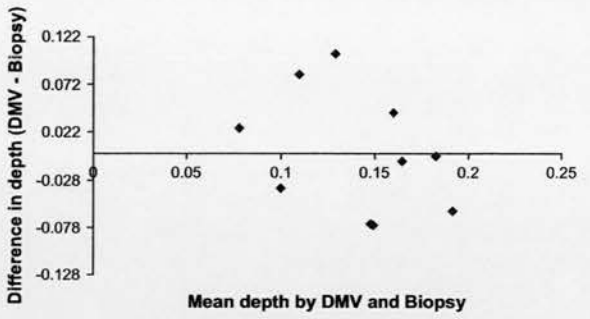

Figure 8 Corrected difference against mean for depth measurements (+1.02). d + 2 SD = 0.128, d − 2 SD = −0.128.

For the second part of this study, to investigate the pressure required to give anomalous results using the DMV a single patient was assessed using the modified Schubert equipment seen in Fig. 1. This gave a mean pressure required to change the DMV recordings of 62 mmHg (range, 61.2–64.8; SD, 2.9).

Discussion

There is a need to devise better methods of assessing CMs.[21,25,28–33] Currently patients commence a series of treatments with no indication of how long this treatment regime may take. Prognosis following treatment is also hard to quantify with only a minority of patients receiving full clearance of their lesion. Treatments tend to persist until the patient or physician decides that no further improvement is occurring. This inevitably leads to unnecessary treatments and the difficulty of subjectively assessing photographs to look for continued improvement. After ceasing treatment it is recognised that CMs continue to evolve, leading to darkening or re-emergence of the lesion.[34,35]

In an effort to improve the assessment of CMs a number of objective methods have been devised. Reflectance Spectrophotometry and digital photography aim to provide an objective method of

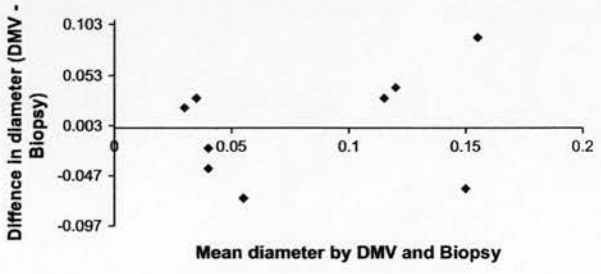

Figure 9 Difference against mean for diameter measurements. d + 2 SD = 0.097, d − 2 SD = −0.107.

assessing CM colour. These techniques, however, give no information as to the vessel structure and perfusion within a CM. Both these properties have been established as important determinants of response to treatment. Previously information on vessel structure has been achieved through the characterisation of these malformations through biopsy studies.

Noninvasive techniques of establishing vessel structure within a CM have been devised. High-resolution ultrasound can determine the maximum depth of a CM.[32] The response of a CM to laser treatment, however, is dependent upon the depth and diameter of the most superficial capillaries within it, as deeper vessels will be shielded from treatment by the scattering and absorption of the incident laser by the more superficial vessels. The technique of Photoacoustic Probing is able to demonstrate the depth to the superficial vessels within a CM, but not to demonstrate their individual diameters.[36] Colour Doppler Optical Coherence Tomography can allow the three dimensional reconstruction of blood vessels within an animal model, but lacks the resolution required to delineate the smallest CM vessels.[29]

The technique of Depth Measurement Videomicroscopy provides a noninvasive in vivo means of determining the depth and diameter of the most superficial vessels within a CM. This study has sought to compare the results obtained with this technique with those obtained histologically. The absolute values obtained with the DMV for vessel depth differ from those obtained from the biopsy specimens. This could be due to a number of factors. The DMV is a noninvasive technique, whereas the biopsy specimens are subject to the trauma of their excision and sectioning and the effect of fixing and staining of the sections. Also, the field of view given by the DMV is approximately 1.5 mm, whereas for this study we have used 3 mm punch biopsy. Due to the heterogeneity of these malformations it is likely that the same vessels have not been measured with each technique. Therefore with the depth measurements there is a significant difference found between the DMV recordings and the histological measurements. However, it would be inappropriate to conclude that the DMV was a less accurate method of assessing capillary depth as neither method can be assured to give completely accurate results and thus the use of the DMV is valid only as long as the measurements are compared with measurements taken using the same technique. This experiment allows one to compare DMV recordings with biopsy values using the correction factor but would not allow comparison with other noninvasive methods of examining CMs, such as optical coherence tomography, unless these techniques were also compared to histological measurements.

The values obtained for vessel diameter are similar with both the DMV and histological measurements. Therefore the values obtained using the DMV can be accurately compared to histological measurements. To further test the validity of the DMV it would be worth performing measurements on more patients and also repeating those measurements at different intervals to test repeatability. However, this would require many more biopsy specimens and this was not agreed with the Local Ethics Committee for this study.

Although the depth of penetration of a laser can be increased by using longer wavelengths of light this is limited by the reduction in specificity for the absorption spectrum of oxyhaemoglobin. Thus, vessel depth may not be as significant a factor as diameter in determining a CM's response to modern lasers, which allow variation of pulse duration. From the theory of Selective Photothermolysis, the pulse duration of the incident laser should ideally be less than the thermal relaxation time of the target vessels within a CM to reduce the incidence of complications from the treatment.[18] The thermal relaxation time is itself dependent upon the diameter of the vessels to be treated. The DMV provides a noninvasive method of assessing this, which is rapid and relatively inexpensive and can be used in a clinic setting.

The second part of this study aimed to evaluate the effect of pressure from the DMV tip on the CM microvasculature. The effect of pressure causing anomalous results is a criticism of many methods of contact assessment of skin lesions, such as reflectance spectrophotometry and ultrasound as well as the DMV. When using the DMV it is essential to rest the tip on the skin so that a skin–oil–glass interface is created. Too little pressure results in loss of this interface and too much pressure is evident from the monitor. The pressure assessment we have carried out gave a mean pressure of 62 mmHg required to alter the results obtained. Previous studies by Schubert and Fagrell found a pressure exceeding 50 mmHg was required to reduce skin microcirculation perfusion in normal skin in the sacral area.[26] Lindan et al. found that the normal pressure on pressure points in a sitting position was between 10 and 60 mmHg.[37] The pressure thus required to alter the values recorded using the DMV are significant and much greater than the pressure applied in practice.

We believe the DMV provides a useful tool for the assessment of CMs both for research and to

guide treatment practically. The ability to assess CM structure may allow newer Pulsed Dye Lasers to treat CMs more efficiently, as pulse duration and wavelength could be matched to measure vessel diameter and depth, respectively. Also, by determining the depth of CM vessels in relation to the epidermal layer it may be possible to alter the cooling applied to the skin, for instance by changing spray timings of Cryogen Spray Cooling Systems (Candela Corp., Wayland, MA, USA), so to improve epidermal protection.[36] By measuring the depth and diameter of vessels within a CM it may be possible to determine more accurately when it becomes resistant and hence prevent patients receiving unnecessary treatments.

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The Relationship Between Location, Color, and Vessel Structure Within Capillary Vascular Malformations

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Abstract: The treatment of capillary vascular malformations by laser is well established. Despite this, only a minority of patients obtain full clearance of their lesion after treatment. Both anatomic location and color have been proposed as prognostic factors determining the amount of improvement seen after laser treatment. This study uses the technique of depth measuring video microscopy to examine the hypothesis that smaller and more deeply placed capillaries may be responsible for the poorer response seen in certain anatomic locations. Fifty sites on 44 previously untreated patients were examined resting in a temperature-controlled room at 28°C. No statistically significant correlation was found between color, based on a Munsell color chart recording and capillary depth or diameter. Also, no correlation was found between diameter, depth, or type of capillary ectasia and anatomic site. The authors believe that other factors, such as alteration in blood flow between different anatomic regions, and not vessel morphology alone, may be responsible for this variation in response.

Key Words: laser, Munsell color chart, port wine stain, videomicroscopy

(*Ann Plast Surg* 2004;53: 378–381)

The treatment of capillary vascular malformations by laser treatment is well established.[1–3] A number of different laser systems have been used to treat capillary vascular malformations, including argon, copper vapor, and more recently dye and KTP lasers. Despite this, complete fading of the lesion is only achievable in a minority of patients.[4–6] This is believed to be due in part to the structure of the ectatic capillaries within the dermis that characterizes capillary vascular malformations.[4,7,8] Previous studies have suggested that both vessel diameter and depth are important determinants of response of capillary vascular malformations to laser treatment.[4,8–12]

It has been observed that capillary vascular malformations in some locations tend to improve more than in others. Nguyen et al[13] divided facial capillary vascular malformations into central forehead lesions; central lesions consisting of the nose, upper lip, and medial cheek; and peripheral lesions. They found that central forehead lesions cleared best, followed by peripheral lesions, and central lesions did worst. Renfro and Geronemus[14] found that lesions in the central facial area responded less well to laser treatment than more peripheral lesions. They also split patients depending on the dermatomal distribution of the capillary vascular malformations and found that those involving V2 tended to do less well than those involving V1 and V3.

A number of tools have been used to determine capillary structure noninvasively within a capillary vascular malformation. Among these is a new technique of depth measuring video microscopy (DMV; PW Allen, Tewkesbury, UK). This tool is similar to a traditional video microscopic unit but allows a recording to be made of vessel depth and diameter in the dermis.

Using traditional video microscopy has allowed capillary vascular malformations to be characterized into 2 different types[7]: those involving predominantly ectasia of the superficial papillary plexus (termed type 1) and those involving ectasia of the deeper reticular plexus (type 2). A minority of capillary vascular malformations also displays a mixed pattern. It has been found that those capillary vascular malformations displaying a mainly type 1 pattern tend to respond better to laser treatment than those with a type 2 pattern. It has also been suggested that capillary vascular malformation type varies depending on location.[15]

Poor response to laser treatment has been observed in capillary vascular malformations with small and deeply located capillaries[10,11] and in different locations.[13,14] This study aims to examine the hypothesis that the poor response in certain anatomic locations is the result of a preponderance of smaller and more deeply located (type 2) vessels in these areas.

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METHOD

Fifty sites in 44 patients were studied. Multiple sites in the same patient were only examined in large capillary vascular malformations covering more than 1 anatomic unit. Anatomic units were chosen based on existing criteria in previous studies[14] (Table 1). None of the patients examined had received previous treatment of their capillary vascular malformation.

Patients were rested in a temperature-controlled room at 28°C for 20 minutes. This was done to reduce the effect of temperature[16] and exercise on capillary vascular malformation capillaries. Color of the capillary vascular malformation was recorded by 2 observers using a Munsell color chart (GretagMacbeth, New Windsor, NY).[17] This color chart result was then converted to a scale based on paleness, to allow statistical interpretation. Patients were then asked to complete a combined skin type test questionnaire to determine their skin type.

The DMV consists of a compact video microscope (PW Allan) connected to a 200× Cy-Scope Lens. A DMV recording was taken of the unaffected contralateral side and 3 recordings were taken of the capillary vascular malformation to reduce the error from the heterogeneity of the lesion. The DMV recording gave a measure of the depth of the capillaries within the lesion. The DMV measurements were captured to film using a color video printer (Mitsubishi Color Video Copy Processor). From the images taken, vessel diameters were recorded based on 3 vessels in each image using the image of a 1-mm graticule (Graticules Ltd, Tunbridge, UK). A mean value was thus attained for capillary vascular malformation vessel depth and diameter. Type of capillary ectasia could be visualized on the images obtained.

TABLE 1. Locations of Capillary Vascular Malformations Studied and Numbers of Patients per Location

Ref	Location	No. of Patients
1	Lower limb, distal	7
2	Lower limb, proximal	4
3	Upper limb, proximal	5
4	Trunk	5
5	V1	6
6	V2	16
7	V2 medial	9
8	V2 lateral	7
9	V3	3
	Other	2
	Upper limb, distal	1

RESULTS

From the combined skin type test results, the majority of patients in the study were Fitzpatrick skin types 1 through 3. One patient was a skin type 4; no darker skin types were encountered. The number of cases in each anatomic location is shown in Table 1. V2 on the face was further divided into medial and lateral lesions because previous studies have found that medial lesions tend to respond less well to laser treatment than lateral ones. In some groups there was insufficient numbers of patients to allow statistical analysis, and groups containing less than 3 patients were excluded.

The Munsell color chart scores were adjusted to represent a scale based on paleness of the capillary vascular malformation. Figure 1 shows the mean and range of Munsell color types for each location. As demonstrated, there is a wide variation in the colors of the capillary vascular malformations in different locations.

There was no correlation found between Munsell color score and either depth or diameter of the capillary ectasia using the Pearson correlation calculation. Statistical analyses were carried out using SPSS version 10.

When different locations were examined there was a wide variation found in both the depth (Fig. 2) and diameter (Fig. 3) of the capillary vascular malformations.

Figure 4 shows the distribution of capillary ectasia type for each location. As evidenced, there is no correlation between type of capillary ectasia and those sites known to do badly from laser treatment—namely, V2 and distal extremities.

DISCUSSION

It has been widely recognized that certain sites for capillary vascular malformations tend to respond better to laser treatment than others.[13] Lesions on the distal extremities

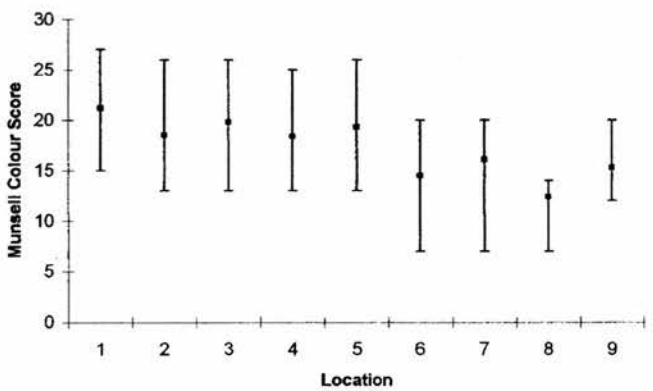

FIGURE 1. Range of Munsell color chart scores per location. Locations: 1, lower limb distal; 2, lower limb proximal; 3, upper limb proximal; 4, trunk; 5, V1; 6, V2; 7, V2 medial; 8, V2 lateral; 9, V3.

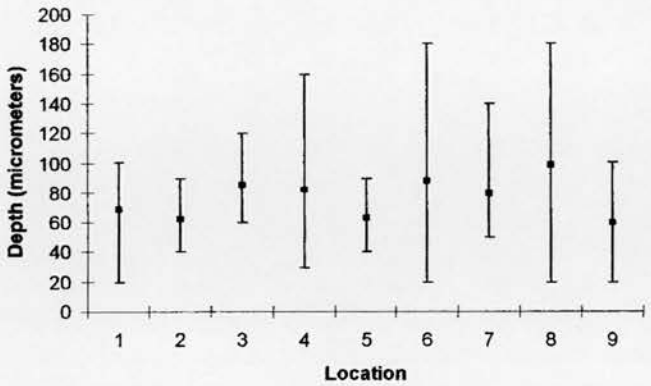

FIGURE 2. Range of depth measurements per location. Locations: 1, lower limb distal; 2, lower limb proximal; 3, upper limb proximal; 4, trunk; 5, V1; 6, V2; 7, V2 medial; 8, V2 lateral; 9, V3.

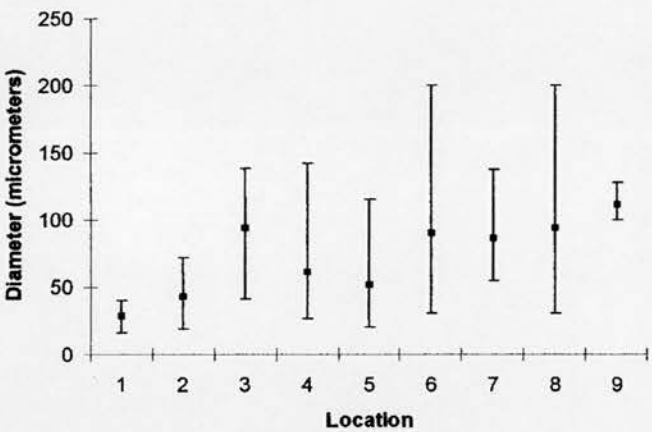

FIGURE 3. Range of diameter measurements per location. Locations: 1, lower limb distal; 2, lower limb proximal; 3, upper limb proximal; 4, trunk; 5, V1; 6, V2; 7, V2 medial; 8, V2 lateral; 9, V3.

and medial face tend to do less well from laser treatment than lesions on the lateral face or trunk. Eubanks and McBurney[15] attributed this difference to type of vessel ectasia seen using traditional video microscopy. Their study, however, looked at only 17 patients, and 12 of them had already received laser treatment before imaging. Previous unpublished work within our unit examining 111 resistant capillary vascular malformation patients (all having had more than 10 dye laser treatments) demonstrated that 98% of patients exhibited a type 2 pattern after treatment. In the study by Eubanks and McBurney[15], 10 of the 12 previously treated patients were found to have a type 2 pattern as opposed to only 1 of the 5 previously untreated patients. Their conclusion that response in different anatomic sites is the result of differences in vessel type is, therefore, more likely to be the result of whether

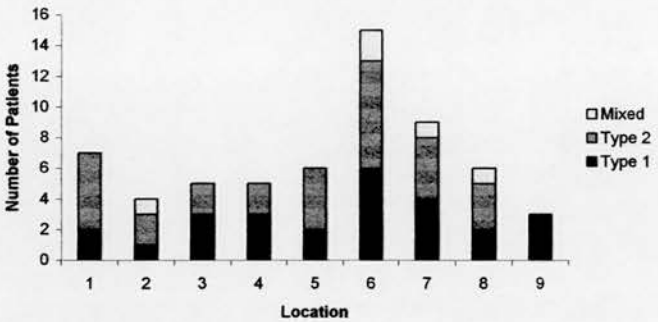

FIGURE 4. Type of vessel ectasia seen per location. Locations: 1, lower limb distal; 2, lower limb proximal; 3, upper limb proximal; 4, trunk; 5, V1; 6, V2; 7, V2 medial; 8, V2 lateral; 9, V3.

patients had received prior treatment. In this study we examined only patients who had received no former treatment by any modality.

We used the DMV to examine capillary vascular malformations and to determine not only type of ectasia, but actual vessel diameter and depth as well. In this study we measured the depth to the most superficial of the capillary vascular malformation capillaries, rather than the mean or maximum depth. With this technique the deeper capillaries are masked by more superficial vessels. The response of an untreated capillary vascular malformation to laser treatment will be dependent on the range of depths and diameters of capillaries comprising the lesion. Biopsy studies by Fiskerstrand et al[10] and Hohenleutner et al[11] have demonstrated that capillary vascular malformations with deeply placed and smaller vessels tend to respond less well to laser treatment than those with superficial and large vessels. We have examined the hypothesis that poor response to laser treatment in certain anatomic locations is related to the presence of small or deeply placed capillaries in these areas.

Color of a capillary vascular malformation has been found to be predictive of outcome after laser treatment, with purple and red lesions tending to respond better than pink ones.[18] This is believed to be the result of the theory that pale-pink capillary vascular malformations have more deeply located or smaller capillary ectasia. Our results have not demonstrated a relationship between capillary depth or diameter and color.

We have found no relationship between the type of capillary ectasia and the site of a capillary vascular malformation. It would seem likely that predominance of one type of capillary ectasia over another is not a reliable indicator as to why one anatomic area will respond better to laser treatment than another. Also, it appears that capillary depth and diameter alone cannot be the only factors responsible for the variation of response between different areas.

This study does not take account of any variation of capillary flow between different locations. Previous studies have demonstrated abnormal flow within capillary vascular malformation vessels in comparison with normal skin.[19,20] In these studies, many of the capillary vascular malformations investigated exhibited increased blood flow in comparison with normal skin. Currently no studies have been performed to evaluate the difference in blood flow to capillary vascular malformations in different anatomic locations. It seems likely that factors such as blood flow, and not just the capillary depth and diameter, may be responsible for the differing responses of certain sites to laser treatment. Further work is required to evaluate how these factors interact.

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Lasers in Surgery and Medicine 34:193–197 (2004)

The Depth Measuring Videomicroscope (DMV): A Non-Invasive Tool for the Assessment of Capillary Vascular Malformations

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Background and Objectives: The response of capillary vascular malformations (CVMs) to laser treatment is believed to be due to the pattern of capillary ectasia, the depth, diameter, and flow through these capillaries and the amount of competing chromophores within the skin. Videomicroscopy has successfully been used to determine CVM capillary pattern and diameter of vessels. The depth measuring videomicroscope (DMV) allows the depth of capillaries to be measured also. The aim of this study is to examine how capillary depths within a CVM are affected by dye laser treatment using DMV.

Study Design/Materials and Methods: Thirteen previously untreated patients were examined in a temperature-controlled room. A DMV examination was carried out prior to and 6 weeks following a treatment with pulsed dye laser. A further cohort of 11 resistant CVM patients, who had all received over five treatments, was also examined for comparison.

Results: Using a Wilcoxon Signed rank test, the results showed that the remaining vessels within the CVM as measured using DMV were more deeply located and smaller ($P < 0.01$ and $P < 0.02$ respectively), following the laser treatment. Also in the resistant patients the vessels were again more deeply placed and smaller.

Conclusions: The hypothesis that smaller and more deeply placed CVM vessels respond poorest to laser treatment is supported by these findings. Moreover, the DMV provides a simple non-invasive technique for demonstrating this. Lasers Surg. Med. 34:193–197, 2004.

Key words: laser; port-wine stain; videomicroscopy

INTRODUCTION

Capillary vascular malformations (CVMs) or port wine stains are congenital vascular abnormalities of the dermal capillaries, which tend to be present at birth and grow with the child [1]. They are believed to have a genetic basis [2] and occur in between 1 and 3 per 1,000 live births [3]. They cause considerable psychological distress [4] and patients and their parents frequently seek treatment. The mainstay of treatment for CVMs remains the pulsed dye laser [5–7].

Despite treatment, complete lightening of the lesion is rare, with most patients obtaining a variable amount of fading without complete resolution [8,9]. At present it is difficult to give a patient an accurate assessment of how much fading they can expect prior to treatment, or to assess whether further fading is possible in a CVM receiving treatment [10].

The response of CVMs to laser treatment is believed to be dependent on a number of factors:

1. The pattern of capillary ectasia, deep versus superficial.
2. The depth of capillaries.
3. The diameter of the capillaries.
4. The flow through these capillaries.
5. The amount of competing chromophores within the skin.

A number of techniques have been used to provide an accurate means of assessing CVM characteristics non-invasively. These include methods for assessing color, such as reflectance spectrophotometry [11,12], digital [13,14] and traditional photography [15], and methods of assessing flow such as laser Doppler flowmetry [16]. None of these techniques allows the delineation of actual ectatic capillary structure. A number of studies have established the importance of capillary depth and diameter in determining the results from laser treatment of CVMs [17–22].

Recently, new techniques have been devised in an attempt to achieve accurate non-invasive evaluation of CVM vessels. Ultrasound and photoacoustic probes may be able to determine maximum CVM depth but do not delineate individual vessel diameter [23,24]. Optical coherence tomography (OCT) uses a light source to scan through the skin and can delineate small vessels from their reflection to down to 10–20 μm resolution [25,26]. At present there are no studies examining series of patients with this technique.

To enable the measurement of capillary depth and diameter within a CVM we have developed a Depth Measurement Videomicroscope in conjunction with PW Allen,

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Tewkesbury, UK. The aim of this study is to examine how capillary characteristics within a CVM are affected by dye laser treatment using DMV.

MATERIALS AND METHODS

During the period of February to August 2002, all new patients attending Canniesburn laser suite were entered into the trial of the DMV. Thirteen, previously untreated patients were assessed with the DMV prior to and 6 weeks following a single treatment with a 0.45 milliseconds 585 nm pulsed dye Laser (SLS Chromos, Wales, UK). For each patient the treatment parameters remained the same: 6.4 J/cm^2 fluence and a 7 mm spot size with topical ice epidermal cooling.

During the DMV examination, each patient was allowed to rest for 20 minutes in a temperature-controlled room at 28°C. They were also asked to carry out a combined skin type test (CSTT), which assesses their skin type and recent sun exposure. The area to be examined was then color matched using a Munsell Color Chart (GretagMacbeth, New Windsor, NY) by two observers.

The DMV consists of a Compact Video Microscope (PW Allan, Tewkesbury, UK) connected to a 200× Cy-Scope Lens (Fig. 1). Images were viewed through a monitor (Sony Trinitron) and image capture was via a color printer (Mitsubishi Color Video Copy Processor).

The normal capillary depth was measured on the contralateral unaffected side of the body prior to the assessment of the CVM test area.

The DMV examination was carried out by first adjusting for zero on the skin surface by focusing onto the reflection from the skin. Oil was then applied to the skin of the test area to reduce reflection and allow the deeper dermal capillaries to be imaged. Reflection with this lens is much less than traditional videomicroscope units.

The handpiece was applied to the skin so that a glass–oil–skin interface was created. It was obvious from the monitor if the lens lifted off the skin as this interface was lost. It was also obvious from the monitor if undue pressure was applied as this caused dermal capillaries to occlude.

The lens was used to pan down through the skin until the ectatic dermal capillaries came into focus, as judged by the clarity of red blood cells passing though them. The image was then captured to film and a depth measurement obtained (Fig. 2). This was then repeated two further times to increase the accuracy of the recording and to allow for heterogeneity of vessel depth within a CVM.

Vessel diameters were measured from the videomicrographs using an image of a 1mm graticule (Graticules Ltd., Tunbridge, UK). Three recordings were taken from each videomicrograph and the mean of these taken.

A further cohort of 11 resistant patients attending the Laser Suite for final assessment was also examined. These patients had all had over five dye laser treatments (mean 11.6, range 5–29).

RESULTS

Following treatment the majority of untreated patients demonstrated some lightening of their CVM as assessed using Munsell color charts. To aid statistical analysis Munsell chart recordings were converted to scores based on paleness (Fig. 3). In this chart, the higher scores represent paler lesions. When color pre and post laser treatment was

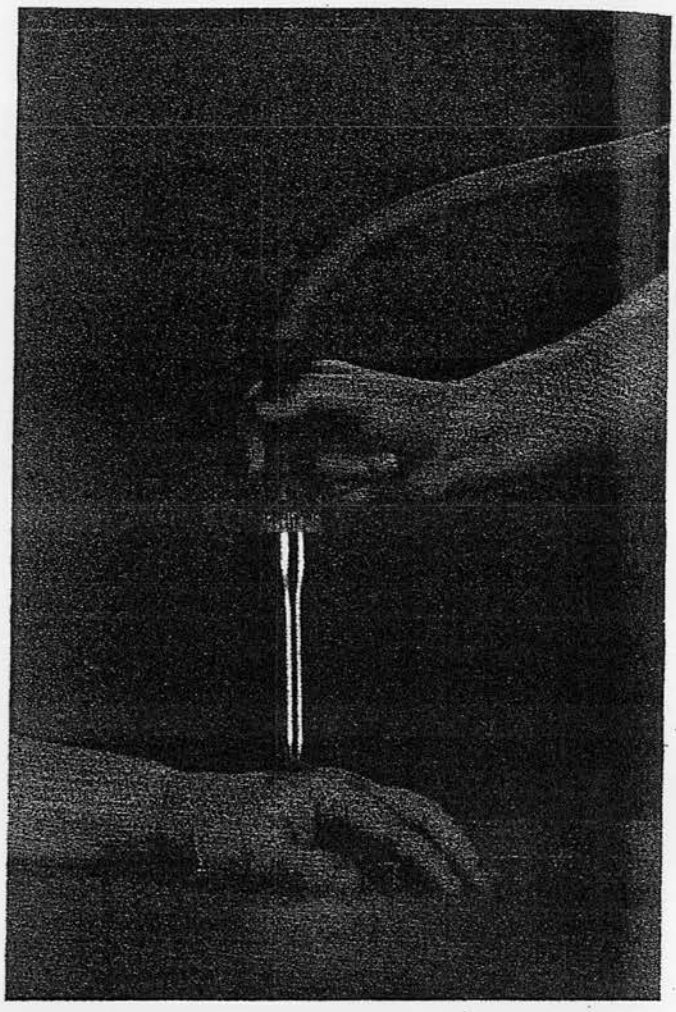

Fig. 1. The depth measuring videomicroscope (DMV) Cy-scope lens.

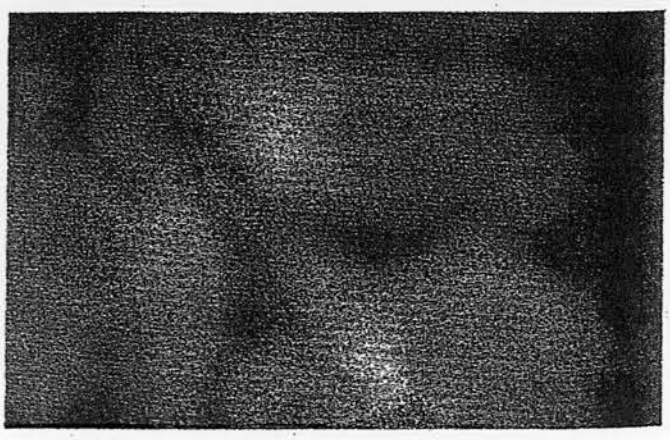

Fig. 2. DMV image of CVM capillaries.

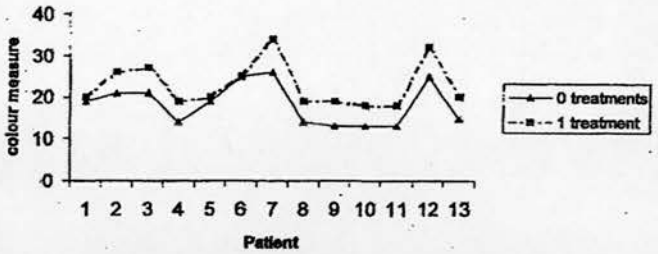

Fig. 3. Color change following pulsed dye laser treatment.

compared using a Wilcoxon Signed rank test, there was a significant lightening of the lesions ($P < 0.01$). All statistics were carried out using SPSS V.10.

All the patients in the study were Fitzpatrick Skin Type 1 or 2. There was no statistically significant change in CSTT scores between the two recordings, indicating that sun exposure within the 6-week gap between DMV measurements was not sufficient to cause confounding results (Table 1).

When vessels were examined prior to and 6 weeks following pulsed dye laser treatment the measured depths were found to be greater following treatment in most cases (Fig. 4), a mean of 68.5 μm (range 20–140 μm) before and 82.3 μm after (range 50–140 μm).

If the depth measurements for the CVM vessels are compared to those obtained for the normal contralateral skin the depth of the measured vessels can be seen to increase following treatment (Fig. 5). Statistical analysis using a Wilcoxon Signed Rank test showed a significant increase in the depth measurements following treatment compared to normal capillary depth ($P < 0.01$). This comparison between treated CVM and normal contralateral vessel depth was made to test the validity between the two sets of measurements and reduce any error from zeroing the DMV. As the patient is rested in a temperature-controlled room, fluctuations in capillary filing are minimized. There was no statistically significant difference seen between the normal capillary depth measurements taken pre and post treatment. In addition, as dermal capillary depth varies depending upon location [27], values compared to normal allow comparison between different CVM sites.

For vessel diameter, mean measurements were found to be smaller following treatment with a mean of 38 μm (range 12–86 μm) versus a mean of 67 (range 24–148 μm) prior to treatment. This result was statistically significant using Wilcoxon signed rank test ($P < 0.02$, Fig. 6).

When compared to the pre-treatment group, the cohort of 11 resistant patients was again found to have deeper vessels ($P < 0.01$, Fig. 5). Although diameter of vessels was not found to be smaller statistically, a trend is evident from the chart (Fig. 6).

DISCUSSION

The treatment of CVMs by pulsed dye laser is based upon the Theory of Selective Photothermolysis proposed by Anderson and Parish [28]. Fundamental to this theory is that vessels will receive sufficient energy to cause photothermolysis. Also, that pulse duration of the incident laser light is less than the thermal relaxation time of the target vessel so as to reduce the risk of complications. In order for incident laser energy to heat a particular vessel to 70°C, sufficient to cause photocoagulative necrosis of the vessel wall, implies that sufficient energy reaches that vessel taking into account the processes of reflection, scattering, and absorption by other vessels and other chromophores within the skin. This is, as such dependent upon depth of the vessel. Thermal relaxation time for a vessel is a consequence of size of this vessel and therefore dictates

TABLE 1. Results for Patients Receiving First Dye Laser Treatment

Patient number	Age	CSTT score[a]		Color change[b]	Contralateral depth		Mean depth CVM		Mean diameter CVM	
		Untreated	Treated		Untreated	Treated	Untreated	Treated	Untreated	Treated
1	11	29	24	1	150	40	140	100	69	24
2	41	13	13	5	30	30	80	90	40	19
3	41	13	13	6	30	30	70	100	34	20
4	37	14	14	5	110	110	60	50	97	86
5	48	26	23	1	100	120	60	100	54	43
6	18	12	12	0	70	70	50	70	24	20
7	14	15	15	6	60	60	120	140	103	20
8	48	22	18	5	80	100	50	70	48	53
9	11	20	20	6	70	70	20	50	31	61
10	19	19	18	13	40	20	40	50	80	34
11	22	16	16	6	110	110	100	120	117	49
12	42	18	18	7	10	10	40	60	29	12
13	43	28	27	5	20	10	60	70	148	57

[a]CSTT, combined skin type test.

[b]Color change—as modified Munsell color chart score.

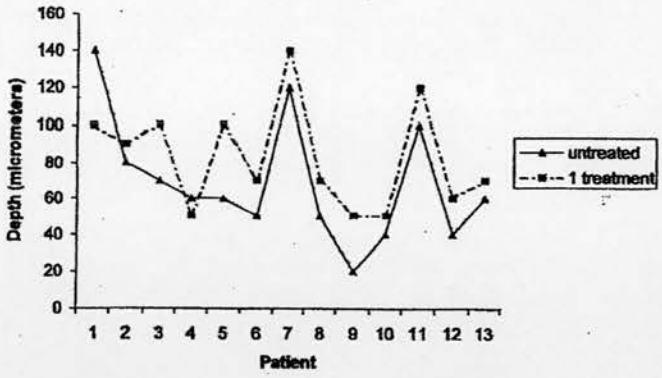

Fig. 4. Capillary depth as assessed by DMV before and after pulsed dye laser treatment.

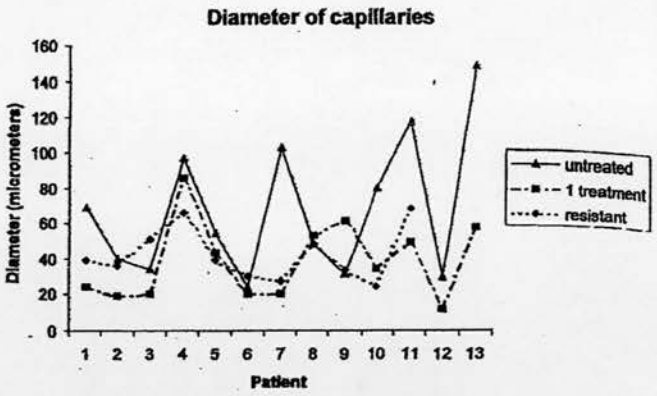

Fig. 6. Diameter of CVM vessels before and after pulsed dye laser treatment including results for the resistant cohort of patients.

the necessary pulse duration of the incident laser light [19]. Pulse duration is therefore dependent upon vessel diameter.

Previous biopsy studies by Fiskerstrand et al. [7] demonstrated capillary diameters within the range of 9–100 μm, with lesions with smaller vessels responding less well to laser treatment than those with larger vessels. These values for vessel diameters correspond closely to our in-vivo measurements. In this study, the measurements taken for capillary diameter before and after pulsed dye laser treatment reduced suggesting that larger diameter vessels are cleared by the treatment leaving the smaller ones still present.

In terms of vessel depth, Fiskerstrand et al. [17] and Hohenleutner et al. [18] demonstrated that following dye laser treatment deeper vessels tended to be left uncoagulated within a CVM, using biopsy studies. From previous studies using videomicroscopy by Motley and Lanigan [9,29], it is established that there may be abnormality within either the superficial papillary dermal plexus (type 1), the deep reticular dermal plexus (type 2), or a mixed pattern. In this study, the measured capillary depth was found to increase following treatment. This suggests that as vessels that are more superficial are treated the deeper underlying vessels are revealed; these vessels being obscured by more superficial vessels prior to treatment. If the CVM is mainly type 1 pattern then the measured depth will be down to the surface of the superficial capillary plexus and if the pattern is type 2 then the measurement will be to the surface of the deep capillary plexus.

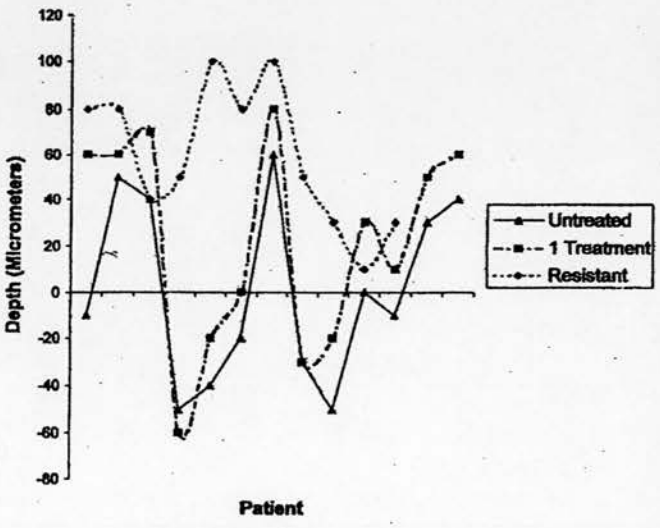

Fig. 5. CVM. Capillary depth compared to normal contralateral capillary depth before and after pulsed dye laser treatment and including a cohort of resistant patients for comparison. Negative values are closer to the skin surface and positive values deeper.

In this study, the vessel diameters correspond well to previous biopsy studies. For vessel depth, however, the values obtained appear smaller than previous studies. We believe the reason for this to be two fold; firstly, our measurements have been calculated to the level of the most superficial ectatic dermal capillaries, whether these are part of the superficial type 1 plexus or deeper type 2. We believe this to be a more realistic measurement to follow response of CVMs to laser treatment rather than total depth of the CVM vessels as these deeper vessels are unlikely to sufficiently improve before the more superficial vessels are cleared. It seems likely that predicting eventual treatment outcome is most likely to depend on the depth of the deeper vessels, and we therefore believe the depth measurements obtained using the DMV are most likely to be useful when taken after the more superficial capillaries have been cleared by four or five dye laser treatments. Such measurements may be useful in guiding changes to laser treatment parameters, for example using longer wavelengths. Secondly, the measurements we have obtained may not have been taken exactly from the surface of the stratum corneum. Because of this, we measured depth of contralateral normal capillaries and compared them between the two examinations. As there was no statistically significant difference between the normal depth measurements before or after the laser treatment, we believe our

depth measurements to be valid. We believe there is no loss of resolution when imaging the deep reticular plexus versus the superficial papillary plexus. We are currently performing a further investigation with the aid of biopsies to study this.

The ability to assess CVM structure may allow newer pulsed dye lasers to treat CVMs more efficiently as pulse duration and wavelength could be matched to measured vessel diameter and depth, respectively. In addition, the use of Cryogen Spay Cooling may be used more accurately when depth to CVM vessels can be calculated [24]. By measuring depth and diameter of vessels it may be possible to determine more accurately when a CVM becomes resistant to further treatment and hence prevent patients receiving unnecessary treatments.

Although ectatic capillary depth and diameter are important factors that determine the response of a CVM to laser treatment, other characteristics also play a role. The flow through the vessels and the presence of high concentrations of other chromophores, such as melanin, will also be important factors governing how a particular CVM responds to treatment. Further work is needed to establish how each factor: depth, diameter, flow, and melanin contribute together to the response of CVMs to laser treatment.

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Noninvasive In Vivo Assessment of Vessel Characteristics in Capillary Vascular Malformations Exposed to Five Pulsed Dye Laser Treatments

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Background: The treatment of capillary vascular malformations by pulsed dye laser results in fading of these disfiguring lesions in the majority of patients. In only a minority, however, is full clearance of the lesion achieved. It is believed that the capillary composition of a capillary vascular malformation is an important determinant of whether it will respond to further laser treatment. Moreover, by determining the type, size, and depth of the ectatic capillaries within a capillary vascular malformation, it may be possible to target these vessels with specific laser parameters.

Methods: The noninvasive technique of depth measurement videomicroscopy was used to delineate the capillary structure of 22 previously untreated capillary vascular malformations and examine how this structure changes after five treatments with a 0.45-msec pulse duration using a 585-nm pulsed dye laser.

Results: After one and five treatments, there was a statistically significant lightening ($p < 0.02$ and $p < 0.001$, respectively) of the lesions, as seen on Munsell Color Chart testing. Before any laser treatment, the majority (59 percent) of capillary vascular malformations displayed a superficial type 1 or mixed capillary pattern, whereas after five laser treatments, the majority displayed a deep type 2 pattern (81 percent). After five laser treatments, there was a statistically significant increase in the depth of the remaining capillaries within the lesion compared with normal skin ($p < 0.02$) and a statistically significant reduction in the vessel diameters ($p < 0.001$).

Conclusions: The authors found that vessels with a diameter greater than 50 μm were adequately treated, whereas those smaller than 50 μm appeared resistant to laser treatment. These data would suggest that pulse durations longer than 0.45 msec are not required to treat large ectatic capillary vascular malformation vessels. The authors suggest that the failure to treat very-small-diameter vessels is attributable to thermal dissipation from the target vessels, whose thermal relaxation time is much shorter than the pulse duration of the laser used. (*Plast. Reconstr. Surg.* 115: 1245, 2005.)

The treatment of capillary vascular malformations, or port wine stains, by pulsed dye laser is widely regarded as the optimum treatment for these disfiguring lesions.[1–7] Capillary vascular malformations tend to appear at or shortly after birth and can have profound psychological effects on both the growing child and the relatives.[8–12] As described by Mulliken and

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Glowacki, these lesions consist of an area of ectatic dermal capillaries, which show normal endothelial cell turnover and grow with the child.[13] Previous studies have demonstrated the efficacy of treatment both for adults and for children.[1,3,4,6,14] However, results are unpredictable, and the majority of patients do not achieve full clearance of their lesions.[15]

The lack of response of capillary vascular malformations to laser treatments is believed to be attributable in part to the morphology of the vessels constituting the malformation.[15–19] Previous work has established that by adhering to the principles of selective photothermolysis, the treatment of capillary vascular malformations can be performed with a low complication rate.[20,21] This theory states that by matching the wavelength of the incident laser light to the absorption spectrum of a particular chromophore within the skin (in this case, oxyhemoglobin), preferential damage can be caused to this compound. In the case of treating capillary vascular malformations, it is the heating of oxyhemoglobin that damages the capillaries within the lesion. Also, by choosing longer wavelengths of light, deeper penetration of the skin can be achieved, although with a commensurate loss of specificity for the chromophore.[22,23]

Furthermore, this theory states that by choosing pulse durations less than the thermal relaxation time of the target vessel (the time taken to lose half the incident energy), diffusion of heat away from the target will be minimized. It is this diffusion of heat into the surrounding structures within the skin that causes the complications of skin pigment change and scarring that marred early laser treatment.[24] The goal of current laser treatment of capillary vascular malformations is to be able to match the wavelength and pulse duration of the incident laser light to the characteristics of the target vessels within the capillary vascular malformation, namely, vessel diameter and depth.[19,25–27]

The aim of this study was, first, to see how the vessel characteristics of untreated port wine stains would alter after five laser treatments, when the majority of any achievable improvement had been achieved.[14,28] By noting the vessel characteristics after treatment, it should be possible to elucidate the ideal laser properties with which to treat these now resistant capillary vascular malformations. Second, we wanted to see whether the eventual outcome could be predicted from the initial capillary characteristics of the untreated capillary vascular malformations.

Patients and Methods

The delineation of capillary vascular malformation vessel characteristics was carried out using the technique of depth measurement videomicroscopy. Between January of 2002 and April of 2003, 22 previously untreated patients were assessed using this technique before and 6 weeks after one laser treatment and 6 weeks after five full treatments or before if the lesion had totally cleared.

The depth measurement videomicroscopy consisted of a 200× Cy-scope lens on a compact videomicroscope (PW Allen, Tewkesbury, United Kingdom). Before the examination was performed, patients were rested in a temperature-controlled room at 28°C for 20 minutes. This was done to reduce the effect of sympathetic stimulation on the capillary vascular malformation microvasculature and also to maximally vasodilate the vessels at a reproducible temperature. The patients were then asked to carry out a combined skin type test that evaluated their Fitzpatrick skin type and any recent sun exposure.

Color was recorded by two individuals using a Munsell Color Chart (GretagMacbeth, New Windsor, N.Y.),[29] and these values were converted to an ordinal scale to aid statistical analysis. The examination involved using depth measurement videomicroscopy to record the contralateral normal capillary depth before taking three recordings from the capillary vascular malformation–affected skin. Multiple recordings were taken to reduce the error incurred from evaluating a heterogeneous lesion. These recordings gave values for the depth of the ectatic capillaries from the skin surface, and images were then taken and diameter measurements obtained by using the videomicrograph of a 1-mm graticule (Graticules Ltd., Tunbridge, United Kingdom), with 10-μm gradations.

From the depth measurement videomicroscopy examination, the type of capillary ectasia was also noted. Previous studies have suggested that type of capillary ectasia (with type 1 being superficial and type 2 being deep) is an important determinant of response.[30,31]

All treatments were carried out using a 0.45-msec, 585-nm pulsed dye laser (SLS Chromos, Wales, United Kingdom). Initial treatment pa-

rameters were the same in all patients: 7-mm spot size, 6.4-j/cm^2 fluence, with topical ice epidermal cooling. Fluences were changed as required during the five treatments to maintain response.

RESULTS

Twenty-two patients entered the trial. Two patients failed to attend their examination after one treatment but did attend the following five and are thus included in the study. Two patients had their final assessment before five treatments, one at three and another at four treatments because their lesions had fully faded. All statistical analyses were performed using SPSS version 10 (SPSS, Inc., Chicago, Ill.).

One patient in the study had Fitzpatrick skin type 4, and all the others had skin types 1 or 2. There was no statistically significant difference in combined skin type test scores between the examinations using a Wilcoxon signed rank test, indicating that sun exposure was not a significant confounding variable within this study. From Figure 1, it can be seen that before any treatment, more of the patients' capillary vascular malformations showed a type 1 or mixed pattern (59 percent) than they did after five treatments (18 percent). This reduction in the proportion of patients demonstrating a superficial type 1 pattern after five treatments is statistically significant using a Wilcoxon signed rank test ($p < 0.02$).

When capillary vascular malformation capillary depth was compared with normal capillary depth, there was a statistically significant increase in the depth of the recorded capillary vascular malformation vessels after five laser treatments ($p < 0.02$) (Fig. 2). Capillary vascular malformation vessel depth was compared with contralateral capillary depth to reduce error incurred because of the zeroing of the depth measurement videomicroscopy on the skin surface.

When capillary diameter was examined, there was a statistically significant reduction in the values using a Wilcoxon signed rank test ($p < 0.001$) (Fig. 3). After treatment, the diameters of the capillaries remaining within the capillary vascular malformations were between 10 and 50 μm, with the larger vessels having been cleared. Figure 4 shows videomicrographs obtained from the same patient before any laser treatment and after five treatments. These images demonstrate the clearance of large-diameter type 1 vessels, leaving smaller diameter type 2 vessels present after five treatments.

For Munsell Color Chart assessment, there was a statistically significant fading in the lesions after one and after five treatments ($p < 0.02$ and $p < 0.001$, respectively) (Fig. 5). If the eight patients who had the greatest fading of their capillary vascular malformation (as evaluated by largest change in modified Munsell Color Chart score) are compared with the eight patients who had the least fading, no statistically significant difference can be found between the two groups in either depth or diameter.

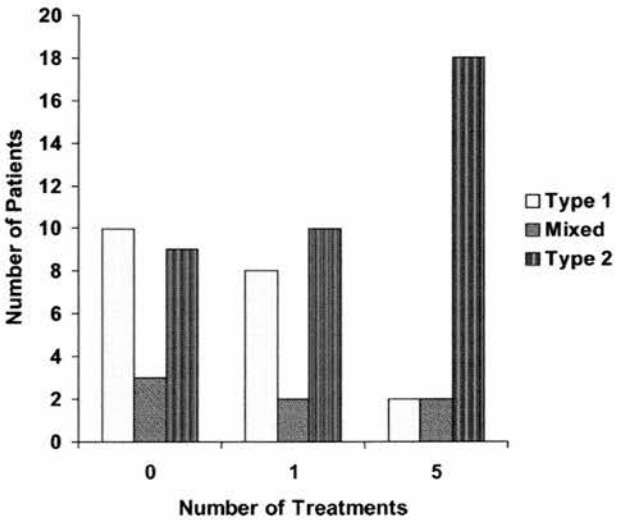

FIG. 1. Histogram type of capillary ectasia found in patients exposed to five pulsed dye laser treatments.

DISCUSSION

In this study, we have attempted to investigate in vivo the change to capillary vascular malformation capillary depth and diameter after five pulsed dye laser treatments. In a majority of cases, most fading of a capillary vascular malformation will occur within the first five treatments, although some longer-term treatment regimens may be able to achieve further modest improvement.[32]

As Fiskerstrand et al. and Hohenleutner et al. have noted in biopsy studies, these data support the hypothesis that it is smaller diameter and deeper capillaries within a capillary vascular malformation that remain untreated by pulsed dye laser treatment.[17,18] This study demonstrates this noninvasively and in vivo by use of the depth measurement videomicroscope.

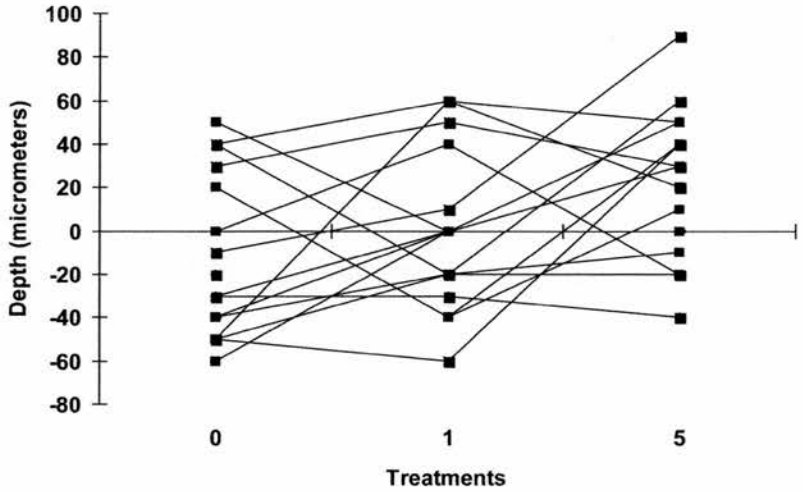

FIG. 2. Line chart showing the depth of capillaries within capillary vascular malformations compared with normal contralateral skin before and after one treatment and after five treatments. Zero on the *x* axis corresponds to normal capillary depth. Positive values are deeper and negative values are closer to the skin surface. *0*, untreated capillary vascular malformations; *1*, after one treatment; *5*, after five treatments.

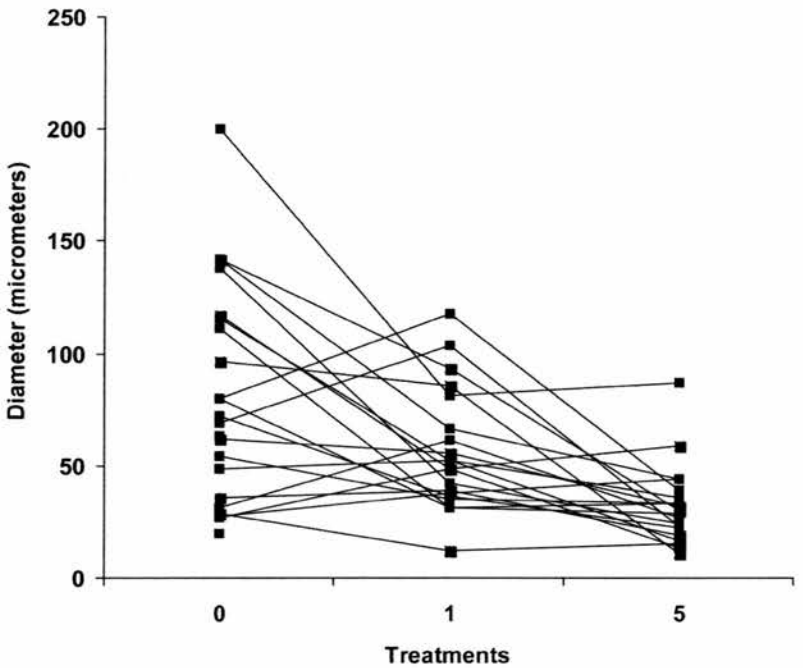

FIG. 3. Line chart showing the diameter of capillaries within capillary vascular malformations before, after one laser treatment, and after five laser treatments. *0*, untreated capillary vascular malformations; *1*, after one treatment; *5*, after five treatments.

Recent advances in the development of pulsed dye lasers have allowed longer wavelengths to be generated than the traditional 585 nm used in this study. It is possible that these longer wavelengths will treat to a greater depth in a capillary vascular malformation despite the less favorable specificity for oxyhemoglobin.[22,23] The use of longer wavelength pulsed dye lasers coupled with more effective skin cooling systems, and thus allowing higher fluence treatment, may treat deeper vessels than the 585-nm laser used in this study.

This study demonstrates that the vessel diameters that are not being cleared by the laser

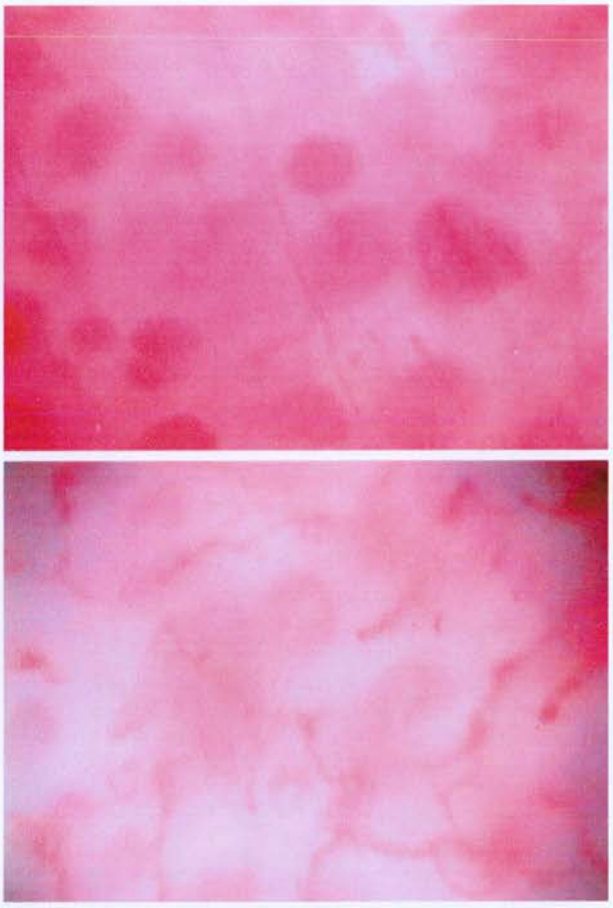

FIG. 4. (*Above*) Depth measurement videomicrograph (200× magnification) of a patient before any laser treatment, demonstrating large-diameter type 1 capillary ectasia. (*Below*) Depth measurement videomicrograph of the same patient as *above* after five pulsed dye laser treatments, demonstrating the clearance of larger vessels; the smaller-diameter type 2 capillaries remain.

treatment are between 10 and 50 μm. The original work carried out by Anderson and Parrish into the theory of selective photothermolysis proposed that, to achieve photocoagulation by means of specific thermal damage and thus limit complications, a laser with a pulse duration less than the thermal relaxation time of the target vessels would be required.[20] For cylindrical vessels, the theoretical thermal relaxation time of a vessel of known diameter based on a gaussian temperature distribution is given by the following equation[20]:

$$Tr = D^2/16_K, \qquad (1)$$

where Tr is thermal relaxation time, D is diameter of the capillary vascular malformation vessel, and $_K$ is thermal diffusivity of blood (1.3×10^{-3} cm^2 second^{-1}).

Previous work by Dierickx et al. using purpura threshold fluence to probe capillary vascular malformation vessels found a close correlation between in vivo measurements and those proposed by Anderson and Parrish, based on a gaussian temperature model.[16] This model predicts maximum heating of the vessel center after irradiation by a uniform collimated beam and temperature toward the periphery of the vessel falling in a gaussian curve. The study by Dierickx et al. proposes pulse durations of between 1 and 10 msec for vessels between 50 and 150 μm. In this study, we have found that larger vessels of between 50 and 200 μm are adequately treated with a 0.45-msec pulse duration laser. It may thus be concluded from these data that longer pulse durations are not required to treat larger diameter capillary vascular malformation vessels.

The in vivo determination of thermal relaxation time by Dierickx et al. examined capillary vascular malformations using two pulsed dye laser pulses, which were both nonpurpuric and separated in time.[16] By considering the fluence required of the second pulse at a known time delay required to cause a purpuric reaction and comparing this with the fluence required to produce purpura by a single pulse, Dierickx et al. were able to determine the thermal relaxation of the target vessels. This would suggest an ideal pulse duration of between 1 and 10 msec, to adhere to the principle of selective photothermolysis. However, in our study, we found that larger vessels are adequately treated despite using a laser with a relatively short pulse duration. It seems that it is unnecessary to treat these large vessels with a pulse duration equal to their thermal relaxation time, and shorter pulse durations would cause sufficient damage to clear the vessel. This would fit with the recent trend toward successful capillary vascular malformation treatment using nonpurpuric fluences.[33,34]

A possible theory for the poor treatment of smaller vessels was initially proposed by Anderson and Parrish[20] and has been predicted through Monte Carlo modeling.[19] This suggests that if the thermal relaxation time of the target vessel is much shorter than the pulse duration of the incident laser, the target may remain untreated because of the greater heat dissipation into the surrounding structures.[20] In our study, 13 of the 22 patients examined were found, after five treatments, to have vessels with diameters that (from the above equa-

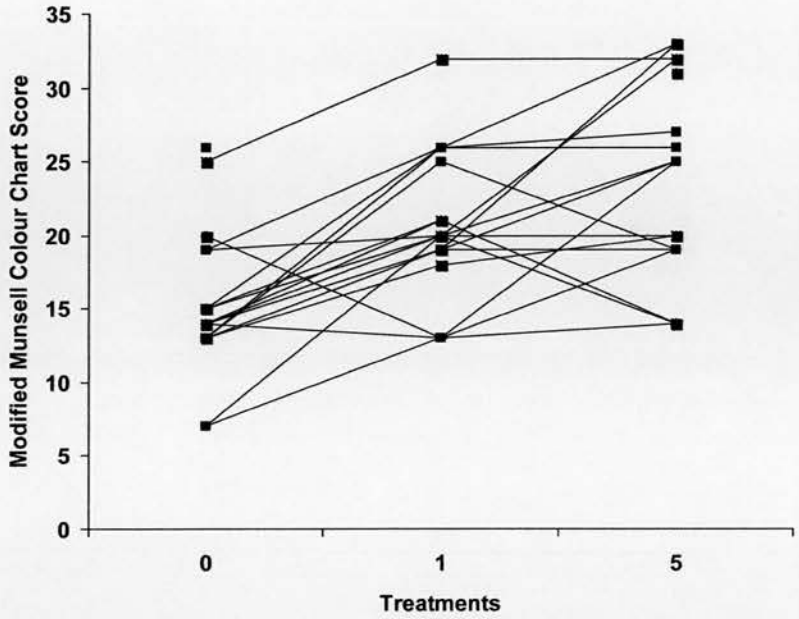

FIG. 5. Line chart color change as evaluated by the Munsell Color Chart before and after one and five laser treatments. The higher values for the modified Munsell Color Chart score represent paler lesions. *0*, untreated capillary vascular malformations; *1*, after one treatment; *5*, after five treatments.

tion) would give a thermal relaxation time less than the 0.45-msec pulse duration of our laser (Fig. 3).

The use of pulse durations of 20 μsec and less not only causes destruction to the target vessels within a capillary vascular malformation but also causes widespread collateral damage by vaporization and mechanical photodisruption.[21,35] Therefore, treatment of smaller diameter capillaries requires a laser with a pulse duration longer than this and less than 450 μsec as used in this study. Further investigation is required to examine the effect of using shorter pulse durations once larger vessels have been cleared. Shorter pulse durations in darker Fitzpatrick skin types should be used with caution to avoid epidermal damage.[36]

Previous work using traditional non–depth-measuring videomicroscopy carried out by Motley et al.[31] suggested that type of capillary ectasia, either type 1 superficial or type 2 deep, found in a particular capillary vascular malformation could give prognostic information as to how it would respond to laser treatment. In our study, we have found a correlation between type of vessel ectasia and color of a capillary vascular malformation, suggesting that type 2 lesions were generally paler in color than type 1 lesions. This would fit with the previous belief that paler lesions tended to be located deeper in the skin than darker lesions. We also found that, after treatment, initially the majority of capillary vascular malformations had a superficial type 1 plexus of capillaries, whereas by the fifth treatment, the majority of lesions consisted of type 2 deeper plexus. We found no statistically significant difference in either final color or degree of color change between the initially type 1 capillary vascular malformations and the initially type 2, using a Wilcoxon signed rank test. Therefore, from these study data, we found no evidence that initial type of capillary ectasia gives prognostic information as to how a capillary vascular malformation will respond to laser treatment.

This study revealed no statistically significant correlation between response and either vessel diameter or depth. The initial capillary characteristics of an untreated capillary vascular malformation are thus not predictive of eventual outcome. A reason for this may be that other factors play a role. This study gives no information as to flow in the capillary vascular malformation vessels. Previous studies have demonstrated that some capillary vascular malformations have greater flow than the surrounding unaffected skin.[37,38] The combined effect of flow and capillary composition requires further study.

The other aim of this study was to evaluate how the vessel characteristics of capillary vascular malformations were altered after pulsed dye laser treatment. This study suggests that the vessels remaining after treatment tended to be located deeper and to be smaller in diameter. The use of longer wave lengths may allow deeper vessels to be treated, and this effect requires further investigation. The lack of response from smaller vessels would tend to suggest that shorter pulse durations would produce further improvement. The latest pulsed dye lasers generate their pulse using a series of very short micropulses, rather than the smooth output of the pulsed dye laser we have used.[39] The effect of this change in beam characteristics on smaller diameter capillaries is unknown.

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The Effect of Varying Pulse Duration, Wavelength, Spot Size, and Fluence on the Response of Previously Treated Capillary Vascular Malformations to Pulsed-Dye Laser Treatment

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Abstract: Modern lasers allow different parameters to be altered in an effort to gain further improvement in otherwise resistant capillary vascular malformations (CMs). The aim of this study was to examine the effect of changing the pulse duration, wavelength, spot size, and fluence on the color and capillary architecture of 585-nm pulsed-dye, laser-resistant CMs. Eighteen patients were assessed with a depth measurement videomicroscope (DMV) before and after 12 test patches with ScleroPlus and V-Beam lasers at specified parameters. In the majority of the test patch areas, there was little improvement after treatment. However, 44% of patients had greater than 75% clearance in at least 1 test patch site. This study demonstrates that both lasers can achieve further lightening in 585 nm 0.45 msec pulsed-dye laser-resistant CMs. However, in CMs consisting of small-diameter deep vessels, further improvement is unlikely.

Key Words: port wine stain, depth measurement videomicroscopy

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Capillary vascular malformations (CMs) or port wine stains can be treated with 585-nm pulsed-dye laser (PDL). The results of this treatment are variable, with only a minority of patients receiving full clearance of their lesions.[1] Previous studies have established that this lack of response is a consequence of the vessel morphology,[1–5] suggesting that mismatch of vessel size and depth with laser pulse duration and wavelength are important considerations. The theory of selective photothermolysis states that for optimal treatment, a wavelength of laser light should be chosen that corresponds to the absorption spectrum of the chromophore to be treated, in this case, oxyhemoglobin within the ectatic dermal capillaries of the malformation.[6] Also, this laser light should be pulsed with a pulse duration less than the thermal relaxation time of the target. The thermal relaxation time is the time taken for the target (dermal capillary) to lose half the energy imparted to it by the laser. This prevents the buildup of heat within the capillaries and its conduction away to neighboring structures, thus reducing complications such as scarring and pigment change.[7] The initial choice of wavelength for the PDL was 577 nm because this corresponds to a peak in the oxyhemoglobin absorption spectrum. It was found, however, that by choosing 585 nm, better results were obtained despite this wavelength having less affinity for the absorption spectrum of oxyhemoglobin.[8] The reason for this is that longer wavelengths penetrate the skin deeper and can thus reach capillaries out with the range of the 577-nm laser. The longer wavelengths are also able to penetrate the ectatic capillary walls better and thus are more effective at heating the center of the vessel.[9] To compensate for the reduction in specificity for hemoglobin, a higher energy (fluence) is required with the 585-nm laser.

Capillary vascular malformations are heterogeneous lesions consisting of abnormal dermal capillaries of differing diameters and depths.[10] The pulse duration of many 585-nm PDLs is fixed at 0.45 msec. Previous studies have suggested that this pulse duration is not adequate to treat many of the vessels within the lesion.[2,11–13]

More recently, third-generation PDLs have become available. Two of these, the ScleroPlus (Candela Corp., Wayland, MA) and V-Beam lasers (Candela Corp.), have the ability to alter the wavelength (ScleroPlus) and pulse duration (V-Beam) they produce. Both lasers also use a cryogen spray cooling (Dynamic Cooling Device [DCD]; Candela Corp.) device to cool the skin and allow increased fluences to be used while providing epidermal protection.[14] The ScleroPlus laser allows the wavelength of the laser to be altered from 585 nm to 600 nm in 5-nm increments. This laser produces higher fluences than the previous 585-nm, 0.45-msec PDLs to compensate for the reduction in specificity for oxyhemoglobin at these higher wavelengths. The pulse duration of the ScleroPlus laser is fixed at 1.5 msec. The V-Beam laser, however,

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allows the pulse duration of the laser to be altered from 1.5 msec to 40 msec with a fixed wavelength of 595 nm.

It has been suggested that these lasers would allow greater fading of CMs that have become resistant to 585-nm PDL.[14–17] The aim of this study was to treat previously resistant patients with these newer lasers and to investigate the influence of changing the spot size, pulse duration, wavelength, and fluence on the capillary characteristics and color of these lesions. Also, by examining the capillary dimensions of resistant CMs, it was hoped that predictive information could be established as to whether a lesion would respond to further laser treatment and what would be the most appropriate settings to use.

METHODS

Twenty-five patients were randomly selected from a database of 250 resistant CMs previously treated with a 585-nm, 0.45-msec pulse dye laser (SLS Chromos, Wales, U.K.). All patients on this database had received a minimum of 5 PDL treatments. They had been assessed with the use of standardized photography and determined to be no longer achieving improvement. The only selection criterion was that their CM was large enough to allow a 3 × 4-cm grid to be placed on an area of uniformly colored malformation.

Each patient was rested within a temperature-controlled room at 28°C for 20 minutes to reduce error from sympathetic stimulation and maximally vasodilate the capillaries. A location was selected on the patients' CM that would allow a 3 × 4-cm grid to be taped onto the skin.

To record the vessel characteristics of diameter and depth, we used the technique of depth measurement videomicroscopy.[18] This technique uses a Cy-scope lens (P. W. Allen, Tewkesbury, U.K.) to measure depth to the superficial vessels within a CM and their diameter.

Three recordings were taken from the normal contralateral side of the patient and then 5 recordings from the area to be covered with the grid. The grid was then taped on to the patient and a digital photograph of the area taken. This photograph was taken to aid the replacement of the grid in the same place when the patient returned for assessment.

The treatment was then carried out by test patching the grid with the parameters outlined in Table 1. These parameters were chosen so that only 1 variable (spot size, wavelength, fluence, or pulse duration) was altered between 2 test patches. For the ScleroPlus laser, 3 of the test patches with the 7-mm spot were carried out with the same fluence (12 j/cm^2) and pulse duration (1.5 msec) and only wavelength altered (585 nm, 590 nm, 595 nm). These were undertaken at 12 j/cm^2 because a higher fluence at a 585-nm wavelength was deemed potentially unsafe. The test patches at 585 and 590 nm were then carried out with a 5-mm spot but with the same fluence (12 j/cm^2) so to give a comparison between a 5-mm and 7-mm spot size. For 595 nm and 600 nm with the 5-mm spot, we used the maximum fluence achievable with the ScleroPlus laser (14 j/cm^2 and 20 j/cm^2, respectively), because longer wavelengths will require higher fluences to be effective as a result of the lack of specificity for oxyhemoglobin. Lastly, a test patch was performed with the ScleroPlus at 595 nm, 14 j/cm^2, and a 7-mm spot so as to give a comparison with the V-Beam laser using exactly the same settings but with a different output profile.[12] The V-Beam laser relies on a series of micropulses rather than the smooth output seen with the ScleroPlus and earlier laser systems.

TABLE 1. Parameters Used for Test Patches

Laser	Wavelength (nm)	Fluence (j/cm^2)	Spot Size (mm)	Pulse Duration (ms)
ScleroPlus	600	20	5	1.5
	595	14	5	1.5
	590	12	5	1.5
	585	12	5	1.5
	585	12	7	1.5
	590	12	7	1.5
	595	12	7	1.5
	595	14	7	1.5
V-Beam	595	14	7	1.5
	595	14	7	3
	595	14	7	6
	595	14	7	10

For the V-Beam treatments, the parameters of fluence and spot size were kept constant and only pulse duration changed. The maximum pulse duration chosen was 10 msec because from previous studies, a longer pulse duration was unlikely to be suitable.[2] With both laser systems, the DCD cryogen spray cooling systems were kept at 30-msec spray duration and 30-msec delay for all the treatments.[19,20]

The patients returned for assessment 3 months later and were again rested in a temperature-controlled room at 28°C and the grid replaced with the aid of the digital photograph. A blinded impartial observer using a standardized outcome scale then assessed the percentage improvement in color in each of the test patch sites. The assessment scale rated the improvement in color of the CM subjectively on a scale of: >75% as excellent, 50–75% as good, 25–50% as moderate, <25% as poor, and zero as no improvement. A DMV examination was then performed at each site to determine any changes to the vessel structure after treatment.

Data Analysis

Two analyses of variance (ANOVAs) were carried out, of depth measurements and of the natural logarithm of diameter measurements, respectively.[21] A logarithmic transform of capillary diameter was used to achieve approximate homogeneity of variance. Patients were treated as a "blocking factor" in the analyses, and the treatment factor had 13 levels, which included a level for "pretreatment measurement" (Figs. 1 and 2). Each ANOVA table yielded 2 F tests: first, a test of the overall effect of laser treatment averaged over all 12 treatments, and second, a test of the null hypothesis of no difference between the effects of these 12 treatments. Regardless of the outcome of the second of these hypothesis tests, various follow-up tests were also carried out comprising 2 F tests of the effects of differing pulse durations for the V-Beam laser and the effects of differing wavelengths for the ScleroPlus laser (at fluence 12 j/cm^2 and spot size 5 mm),

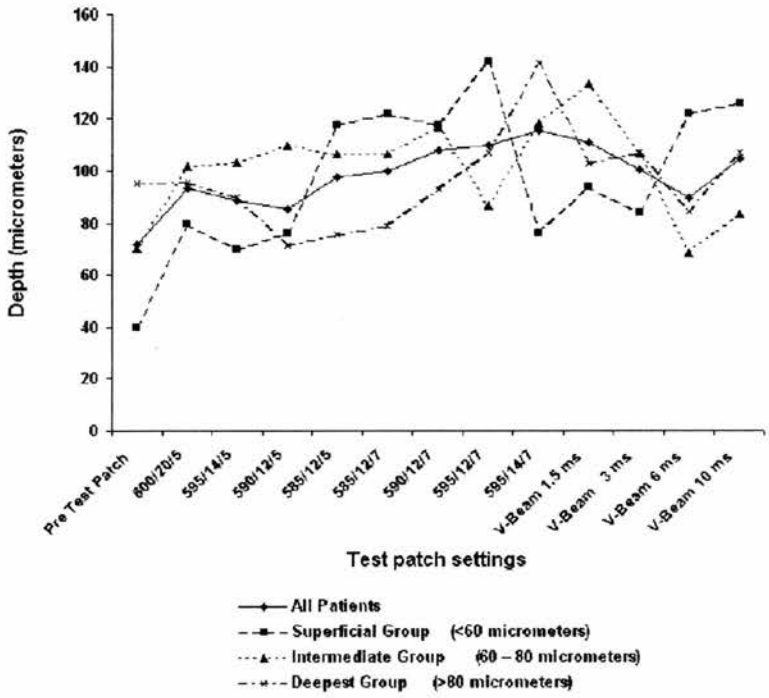

FIGURE 1. Capillary depth measurements after test patches for all patients and 3 groups based on initial capillary depth.

respectively, and various *t* tests of comparisons of interest. Note that all follow-up tests were formulated in advance of data collection. For F tests, the relevant sums of squares were obtained from the full ANOVA sum of squares for treatments, partitioned appropriately, and for *t* tests, the residual mean square from the ANOVA was used as an estimate of error variance.

Differences between the 12 treatments in the percentage improvement in color were tested for statistical significance using Friedman's test.[21]

RESULTS

Five patients failed to attend for their review, 1 patient's CM was insufficiently large to allow the placement of

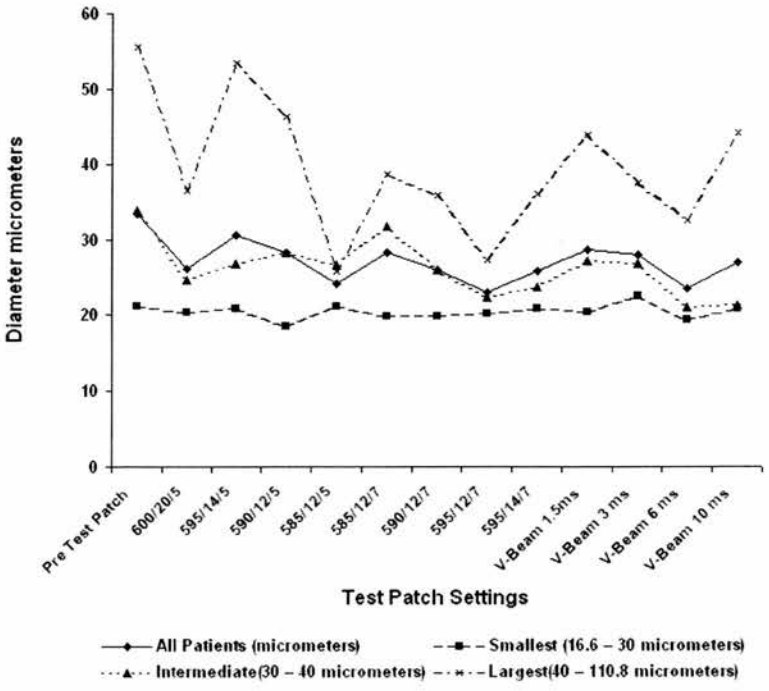

FIGURE 2. Capillary diameter measurements after test patches for all patients and 3 groups based on initial capillary diameter.

TABLE 2. Initial Capillary Measurements and Best Response to Each Laser System

Patient No.	Initial Depth (μm)	Initial Diameter (μm)	Best Response	
			ScleroPlus	V-Beam
1	73.3	15.6	<25	<25
2	42	19.3	25–50	0
3	66	19.3	>75	50–75
4	66	19.3	>75	>75
5	123.3	23.3	0	0
6	50	25.3	<25	0
7	92	29.3	25–50	25–50
8	53.3	30	25–50	25–50
9	96.7	34.4	50–75	50–75
10	16	35	>75	<25
11	38	35.3	>75	50–75
12	80	35.3	>75	>75
13	80	41.1	>75	50–75
14	74	45.3	>75	>75
15	86	48.7	<25	>75
16	66	49.3	<25	0
17	76.7	60	25–50	50–75
18	110	110.8	25–50	25–50
Mean (standard deviation)/ median	71.63 (26.09)	37.59 (21.93)	25–50	Between 25–50 and 50–75

the grid on a uniform area of malformation, and 1 patient had received previous treatment with a Copper Vapor laser and so are excluded from the study. The remaining 18 patients attended for review. These patients had received a mean of 18.8 previous laser treatments with a 585-nm, 0.45-msec PDL (range, 5–30). The mean time since last treatment was 22 months (range, 4–54 months; standard deviation, 13.9). All patients were being seen on a yearly review basis with photographic assessment.

The Effect of Treatment on Color of the Capillary Vascular Malformation

When the effect of treatment on the color of the CMs was analyzed, there was found to be a great variation in the response. Only 8% of the test patches undertaken showed more than 75% improvement in the color of the CM, and only 17% demonstrated a greater than 50% improvement, whereas 61% showed either no improvement or less than 25%. None of the test patch settings managed a median improvement of greater than 50% improvement. Using Friedman's test, there was found to be no statistical difference in the subjective assessment of improvement between the different test patch settings overall. However, 8 patients (44%) had greater than 75% clearance of their malformation in at least 1 of the test patches, and 10 (55%) had more than 50% improvement. Therefore, although no 1 setting was superior to the others statistically in lightening the color of the CMs, more than half the patients received a significant amount of lightening in at least 1 test patch area. There was little difference in the ability of the ScleroPlus or V-Beam lasers in eliciting a significant improvement in a test patch site (8 of 18 and 9 of 18 patients receiving over 50% clearance, respectively).

The initial capillary depths, diameters, and the best response achieved by both laser systems are shown in Table 2. As can be seen, those CMs that did respond to laser treatment tended to respond to both laser systems (Spearman rank correlation coefficient 0.614, $0.002 < P < 0.01$). No correlation can be found between initial capillary measurements and eventual outcome in terms of color change, although CMs with small, deeply located vessels tended to improve least after treatment.

The Effect of Treatment on Capillary Depth and Diameter

When depth of the capillaries within the CM was examined using the DMV and analyzed using ANOVA, there was found to be a statistically significant increase in the vessel depths after treatment ($P = 0.005$, see Fig. 1). The effect of laser treatment, averaged over the chosen parameter settings and the 2 laser systems, was to increase mean capillary depth from 71.6 μm to 100.3 μm. However, when the test patch settings were considered individually, there was no statistically significant difference between them (F test $P = 0.477$).

For vessel diameter, there was a statistically significant effect of treatment overall (Fig. 2, F test $P = 0.008$). Geometric mean capillary diameter decreased from 33.3 μm to 26.5 μm. However, again, there was no statistically significant difference found between the different test patch settings (F test $P = 0.37$).

The Effect of Altering Pulse Duration

Changing the pulse duration of the V-Beam laser demonstrated that the 1.5-msec pulse duration tended to have the

most effect on vessel depth (see Fig. 1). However, an F test of differences between the 4 mean depths was not statistically significant ($P = 0.485$).

The effect of changing the pulse duration had no statistically significant effect on the capillary diameters overall (F test $P = 0.36$).

The Effect of Altering Spot Size

When the effect of increasing the spot size from 5 mm to 7 mm is compared across all the patients (fluence 14 j/cm^2, wavelength 595 nm), the 7-mm spot size tended to have more of an effect on vessel depth than the 5-mm spot size and this approaches statistical significance (t test $P = 0.056$).

There was no statistically significant effect found from altering the spot size on the vessel diameter (t test $P = 0.15$).

The Effect of Altering Wavelength

For the ScleroPlus laser, the effect of changing the wavelength from 585 through 590 to 595 nm (fluence = 12 j/cm^2, spot size 7 mm) on the depth of the capillaries was not significant with no particular wavelength statistically better than any of the others overall (F test $P = 0.75$, t test for trend $P = 0.47$).

For the ScleroPlus laser, there was no evidence that the effect on capillary depth of varying the wavelength from 585 nm to 590 nm depended on whether the spot size was 5 mm or 7 mm (recall that the fluence setting for the 4 patches concerned was 12 j/cm^2). That is, in statistical terminology, there was no evidence of interaction between wavelength and spot size. In view of this result, t tests were carried out of the effect of wavelength averaged over the 2 spot sizes and of spot size averaged over the 2 wavelengths. In both these tests, neither the effect of wavelength nor of spot size was found to be statistically significant.

Changing the wavelength from 585 through 590 to 595 nm (fluence = 12 j/cm^2, spot size 7 mm) appeared to reduce the diameters of the capillaries, and the trend, although strictly not statistically significant, was suggestive of a possible effect (t test for trend $P = 0.076$).

No significant interaction was found between wavelength (585 and 590 nm) and spot size (5 and 7 mm) in respect of their effects on diameter at a fluence setting of 12 j/cm^2 (t test $P = 0.14$). Furthermore, the effects on diameter of wavelength averaged over the 2 spot sizes, and of spot size averaged over the 2 wavelengths, were not significant (t test $P = 0.64$, 0.66, respectively).

The Effect of Increasing Fluence

For increasing the fluence of the ScleroPlus laser from 12 to 14 j/cm^2 (wavelength 595 nm, spot size 7 mm), there was no statistically significant effect seen, although the mean depths increased slightly after treatment with higher fluences (Fig. 1).

There was no statistically significant effect found of increasing the fluence on the vessel diameters (t test $P = 0.30$).

Comparison of 2 Laser Systems

When the 2 lasers were used on the same settings (595 nm, 14 j/cm^2, spot size 7 mm, 1.5-msec pulse duration), there was no statistically significant difference found between the results obtained either on vessel depth (t test $P = 0.72$) or on vessel diameter (t test $P = 0.41$).

The Effect of Vessel Morphology on Outcome

Vessel Depth

The second part of this study was to examine if the ideal laser parameters for a particular CM could be selected from the vessel characteristics measured using the DMV. For the analysis of capillary depth, the patients were grouped into 3 similar-sized groups on the basis of initial capillary depth (Fig. 1). Thus, the 3 groups consisted of a "superficial" group of 5 patients with vessels less than 60 μm from the skin surface, an "intermediate" group of 6 patients with vessels between 60 to 80 μm, and a "deep" group of 7 patients with vessels deeper than 80 μm.

Capillary Vascular Malformations With Superficial Vessels

From Figure 1, it can be seen that for the superficial group, all the test patch sites showed an increase in vessel depth after treatment, and this is statistically significant (F test $P = 0.005$). However, differences between treated patches were not statistically significant (F test $P = 0.162$). For these superficial vessels, there was no statistically significant difference found between using a 7-mm spot size and a 5-mm spot size with the ScleroPlus laser (wavelength 595 nm, fluence 14 j/cm^2). For the V-Beam laser, the longer pulse durations tended to increase capillary depth more than the shorter pulse durations for this group of patients, although this was not statistically significant ($P = 0.13$). Increasing the fluence from 12 j/cm^2 to 14 j/cm^2, at a wavelength 595 nm and a spot size of 7 mm, had a statistically significant effect on the results achieved in this group ($P = 0.025$).

Capillary Vascular Malformations With Intermediate-Depth Vessels

In the intermediate-depth group, there was again a significant increase in vessel depth with treatment (F test $P = 0.033$). However, like for the superficial group, an F test showed no significant differences between treated test patches. This result is consistent with Figure 1, in which there appears to be little difference between the results obtained for any of the ScleroPlus test patch settings, although 595 nm, 14 j/cm^2, 7-mm spot had the greatest effect on vessel depth. Again, for this wavelength and fluence, there was no statistically significant difference found between the 5 mm and 7-mm spot sizes. When the V-Beam test patches are examined, the shorter pulse durations tended to produce more of an effect than the longer pulse durations, and this is a statistically significant trend (t test for trend, $P = 0.013$).

Capillary Vascular Malformations With Deep Vessels

For the "deep" group of patients, whose vessel depths ranged from 80 to 123.3 μm, there was found to be no statistically significant effect of treatment overall (F test $P = 0.960$). However, an F test of differences between treated patches approached the conventional level of significance ($P = 0.084$). At fluence 12j/cm^2, and wavelengths of 585 and

590 nm, the 7-mm spot size treatments appeared to increase vessel depth more than the 5-mm spot size, but *t* tests showed no significant effects. However, comparison between spot sizes 5 and 7 mm for wavelength 595 nm and fluence 14 j/cm^2 gave a statistically significant result (*t* test, $P = 0.014$), and it is notable that of all the settings, 595 nm, 14 j/cm^2, 7 mm appeared to have the most effect on vessel depth. Varying the pulse duration with the V-Beam laser appeared to have little effect on these deeper vessels.

Vessel Diameter

Patients were also grouped according to their capillary diameters as measured using DMV. Three groups were defined: a small diameter group of 7 patients with diameters in the range 16.6 to 30 μm, an intermediate group of 5 patients with diameters between 30 and 40 μm, and a large diameter group of 6 patients with diameters in the range 40 to 110.8 μm. Figure 2 shows the effect on initial mean capillary diameter of the different test patch settings for the 3 groups.

Capillary Vascular Malformations With Large-Diameter Vessels

For the large-diameter vessel group, there was a statistically significant effect of treatment over all the test patches (F test $P = 0.05$). However, there were no statistically significant differences between the means for each of the different test patch settings (F test $P = 0.32$).

Capillary Vascular Malformations With Intermediate-Diameter Vessels

When the intermediate group is examined, the effect of treatment approaches significance at the conventional level of 5% (F test $P = 0.07$), but again, differences between test patches are not significant (F test $P = 0.80$). When Figure 2 is examined, it can be seen that for this intermediate group, there is little difference between the results obtained with the ScleroPlus laser test patch settings. The longer pulse durations of the V-Beam laser tended to be most successful at reducing vessel diameter, but a test for trend was not statistically significant (*t* test $P = 0.18$).

Capillary Vascular Malformations With Small-Diameter Vessels

When the group with the smallest diameters is examined, there is no longer any statistically significant effect of treatment (F test $P = 0.62$), and there is no evidence that any of the test patch settings reduced capillary diameter more than the others (F test $P = 0.96$). There appeared to be no statistically significant difference in the results obtained by changing the pulse duration (*t* test $P = 0.60$), the wavelength (*t* test $P = 0.35$), the spot size (*t* test $P = 0.98$), or the fluence (*t* test $P = 0.77$).

DISCUSSION

The recent advent of PDLs with variable pulse durations and wavelengths has provided hope that further fading of 585 nm, 0.45 msec, PDL-resistant CMs may be possible. Previous studies have demonstrated that the use of longer wavelength lasers may allow deeper ectatic capillaries to be treated.[16,17] The use of longer wavelengths, however, necessitates the use of higher fluences as the selectivity of the laser to the absorption spectrum of oxyhemoglobin is reduced. This increased fluence requires adequate epidermal protection through more efficient methods of cooling the skin. The lasers used in this study both use cryogen spray cooling devices to provide epidermal cooling. Previous studies by Chang et al and Edstrom et al have established the role of longer wavelength PDLs in the treatment of some patients.[16,17] Both these studies, however, find that for the majority of patients undergoing early treatment of their CM, 585 nm is the wavelength of choice. Previous biopsy studies[3,4] and noninvasive assessment within our unit[18] have established that after laser treatment, the deeper and smaller capillaries within a CM tend to be left untreated. Kimel et al's study examining the effect of varying wavelengths produced by a ScleroPlus laser on chick chorioallantoic membrane has found that smaller caliber vessels tend to be better treated by shorter wavelengths.[22] However, in previously treated CMs, it is likely that the longer wavelengths may be required to allow for greater tissue penetration.

In this study, we have sought to investigate the effect of a variable wavelength and variable pulse duration laser on previously treated CMs. In terms of response to these laser systems, both have been found to be effective in the treatment of these resistant patients. All these patients had previously been treated with a 585 nm, 0.45-msec PDL (SLS CHROMOS, Wales, U.K.) up to fluences of 7.7 j/cm^2 with a 7-mm spot size and topical ice epidermal cooling. These newer laser systems allow much higher fluences to be used because their cryogen spray cooling systems are much more efficient at ensuring epidermal protection.[23] The majority of patients within this study have responded favorably to some test patches. It is possible that the increase in fluence alone could account for this. This may also explain why little difference in efficacy was seen between the 2 lasers.

It was hoped that by examining initial capillary characteristics of depth and diameter using the DMV that proposed ideal treatment parameters might have been indicated. From our results, it can be seen that patients whose CMs contain superficial large-diameter capillaries are likely to receive the greatest further fading of their lesions with further treatment. Only patients with deep and small-diameter vessels are unlikely to receive further fading. However, it is not possible from this data to predict the optimum treatment parameters for an individual patient. This may be for a number of reasons. This study has a relatively small sample size and relies on test patch information only. It is possible that prolonged treatment of a larger group of patients may have provided more predictive information. Also, other factors may play a role in selecting ideal treatment parameters. All these patients were Fitzpatrick skin type 1 or 2, so it is unlikely that melanin content is a confounding variable within the study. This study takes no account of capillary perfusion, and previous studies have suggested that this may be an important consideration when choosing treatment parameters.[24,25]

This study found no statistically significant difference in the results obtained by both laser systems at the same parameters (595 nm, 14 j/cm^2, 1.5 msec). This comparison was carried out to investigate whether there was any demonstrable difference in the results obtained because both these lasers use differing methods of producing their laser pulse.[12] For the ScleroPlus (and the SLS Chromos 585-nm laser used previously), a smooth energy output is achieved during the pulse, whereas the V-Beam uses a series of micropulses. No practical difference was found in the results obtained, however.

This study used the DCD cryogen spray cooling devices on a uniform setting, 30-msec spray duration and 30-msec delay, across all the test patches. Recent studies have suggested that these settings should be altered to match the capillary characteristics of the CM to be treated.[19,26,27] Therefore, for deeper vessels in which a higher fluence and possibly a longer wavelength is to be used, it would be appropriate to increase the cooling system to provide more cooling to the epidermis without risking cooling the abnormal vessels excessively. The role of epidermal cooling and vessel depth requires more clinical study.

Our previous studies have indicated that as treatment progresses, the capillaries within a CM tend toward being small and deeply located as the larger and more superficial vessels are cleared.[18] This study also demonstrates this because patients with more superficial and larger vessels tended to have more of a change in these characteristics after treatment than patients with deeper and smaller vessels. It is noticeable in this study that there was a wide variation in the initial capillary depths and diameters measured. This may be the result of a number of factors. It is likely that response to treatment is a multifactorial phenomenon, with capillary depth, capillary diameter, capillary flow rates, and epidermal melanin concentration all interacting to influence nonresponse to treatment. Also, a number of these patients had not been treated for over 3 years. Because it is well established that CM vessels continue to evolve with age, and even completely treated lesions may recur, it is possible that more superficial and larger vessels had developed in these malformations since their last treatment.[28,29]

A number of other studies have also examined the effect of varying laser wavelength and pulse duration on the response to treatment of CMs. Greve et al examined 15 patients previously untreated patients and varied both wavelength and pulse duration.[30] They found 585 nm to be the ideal wavelength to treat previously untreated patients. Scherer et al examined the response of 62 previously untreated patients and also found 585 nm to be the most effective wavelength.[31] Both these studies examine the treatment of new patients. Our previous work[32] demonstrated that the depth of vessels within CMs undergoing treatment tends to increase and their diameter reduce. It seems likely that 585 nm with its higher specificity for oxyhemoglobin is an effective wavelength for the initial treatment of CMs, but as the ectatic capillaries become deeper and smaller, longer wavelengths may become more important. Laube et al examined the treatment of 15 resistant CMs using the V-Beam laser and found further lightening in 67% of patients and a similar percentage to this study (55%).[33] Interestingly, they found the settings 595 nm, 14j/cm^2, 1.5-msec pulse duration to be the most effective when treating resistant CMs, as was also the case within this study. Bjerring et al examined the treatment of resistant CMs with an intense pulsed light (IPL) system and demonstrated improvement in just under half the patients.[34] However, this study gives little information as to the nature of the previous PDL treatment the patients had undergone nor how resistance to laser treatment was defined.

In general, our results suggest that patients whose CM vessels are not too deep and not too small, as assessed by DMV, can expect a good response from treatment with these newer laser systems. This study demonstrates trends that conform to the commonly accepted belief that increasing the fluence, spot size, and using longer wavelengths improves the results when treating resistant CMs. For example, 595-nm wavelength, 7-mm spot size, and 14-j/cm^2 fluence appeared to be the most effective parameters when treating the malformations with the deepest vessels. It must be borne in mind, however, that the majority of test patches within this study produced less than 25% improvement in the color of the CM. A previous study by Woo et al showed only 5 of 22 patients with resistant CMs improved after treatment with a V-Beam laser.[35] Their study, however, used only 2 different treatment settings, and it is possible that these settings were not well matched to the majority of the CMs. This illustrates the need for a technique such as depth measurement videomicroscopy that would allow those patients most likely to respond to treatment (ie, those with large and superficial vessels) to be identified and thus reduce the likelihood of excessive unnecessary treatments. This study emphasizes that patients whose CM may have become resistant to PDL may derive further improvement with laser treatment a few years later.

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