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A Comparison of Laser Doppler Fluxmetry and Transcutaneous Oxygen Pressure Measurement in the Dysvascular Patient Requiring Amputation

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Objective: *To determine the predictive power of laser Doppler fluxmetry (LDF), both heated and unheated, as a preoperative investigation of wound healing potential in dysvascular patients requiring amputation, by comparison with transcutaneous oxygen pressure measurement ($TcpO_2$) and the limb to chest $TcpO_2$ index.*

Methods: *Thirty-five non-diabetic patients with peripheral vascular disease were investigated before amputation. Heated and unheated LDF and heated $TcpO_2$ measurements were taken on the chest wall and at the routine above-knee, below-knee and mid-foot amputation levels. Wound healing potential was evaluated against a $TcpO_2$ index value of 0.55 and on clinical outcome.*

Results: *A heated LDF value of 4.9 arbitrary units (au) was shown by receiver-operator characteristic curve to have the best predictive power, with an overall accuracy for preoperative prediction of wound healing of 91.4%, and a predictive value for wound failure of 89%. Based on the heated LDF of 4.9 au, review of 26 amputations performed shows the overall accuracy for preoperative prediction of wound healing of 92.3%, a predictive value for wound healing of 100%, and a predictive value for wound failure of 62.5%.*

Conclusion: *A heated LDF value of 4.9 au appears to be a useful predictor of the potential of an amputation site to heal.*

Key Words: Laser Doppler fluxmetry; Blood gas transcutaneous; Amputation.

Introduction

Peripheral vascular disease is the most common cause of lower limb amputation.[1,2] Amputation in these patients is associated with high morbidity and mortality.[3] Primary wound healing is more likely the more proximally the amputation is performed. While proximal amputation with primary wound healing reduces initial morbidity, the additional energy cost required to use a larger and heavier prosthesis makes total rehabilitation more difficult.[4] Preservation of the knee or ankle joint improves the chance of successful rehabilitation, but increases the risk of delayed or failed wound healing with consequent morbidity and mortality. The problem facing the surgeon is determination of the most distal site at which an amputation will heal.

The benefits to both patients and hospital administrations of using routine preoperative evaluation of amputation wound healing potential have been reported.[3,5] Despite this, routine evaluation of preoperative wound healing potential has not been widely implemented. A reason for this is that there is no single investigation which has gained universal acceptance. This is not surprising as there are many factors in addition to the adequacy of regional blood flow which influence wound healing. Investigations such as Doppler ankle-brachial pressure indices, $Xenon^{133}$ skin clearance, thermography, fluorescein dye angiography, transcutaneous oxygen pressure measurement ($TcpO_2$) and laser Doppler fluxmetry (LDF) have been tried. While each test has its proponents, it has not always been possible to reproduce the results reported in different settings.

Of these tests, $TcpO_2$ measurement is presently held to be the most useful investigation of preoperative wound healing potential.[6,7] The use of the ratio of $TcpO_2$ measurement obtained at the amputation level to that of the anterior chest wall, the $TcpO_2$ index, has improved the sensitivity and specificity of the test.[3] The index gives a better indication of oxygen delivery as it takes into account variation caused by central factors of oxygen delivery, such as cardiac and respiratory function. It has been our practice to use the

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$TcpO_2$ index to select the most distal amputation site which will heal by primary intention. An amputation site with a $TcpO_2$ index greater than 0.55 is considered likely to heal and is associated with an amputation revision rate of less than 5%.[3]

Transcutaneous oxygen pressure measurement is however time consuming. With patient acclimatisation to the environmental temperature of the laboratory, probe calibration, and measurement at the four routine sites, the average test time is approximately 2 h. A test is required which is as sensitive and specific as the $TcpO_2$ index, but which can be performed more rapidly.

Laser Doppler fluxmetry (LDF), a test of skin microperfusion, has found a place in the evaluation of diabetic microangiopathy.[8] It has also been proposed as a test of amputation wound healing potential.[9–11] If the test is performed on unheated skin, readings can be obtained 3 min after application of the probe and with the heated probe after 5 min. Routine testing at the four sites would then take less than 30 min. There is however controversy as to whether skin heating is required.[10–14] In addition, several shortcomings of LDF are documented.[15,16] These include problems with zeroing, variations in biological zero and the various ways in which the output of the LDF is expressed, which range from voltage (mV), arbitrary units, flux units, to blood flow per cm^3. In view of this it is probably necessary for each laboratory to validate the use of LDF in its own setting.[17]

The aim of this study was to compare readings obtained from both an unheated and heated LDF with $TcpO_2$ measurement in non-diabetic patients with peripheral vascular disease requiring amputation.

Materials and Methods

Thirty-five patients with atherosclerotic peripheral vascular disease undergoing routine evaluation of amputation wound healing potential were studied at the Non-invasive Vascular Laboratory, King Edward VIII Hospital. The patients were not diabetic and were not on vasoactive medication. Informed consent was obtained and the study was performed with the approval of the University of Natal, Faculty of Medicine Bioethics Committee.

Prior to testing, each patient lay supine for 20 min to acclimatise to the ambient temperature of the laboratory, which ranged from 20 to 23 °C. To avoid the possible confounding effects of previous heating of the skin, laser Doppler measurements were made before $TcpO_2$ measurement.

LDF was performed with a Laserflo BPM2 Blood Perfusion Monitor, Vasamedics, St Paul. The laserflo monitor does not offer a zeroing procedure, but rather a "user confidence test" which checks that the laser and photodetector are within specifications. This test was performed before each series of LDF measurements were made.

Measurement sites were shaved and cleaned with an alcohol solution when necessary. The laser probe was attached to the skin by means of a double sided adhesive ring and the unheated LDF measurements recorded 3 min after probe application. For the heated readings, the probe was then heated to 45 °C and recordings made after 5 min of heating. The LDF index, the ratio of limb to chest LDF reading was calculated for the amputation sites.

The chest and the below-knee sites were measured in all patients. Measurements at the foot or above-knee site was based on the clinical decision required. Where the choice was between below-knee amputation and an amputation about the foot, the foot site was measured and similarly, the above-knee site was measured when clinically the choice was between a below-knee and an above-knee amputation. Data were obtained from the foot ($n=17$), the below-knee site ($n=35$), the above-knee site ($n=17$) and the anterior chest wall ($n=35$). One patient presenting with a gangrenous forefoot, had only a below-knee measurement taken, and no measurement made at the foot.

Transcutaneous oxygen pressure measurement was made using a Hewlett Packard Transcutaneous Oxygen Monitor. The monitor was calibrated against air and a zeroing solution, according to the manfacturers' instructions. Calibration was performed before use on each patient. The probe was attached to the skin by a double sided adhesive ring, over a drop of contact solution. The heating thermistor was set to 45 °C. Hyperaemic stabilisation occurred within 20 min and readings were taken after 20 min. $TcpO_2$ measurements were taken at the same sites as the LDF measurements. The $TcpO_2$ index, the ratio of limb to chest $TcpO_2$ was calculated for the amputation sites.

Amputations were performed according to standardised procedures by members of the Vascular Service. Although guided by the preoperative assessment of wound healing potential, the final decision as to the level of amputation in patients in this study, was made intraoperatively by the surgeon.

Statistical analysis was performed using Spearman's rank correlation. The sensitivity and specificity, and positive and negative predictive value of LDF relative to $TcpO_2$ were calculated. Sensitivity was defined as the ability of LDF to predict wound healing failure and specificity as the ability of the test to predict

Table 1. $TcpO_2$ and laser Doppler values at the chest and amputation levels.

	Chest $n=35$	Above-knee $n=17$	Below-knee $n=35$	Foot $n=17$
$TcpO_2$ (mmHg)	55.7 ± 11.2	39.2 ± 19.0	36.3 ± 20.3	17.3 ± 13.3
Laser unheated (au)	3.9 ± 2.1	1.7 ± 1.1	1.6 ± 0.9	1.9 ± 1.3
Laser heated (au)	18.1 ± 4.1	10.0 ± 4.8	7.9 ± 5.0	5.2 ± 5.2
$TcpO_2$ index		0.73 ± 0.37	0.62 ± 0.31	0.31 ± 0.23
Laser index unheated		0.61 ± 0.41	0.52 ± 0.41	0.53 ± 0.63
Laser-index heated		0.58 ± 0.33	0.46 ± 0.33	0.29 ± 0.30

$TcpO_2$, unheated and heated laser Doppler fluxmetry, $TcpO_2$ index, and unheated and heated laser Doppler indices expressed as means and one standard deviation are shown for the different measurement sites. The indices are the limb to chest ratios. Correlation of the results at each site was by Spearman's rank correlation which was considered significant when $p<0.05$. Significant correlation was noted between heated LDF and both absolute $TcpO_2$ and the $TcpO_2$ index and between the heated LDF index and the $TcpO_2$ index at the foot and below-knee sites.

wound healing. Receiver-operator characteristic (ROC) curves were constructed to determine the LDF value which would be most useful for preoperative prediction of wound healing.

Results

The absolute LDF and $TcpO_2$ measurements, and the LDF index and the TcpO2 index at the different sites are expressed as means and one standard deviation are shown in Table 1. The ranges of the readings were, unheated LDF 0–12 arbitrary units (au), heated LDF 0–27.8 au, $TcpO_2$ 0–77 mmHg, the unheated LDF index 0–2.9, the heated LDF index 0–14, and the $TcpO_2$ index 0–1.43. The highest mean readings for LDF and $TcpO_2$ were measured at the chest. Significant correlations were found between heated LDF and $TcpO_2$ absolute and index values at the foot and below-knee sites.

Pooling the heated LDF data for the various sites there was a significant correlation between heated LDF and $TcpO_2$ ($n=104$), $r=0.63$, ($p<0.0001$), heated LDF and the $TcpO_2$ index, ($n=69$), $r=0.72$ ($p<0.0001$), heated LDF index and the $TcpO_2$ index, ($n=69$), $r=0.68$, ($p<0.0001$) and between unheated LDF and $TcpO_2$, with a low correlation co-efficient ($n=104$), $r=0.31$, $p<0.001$. There was poor correlation between the unheated LDF index and the $TcpO_2$ index, ($n=69$), $r=0.21$, $p=0.08$ and between unheated LDF and the $TcpO_2$ index ($n=69$), $r=0.18$, $p=0.21$. The relationship between heated LDF and $TcpO_2$ index is shown in Fig. 1.

Various $TcpO_2$ values have been suggested as predictive of amputation wound healing. These range from 0–40 mmHg.[18–20] Despite a significant correlation between absolute unheated LDF and $TcpO_2$ readings, the correlation co-efficient is low and there is poor linearity. There is no unheated LDF level which has a high predictive power when compared to $TcpO_2$.

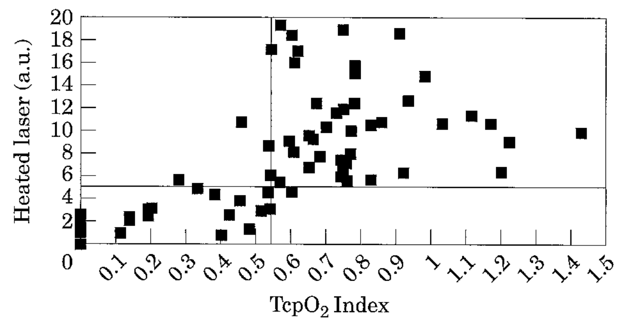

Fig. 1. Scattergram showing the relationship between the $TcpO_2$ index and heated LDF ($n=69$). The Spearman rank correlation coefficient is $r=0.72$, ($p<0.0001$). The linear regression equation is $y=2.38+9.4x$. No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. A heated LDF value of 4.9 au is shown as a potential predictive value.

Receiver-operator characteristic (ROC) curves were constructed to determine the LDF value which would be most useful for preoperative prediction of wound healing.[21] This is a graphic way of portraying the trade-offs involved between improving either the LDFs sensitivity or its specificity. Sensitivity and specificity were calculated for unheated and heated LDF at $TcpO_2$ indices of 0.50, 0.53, 0.55, 0.57 and 6. Sensitivity was then plotted against 1-specificity for unheated and heated LDF against each of the $TcpO_2$ indices. The diagnostic accuracy based on the area under the curve was also calculated.[22] For the $TcpO_2$ index of 0.55 the best predictive level was found to be at a heated LDF of 4.9 au (Fig. 2).

Twenty-five readings fall below a heated LDF$=$4.9 au and all but one have a $TcpO_2$ index of less than 0.55. Of the 44 readings above LDF$=$4.9 au, 40 are above the $TcpO_2$ index of 0.55 and would be expected to heal. The four sets of readings above LDF$=$4.9 au and below a $TcpO_2$ of 0.55 would be expected to fail. This has a sensitivity of 97.6%, specificity of 82.8%, an LDF >4.9 au has a predictive value of 88.9% in identifying sites that will heal and an LDF <4.9 au has

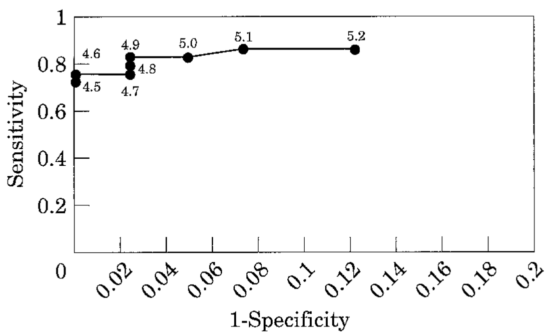

Fig. 2. Receiver-operator characteristic curve displaying sensitivity and 1-specificity of absolute LDF values from 4.5 to 5.2 based on a $TcpO_2$ index of 0.55.

a predictive value of 96% in identifying sites that will fail to heal, with an overall accuracy for preoperative prediction of wound healing or failure of 91.4%.

Clinical outcome

Of the thirty-five patients, two underwent bypass graft surgery and were excluded from follow-up, one patient refused surgery and three patients died perioperatively. Three patients were transferred back to rural hospitals before wound healing was complete and have been lost to follow-up. Twenty-six amputations, four transmetatarsal, 15 below-knee and seven above-knee amputations were available for evaluation. Six of these amputations were performed at sites with a $TcpO_2$ index of less than 0.55, five of which failed to heal and required revision to a more proximal site. One amputation with a $TcpO_2$ index of 0.54 and a heated LDF of 4.7 au healed after four weeks.

A heated LDF of 4.9 au had a sensitivity of 100%, specificity of 90%, and LDF >4.9 au has a predictive value of 100% in identifying sites that will heal and an LDF <4.9 au has a predictive value of 62.5% in identifying sites that will fail to heal, with an overall accuracy for preoperative prediction of wound healing or failure of 92.3%.

Discussion

The microvascular bed of the skin is composed of superficial nutritional capillary loops containing approximately ~5% of the cutaneous bloodflow and the subpapillary, thermoregulatory vascular bed comprised mainly of venules, containing ~95% of the skin bloodflow.[23] The $TcpO_2$ reading is the reduction current produced when surplus oxygen molecules diffuse from the cutaneous nutritional capillary loops under maximal hyperaemia.[24] LDF has a measuring depth of 1–2 mm and the predominant part of the signal comes from the subpapillary thermoregulatory vessels. LDF therefore evaluates total skin microcirculation while $TcpO_2$ evaluates skin nutritional circulation.

The findings of the present study show a significant but poor correlation between unheated LDF and $TcpO_2$ ($r = 0.31$). Belcaro *et al.*, reported similar results with the correlation of $TcpO_2$ to resting LDF being $r = 0.4$.[8] These findings contradict those of Matsen *et al.*, who in a relatively small sample found that non-heated LDF measurements did not correlate with local skin perfusion.[25]

The signs and symptoms of peripheral vascular disease, intermittent claudication, rest pain, ulceration and finally gangrene are all manifestations of insufficient oxygen and nutrient delivery at a cellular level, secondary to relative degrees of arterial occlusion. It is therefore expected that tests of perfusion of both the macro- and microcirculation should reflect a fall in perfusion as the tests are performed more distally in the atherosclerotic patient. The magnitude of the segmental fall in perfusion parameters is dependent on the site and severity of the disease process. While both the absolute $TcpO_2$ measurements and the $TcpO_2$ index and the heated LDF reflect a fall in mean values as the site of measurement moves distally, the reduction in unheated LDF results is less apparent because of the large range of readings.

Unheated LDF readings may be high around the margin of diabetic ulcers.[26,27] The rise in LDF in these patients with microangiopathy has been attributed to shunting of blood to the thermoregulatory plexus or the dermal capillary loops. The very wide range of unheated LDF readings obtained at the foot, 0–10.9 au may be due to a similar phenomenon. Ischaemia is associated with increasing peripheral vasodilatation in an attempt to improve oxygen and nutrient supply to the tissues. In the ischaemic foot the LDF value may be high when compensation is successful — maximal vasodilatation in the presence of an adequate inflow, or it may be low if the inflow is insufficient despite maximal vasodilatory compensation.

A large variability in resting unheated LDF has been reported.[28,29] This variability has been attributed to physiological variation in skin perfusion, the structure of the anatomical microvasculature under the probe,[30] methodological factors (e.g. penetration depth of the laser, and probe configuration) and environmental factors.[29] This wide variation is seen in the values obtained at an essentially normal segment of the vasculature, the anterior chest wall, where the co-efficient of variation of the unheated LDF was 74.4%. Heating reduced the co-efficient of variation to 22.7% which is similar to that of $TcpO_2$, 23.6%.

Another factor which influences LDF variability is the biological zero.[31] The biological zero is the residual reading, above zero, obtained with LDF when the blood flow to a limb is occluded with an arterial tourniquet. The biological zero is thought to be due to Brownian motion, but may result in part from retrograde osseous bloodflow. Biological zero varies with perfusion, vasodilatation, skin temperature and oedema formation.[31] While the interpretation of LDF readings may be improved by subtraction of the biological zero from the LDF value, or perhaps by the evaluation of biological zero itself, this was not done in this study. In a pilot study, attempts at measuring biological zero in patients with severe peripheral vascular disease caused pain, and the movement artefacts which resulted, affected the readings obtained in most patients.

The addition of cutaneous heating has been reported to improve the predictive power of LDF when evaluating wound healing.[9,10,14,25] Heating of the skin "arterialises" the capillary bed by local vasodilation[12] causing maximal hyperaemia. Direct comparisons of reported LDF values upon which clinical decisions are made are not possible, because of differences in temperature protocols and the different units in which the output of different laser Doppler fluxmeters are expressed.

In the present study heating the LDF probe to 45 °C improved the preoperative prediction potential for evaluating wound healing in PVD patients. Significant correlation was found between heated LDF and $TcpO_2$; heated LDF index and $TcpO_2$ index, and heated LDF and $TcpO_2$ index, $r=0.72$, $p<0.0001$. The degree of the significance obtained is probably due to the comparison being made over the wide range of heated LDF values obtained.

While there is a low but significant correlation between unheated LDF and $TcpO_2$, unheated LDF does not appear to be clinically useful. Heating adds 5 min to each test and using a heated LDF value of 4.9 au gives a theoretical overall accuracy for preoperative prediction of wound healing or failure of 91.4%. In the clinical study, 92.3% accuracy was achieved.

The theoretical predictive value for wound healing is 89%, which was exceeded in the clinical study (100%). Similarly the predictive power of the $TcpO_2$ index for wound healing was 100%, which differs from our previous experience, of 270 amputations in which the $TcpO_2$ index of >0.55 was associated with $\pm$90–95% predictive power of wound healing over 4 consecutive years.[3] This difference is most probably due to the relatively small sample size.

The theoretical predictive value for wound failure of 96% would mean that in 4% of cases a limb would undergo ablation at a site higher than that which has potential to heal. In practice the predictive power for wound failure was found to be 62.5%. Again this may be artificially low because of sample size.

These data suggest that a heated LDF >4.9 au would be a relatively quick and clinically useful preoperative evaluation of an amputation sites' potential to heal. However, basing evaluation of potential wound failure at a site on a heated LDF of <4.9 au could result in up to a third of amputations with a heated LDF <4.9 au being performed at a site more proximal to that which has potential to heal.

It must be remembered, that the predictive LDF value of 4.9 au is based on a $TcpO_2$ "gold standard", which has its own measurement error. Despite this limitation, this study suggests that widespread implementation of heated LDF may be as useful as limited use of $TcpO_2$ measurement for preoperative evaluation of wound healing potential in patients with peripheral vascular disease.

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References

1 Krupski WC, Skinner HB, Effeney DJ. Amputation. In: Way LW, ed. *Current Surgical Diagnosis and Treatment*. San Mateo, CA: Appleton and Lange, 1988; 704–714.
2 McColl I. Review of artificial limb and appliance centre services. Department of Health and Human Services Report. London, Her Majesty's Stationery Office, 1986.
3 Mars M, Mills RP, Robbs JV. The potential benefit of preoperative assessment of amputation wound healing potential in peripheral vascular disease. *S Afr Med J* 1993; **83**: 16–18.
4 Durham JR. Lower extremity amputation levels: indications, methods of determining appropriate level, technique, prognosis. In: Rutherford RB, ed. *Vascular Surgery*, 4th edition. Philadelphia: W. B. Saunders Company, 1995: 1960–1965.
5 Malone JM, Moore WS, Goldstone J *et al.* Therapeutic and economic impact of a modern amputation program. *Ann Surg* 1979; **189**: 798–802.
6 Wyss CR, Harrington RM, Burgess EM *et al.* Transcutaneous oxygen tension as a predictor of success after an amputation. *J Bone Joint Surg Am* 1988; **70**: 203–207.
7 Oishi CA, Fronek A, Golbranson FL. The role of non-invasive vascular studies in determining levels of amputation. *J Bone Joint Surg Am* 1988; **70**: 1520–1530.
8 Shore A, Tooke JE. Assessment of diabetic microangiopathy. In: Belcaro G *et al.*, eds. *Laser Doppler Flowmetry: Experimental and Clinical Applications*. Cyprus: Med Orion, 1994: 119–128.

9 Holloway GA, Burgess EM. Preliminary experiences with laser Doppler velocimetry for the determination of amputation levels. *Prost Ort Inter* 1983; **7**: 65–68.
10 Padberg FT Jr, Back TL, Hart LC *et al.* Comparison of heated-probe laser Doppler and transcutaneous oxygen measurements for predicting outcome of ischemic wounds. *J Cardiovasc Surg* 1992; **33**: 715–722.
11 Adera HM, James K, Castronuovo JJ *et al.* Prediction of amputation wound healing with skin perfusion pressure. *J Vasc Surg* 1995; **21**: 823–829.
12 Karanfilian RG, Lynch TG, Zirul VT *et al.* The value of laser Doppler velocimetry and transcutaneous oxygen tension determination in predicting healing of ischemic forefoot ulcerations and amputations in diabetic and non-diabetic patients. *J Vasc Surg* 1986; **4**: 511–516.
13 Kram HB, Appel PL. Prediction of below-knee amputation wound healing using non-invasive laser Doppler velocimetry. *Am J Surg* 1989; **158**: 29–31.
14 Lantsberg L, Goldman M. Laser Doppler flowmetry, transcutaneous oxygen tension measurements and Doppler pressure compared in patients undergoing amputation. *Eur J Vasc Surg* 1991; **5**: 195–197.
15 Kvernebo K, Slagsvold CE, Stranden E. Laser Doppler flowmetry in evaluation of skin post-ischemic reactive hyperemia. *J Cardiovasc Surg* 1989; **30**: 70–75.
16 Bircher A, de Boer EM, Agner T *et al.* Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry. *Contact Dermatitis* 1994; **30**: 65–72.
17 Mars M. Laser Doppler and transcutaneous oxygen tension in the evaluation of cutaneous microcirculation. *Hospital Supplies* 1995; 30–39.
18 Malone JM, Anderson GG, Lalka SG *et al.* Prospective comparison of non-invasive techniques for amputation level selection. *Am J Surg* 1987; **154**: 179–184.
19 Wagner WH, Keagy BA, Kotb MM *et al.* Non-invasive determination of healing of major lower extremity amputations: the continued role of clinical judgement. *J Vasc Surg* 1988; **8**: 703–710.
20 Sarin S, Shami S, Shields DA *et al.* Selection of amputation level: a review. *Eur J Vasc Surg* 1991; **5**: 611–620.
21 Altman DG, Bland JM. Diagnostic tests 3: receiver-operating characteristic plots. *Br Med J* 1994; **309**: 188.
22 Zweig MH, Campbell G. Receiver-operating characteristics (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clinical Chemistry* 1993; **39**: 562–577.
23 Ostergen J. Studies on skin Capillary blood cell velocity by videophotometric capillaroscopy, Thesis Repro Print A B, Stockholm, Sweden 1984.
24 Bender E, Trojan A, Bucher HU *et al.* Control of skin blood flow in pre- and full-term infants. *Biology of the Neonate* 1994; **65**: 7–15.
25 Matsen FA, Wyss CR, Robertson CL *et al.* The relationship of transcutaneous PO_2 and laser Doppler measurements in human model of local arterial insufficiency. *Surg Gynecol Obstet* 1984; **159**: 418–422.
26 Belcaro G, Vasdekis S, Nicolaides AN. Evaluation of skin blood flow and venoarteriolar response in patients with diabetes and peripheral vascular disease by laser Doppler flowmetry. *Angiology* 1989: 953–957.
27 Sindrup JH, Avonstorp C, Steenfos H *et al.* Transcutaneous PO_2 and laser Doppler blood flow measurements in 40 patients with venous leg ulcers. *Acta Derm Venereol (Stockh)* 1987; **67**: 160–182.
28 Tenland T, Salerud G, Nilsson GE *et al.* Spatial and temporal variation in laser Doppler flowmetry. *Int J Microcirc Clin Exp* 1983; **2**: 81–90.
29 Wahlberg E, Fagrell B. Spatial and temporal variation in laser Doppler flux values in healthy lower limbs: comparison between the standard and the multiprobe. *Int J Microcir* 1994; **14**: 343–346.
30 Braverman IM, Keh A, Goldminz D. Correlation of laser Doppler wave patterns with underlying microvasculature anatomy. *J Invest Dermatol* 1990; **95**: 283–286.
31 Caspary L, Creutzig A, Alexander K. Biological zero in laser Doppler fluxmetry. *Int J Microcirc Clin Exp* 1988; **7**: 367–371.

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