Prosthetics and Orthotics International, 1994, 19, 139–147

Amputation level assessment using lightguide spectrophotometry

D.K. HARRISON*, P.T. McCOLLUM**, D.J. NEWTON*, P. HICKMAN** and A.S. JAIN***

*Vascular Laboratory, Departments of *Medical Physics and **Surgery*
Ninewells Hospital and Medical School, Dundee DD1 9SY, UK
****Department of Orthopaedic Surgery, Dundee Royal Infirmary, Dundee DD1 9ND, UK*

Abstract

The aim of this experimental study was to investigate whether lightguide spectrophotometry in the visible wavelength range in skin could be used to predict stump healing viability in patients with critical lower limb ischaemia.

Remission spectra recorded at two sites (medial and lateral) on the line of a proposed trans-tibial amputation (TTA) and at 10mm intervals along the leg were analysed to give haemoglobin oxygenation (SO_2). Degree of tissue hypoxia (DTH) along the leg was defined as the percentage of values along the leg less than 10% SO_2. DTH and mean SO_2 values were compared with skin blood flow values ((I^{125}) 4-Iodoantipyrine clearance technique) and clinical outcome of trans-tibial amputation, (TTA) or trans-femoral amputation (TFA), in 41 patients. SO_2 histograms were also measured in 12 normal subjects for comparison.

The results of the study allowed the establishment of criteria for the accurate prediction of flap healing potential. Successful TTAs all displayed a minimum mean SO_2 at the medial and lateral measurement sites of 30%, together with a maximum degree of tissue hypoxia of 15% along the limb. The combination of these criteria gave a sensitivity and selectivity of 1.0 for prediction of a successful outcome of TTA.

Introduction

Peripheral arterial disease afflicts some 5% of British males over 50. Its most common symptom is intermittent claudication which tends to stabilise soon after onset; nonetheless about 2% of such patients eventually need to undergo an amputation of the lower limb because of critical limb ischaemia (CLI) (Dormandy, 1991). Patients who have a trans-tibial amputation (TTA) rehabilitate better and have a better chance of leading an independent life than those who have a trans-femoral amputation (TFA) (Cumming *et al.*, 1987).

The Dundee vascular laboratory has over ten years' experience in using a combination of infrared thermographic imaging, skin blood flow recordings by a radioisotope clearance method at the site of a proposed skin flap, and Doppler pressure measurements in the pre-amputation assessment of stump viability (McCollum *et al.*, 1985). These combined methods have achieved a success rate of 93% for TTAs and a 75% TTA/TFA amputation ratio (McCollum *et al.*, 1988).

Despite the development of new techniques for the assessment of skin blood flow and metabolism, the Dundee group has not been able to improve on the TTA/TFA prediction technique since the above reports. Furthermore, there is still no reliable method for predicting the outcome of amputations in patients with CLI who may benefit from an amputation more distal than TTA. This applies particularly in patients who might be suitable for an amputation through the foot, which would allow greater mobility.

It has been shown that oxygen is one of the most important nutrients for wound healing

All correspondence to be addressed to Dr. D.K. Harrison, Vascular Laboratory, Ninewells Hospital, Dundee DD1 9SY, UK.

(Forrester, 1988). Whilst skin blood flow may be an indicator of the rate of delivery of those substrates required for the healing process, it is not necessarily a direct indicator of the adequacy of the rate of delivery of oxygen to the tissue cells (Harrison *et al.*, 1992). Furthermore, its direct measurement is invasive and exposes the patient, medical and technical staff to the hazards of ionising radiation.

Historically, hypoxia has been shown to impair both the synthesis of collagen and differentiation of fibroblasts in healing wounds (Niinikoski, 1969; Stephens and Hunt, 1971). Wound chamber investigations have revealed that the partial pressure of oxygen (pO_2) in tissue falls steadily from 6.0 kPa (45mmHg) in normal undamaged tissue to levels close to zero in the centre of wounds (Silver, 1980). Furthermore, the oxygen supply to the advancing edge of granulation tissue is diffusion-limited (Silver, 1969). Fibroblast activity is maximal up to 50-80μm from nutrient capillaries where pO_2 is between 1.3 and 2.6 kPa (10 and 20 mmHg). Macrophages have a lower oxygen requirement and are to be found in the free edge of granulation tissue within wounds (Silver, 1980). However, despite being able to ingest bacteria in areas of low oxygen tension, it is uncertain whether they can kill ingested bacteria under hypoxia (Hohn, 1977). Thus it would appear that assessment of tissue oxygenation would help to predict the healing potential of ischaemic tissues.

The current method of choice for measuring tissue oxygenation is the polarographic pO_2 electrode. Two commercially available techniques come into consideration for measurement in human skin: the transcutaneous ($tcpO_2$) technique and needle electrodes. The former method relies on heating the skin in order to cause maximal hyperaemia and increase the permeability of the skin for O_2. The technique is time-consuming in that each measurement requires at least 20 (and ideally 40) minutes in order to obtain a stable reading and the results can be instrument dependent (Spence *et al.*, 1985). Furthermore, a reliable value for predicting viability of wound healing has not been established (Hauser, 1987). Despite the development of a skin pO_2 microelectrode almost 20 years ago by Spence and Walker (1976), the only readily available system for measuring tissue pO_2 with a needle electrode employs a needle which, at 300μm in diameter, is too large and invasive for use in skin. It has, however, been quite widely applied to the measurement of pO_2 in human muscle (Heinrich *et al.*, 1989).

A recent development has been the application of near infrared spectroscopy for the monitoring of brain oxygenation (Delpy *et al.*, 1987). Spectrophotometry has also been developed in the visible region in order to measure haemoglobin oxygenation in inflamed skin (Harrison *et al.*, 1992), and in the skin of claudicant patients during treadmill exercise (Hickman *et al.*, 1994). The possible advantages of the technique would be the fast, non-invasive measurement of tissue oxygenation using a parameter upon which local pO_2 depends (via the haemoglobin dissociation curve) but which does not necessitate heating the skin. The aim of the present investigation was to determine whether lightguide spectrophotometry could be used to predict amputation level in terms of mean oxygen saturation or critical level of oxygen supply in skin and to compare the technique with the "gold standard" skin blood flow method.

Methods

Tissue spectrophotometry

The principle of lightguide spectrophotometric measurement of haemoglobin saturation in skin is illustrated in Figure 1. Light emitted from a transmitting fibre passes into the skin where multiple scattering events occur as photons encounter cellular and subcellular particles. The types of scattering

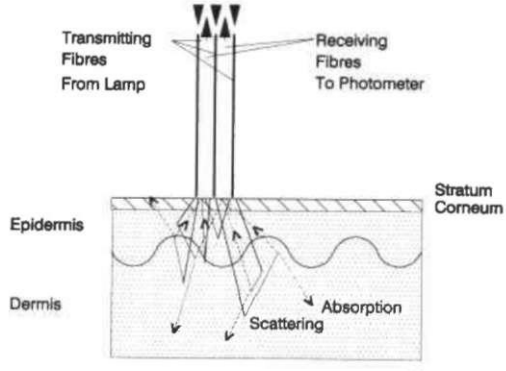

Fig. 1. Schematic diagram of light remission from skin in lightguide spectrophotometry. 3 transmitting and 2 receiving fibres are shown.

involved are discussed in detail elsewhere (Frank and Kessler, 1992). However, as seen in the figure, light will be absorbed by the haemoglobin present in the blood flowing within the catchment volume of the lightguide bundle before being eventually scattered back to a receiving fibre. Analysis of the remitted spectra (the term "reflected" is avoided due to the multiplicity of scattering events) enables the oxygen saturation (SO_2) to be measured.

An MCPD-1000 (Otsuka Electronics, Osaka) lightguide spectrophotometer, employing a Y configuration lightguide consisting of 18 transmitting and 12 receiving fibres (each 200μm diameter), was used for the investigations. The light source was a 150 W Xenon lamp. Light entering the photometer is split by a diffraction grating before falling on a photodiode array. The signal from the detector, now split at 2nm intervals over the wavelength range 300-1100nm, is amplified, digitalised and transferred to a personal computer for storage and processing.

Analysis of the spectra was carried out in the visible range (500-586nm) using a 6 wavelength technique described in detail by Harrison *et al.*(1992). Briefly, the gradients between 5 experimentally determined isosbestic wavelengths (500, 520, 548, 575 and 586nm) were added to give an index which was related to the haemoglobin concentration. This index was used to normalise the measured tissue spectra. SO_2 was calculated from the gradients between the absorption peak for deoxygenated haemoglobin (560nm) and the two adjacent isosbestic wavelengths (548 and 575nm) of the normalised spectra. The index thus obtained was calibrated in both *in vitro* and *in vivo* experiments to enable an accuracy of $\pm 5\%$ to be achieved (Harrison *et al.*, 1992).

IAP clearance

(I^{125}) 4-Iodoantipyrine (IAP) has been in routine use for amputation level assessment for over 10 years and is preferred for skin blood flow (SBF) measurement because of its low fat solubility compared with, for example, Xe^{133}.

0.02 ml (0.1 MBq) IAP in isotonic saline was injected at two sites 100mm distal from and 30mm medial and lateral to the tibial tuborosity, along the flap line of a proposed TTA. A sodium iodide scintillation detector and photomultiplier (PM) tube was placed over the injection site. The output from the PM tube was fed to a charge amplifier, energy analyser and ratemeter (Nuclear Enterprises, Edinburgh) before being plotted semi-logarithmically using a pen-recorder (Servoscribe). The time taken for the count rate to reach one half of its initial value ($T_{1/2}$ method) was used for calculating skin blood flow values in ml/100g/min.

Patients

41 consenting patients undergoing regular amputation level assessment were investigated. Spectrophotometric measurements were carried out at both sites of IAP injection (see above) immediately prior to the injection. Nine spectra were recorded at 10mm intervals in a 30 x 30mm matrix centering on each site of injection. Further measurements were made at 10mm intervals along the critically ischaemic limb from approximately 30mm medial to the tibial tuborosity to as far as the big toe. The protocol was approved by the local ethical committee.

Normal subjects

Although the use of a radioactive substance for the measurement of skin blood flow in CLI is associated with minimal risk and is thus entirely justified on the grounds of its diagnostic efficacy, it was not considered appropriate for use in young, healthy volunteers for comparisons with the photometric measurements. For investigations in normal subjects, therefore, only photometric measurements were carried out, as described above, along the legs of twelve healthy adult volunteers (age range 21-42).

Treatment of results

The spectrophotometric data at each location were analysed as described above to give SO_2 values. The 9 values measured around the injection site were averaged (mean) whereas the values measured along the limb were analysed in terms of frequency histograms representing the real distribution of tissue SO_2 (cf Fig. 2).

Statistical analysis of the results, carried out with the aid of Lotus 123 and SPSS for Windows software, involved the Mann Whitney U and Wilcoxon tests for non-parametric data. Values of $p < 0.05$ were considered to be statistically significant.

Results

Histograms including all of the measurements along the lower limbs of normal volunteers, patients who went on to have successful TTAs and patients who had TFAs are shown in Figure 2. Of the 41 patients studied, 32 patients were predicted from SBF measurements to be TTA and 9 to be TFA cases. One successful TTA was carried out on a recommended TFA case as the result of clinical judgement: this was re-classified here as TTA. Four of the recommended TTAs were clinically judged to be more suitable for, or subsequently revised to, TFAs. These patients were thus excluded from the sample as the eventual TFA was not primarily due to critically low skin blood flow, the clinical reasons being existing infection at site of proposed TTA or subsequent wound infection. The final sample thus consisted of 29 TTA and 8 TFA cases.

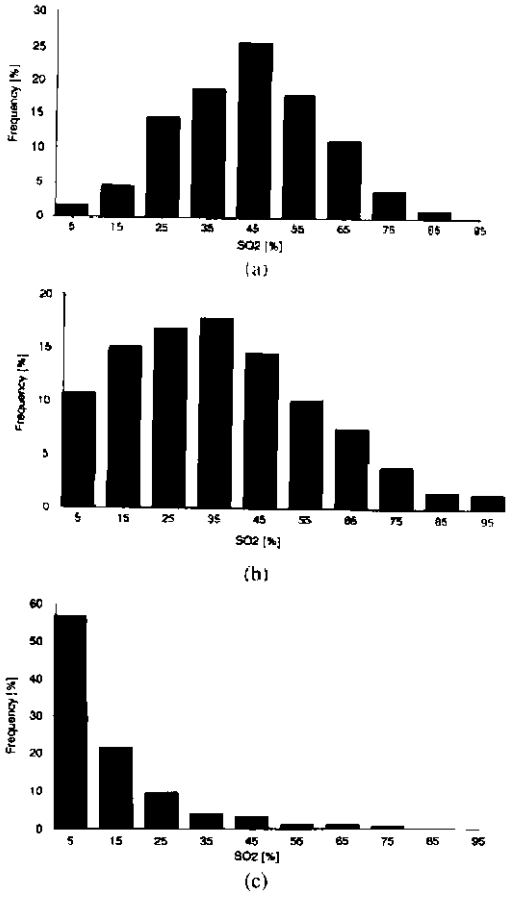

Fig. 2. SO_2 histograms recorded along the legs of (a) normals (N=12, n=547); (b) TTAs (N=29, n=1103) and (c) TFAs (N=8, n=286).

It can be seen (Fig. 2a) that the distribution of SO_2 values along the legs of normal volunteers was normal with a mean value of 44.7% (SD 15.4%) and with very few values (less than 2%) in the lowest class (0-10% SO_2). Figure 2b shows that the summary histogram for the TTA patients is shifted somewhat to the left with a mean of 37.6% SO_2 (SD 21.9%, median 33.5%, interquartile range (IQR) 21%). The summary histogram from the trans-femoral amputees is shifted even more to the left with 57.6% of SO_2 values less than 10%. The mean SO_2 of this group was 14.3 (SD 16.5%, median 10.2%, IQR 11.5%).

On the basis of these summary histograms, the recorded data was interrogated for the degree of skin ischaemia in terms of the percentage of SO_2 values in the classes 0-5%, 0-10%, and 0-20% SO_2. These are given in Table 1. The table also gives the statistical summary of SBF and SO_2 values at the medial and lateral sites in the patients together with the values along the leg for both patient groups and normal volunteers. The significance levels (p values) of the differences between the groups are also given in Table 1. It can be seen that significant differences were observed between all parameters (except lateral site SO_2) between TTA and TFA patients. Highly significant differences were also observed between normal subjects and TFAs in all SO_2 parameters, but the difference between the mean leg SO_2 measured in normals and TTA patients just failed to reach significance.

Differences between SBF and SO_2 values at the medial (SBF_m, SO_{2m}) and lateral sites (SBF_l, SO_{2l}) were also investigated in the TTA and TFA groups and the whole (TTA + TFA) patient sample. Significant differences between SBF at the two sites were observed in the whole patient group (SBF_m-SBF_l=2.8ml/100g/min; z=3.04, 34 df, $p < 0.01$) and the TTA group (SBF_m-SBF_l=3.9ml/100g/min; z=3.21, 26 df, $p < 0.01$). There was no difference in SBF between the sites in the TFA group (z=0.05, 14 df, p=0.96). On the other hand, the only difference between SO_2 values at the two injection sites (SO_{2l}-SO_{2m}=9.8%; z=1.94,14 df, p=0.04) was found in the TFA group: note that SO_{2l}>SO_{2m}. The mean difference between SO_{2l} and SO_{2m} of 1.5% was not significant (z=0.24,51 df, p=0.81) in the TTA group. Table

Table 1. Statistical summary of results

		Mean	Median	IQR	95% CI for Mean Upper, Lower	Difference	Significance p <
Mean	Normal					TTA v Normal	
(ml/100g/min)	TTA	7.9	7.7	6.3	6.1, 9.7	TFA v Normal	
	TFA	1.5	1.5	0.8	1.2, 1.9	TFA v TTA	.0000
Lat SBF	Normal					TTA v Normal	
(ml/100g/min)	TTA	6.0	6.0	4.0	4.8, 7.2	TFA v Normal	
	TFA	1.6	1.5	0.8	1.2, 1.9	TFA v TTA	.0000
Med SBF	Normal					TTA v Normal	
(ml/100g/min)	TTA	9.8	8.3	8.4	6.9, 12.7	TFA v Normal	
	TFA	1.5	1.6	0.8	0.9, 2.1	TFA v TTA	.0000
Mean SO_2	Normal	44.7	44.3	17.6	38.2, 51.3	TTA v Normal	.06
(%)	TTA	37.6	33.5	21.0	32.8, 42.4	TFA v Normal	.0002
	TFA	14.3	10.2	11.5	5.3, 23.2	TFA v TTA	.0001
Lat SO_2	Normal					TTA v Normal	
(%)	TTA	33.9	32.7	24.4	27.0, 40.8	TFA v Normal	
	TFA	20.6	23.8	23.3	9.8, 31.6	TFA v TTA	.07
Med SO_2	Normal					TTA v Normal	
(%)	TTA	35.4	36.9	27.1	29.0, 41.7	TFA v Normal	
	TFA	10.4	9.5	16.3	3.5, 17.3	TFA v TTA	.0001
SO_2<20%	Normal	3.5	0.0	5.3	-0.7, 7.7	TTA v Normal	.0001
(%)	TTA	23.7	24.0	26.6	17.4, 29.9	TFA v Normal	.0000
	TFA	79.7	89.3	27.7	60.2, 99.2	TFA v TTA	.0000
SO2<10%	Normal	0.9	0.0	2.3	-0.2, 2.0	TTA v Normal	.0001
(%)	TTA	9.8	8.5	12.0	6.9, 12.7	TFA v Normal	.0000
	TFA	57.6	68.5	51.7	32.9, 82.4	TFA v TTA	.0000
SO2<5%	Normal	0.1	0.0	0.0	-0.2, 0.4	TTA v Normal	.0004
(%)	TTA	5.4	3.0	8.0	3.0, 7.6	TFA v Normal	.0000
	TFA	30.3	28.0	34.4	15.1, 45.4	TFA v TTA	.0000

1 shows that there was a significant difference ($p < .01$) in SO_{2m} between the TFA and TTA groups but that the difference in SO_{2l} just failed to reach significance ($p=0.07$). Taking the mean SO_2 value from the two sites for each patient, it was found that there was a significant difference ($z=3.15,32$ df, $p < 0.01$) between the TFA group (15.5%) and TTA (34.3%).

Figure 3 shows the scatter of the mean SO_2 values along the legs in the three groups. It can be seen that there is a considerable overlap between the three groups. However, taking arbitrary mean SO_2 values of 22% and 17%, in 7/8 TFAs but only 1/29 TTAs, the mean SO_2 was less than or equal to 22%. On the other hand in 29/29 TTAs, but only 2/8 TFAs, the mean SO_2 values were greater than or equal to 17%.

The scatters of SO_2 values in the classes 0-5%, 0-10% and 0-20% SO_2 are shown in Figure 4. The three measures of degree of ischaemia (i.e. the percentage of SO_2 values in the classes) were tested for various upper and lower limits in order to determine the most reliable parameters for discriminating between the TFA and TTA groups. The 0-10% class was found to be the most sensitive and specific for determining degree of ischaemia between the

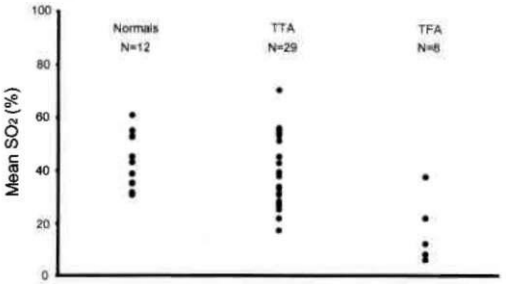

Fig. 3. Mean SO_2 values measured along the legs of the three groups.

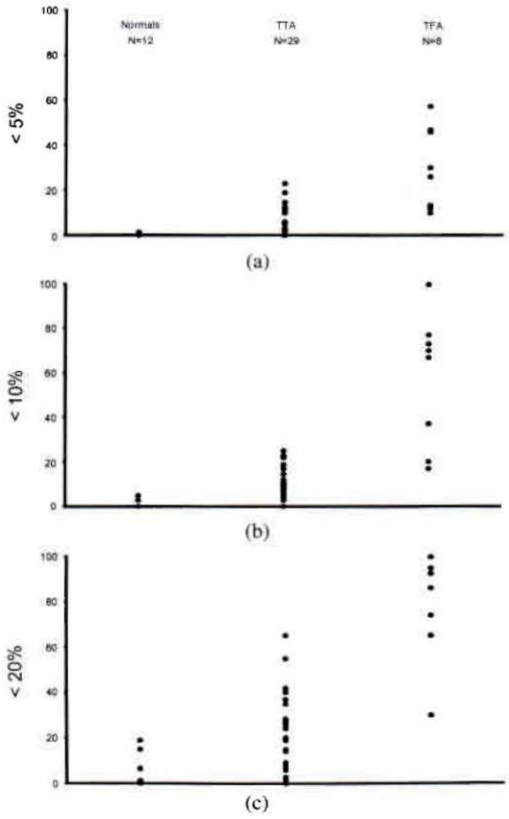

Fig. 4. Percentage of SO_2 values in the range (a) 0-5%; (b) 0-10% and (c) 0-20% along the legs in the three groups.

groups and the value 10% SO_2 was designated as being the value, SO_{2crit}, below which tissue could be considered as being critically hypoxic. Furthermore, the degree of tissue hypoxia (DTH) is defined in this paper as the percentage of SO_2 values less than 10% SO_2 measured along the limb. A test for predicting a TFA versus TTA using the criterion DTH·30% gave a sensitivity of 0.75 and specificity of 1.0. A test for predicting a TTA versus TFA using the criterion DTH μ 15% gave a sensitivity of 0.76 and specificity of 1.0.

The results therefore demonstrate a DTH less than 15% is indicative of a successful TTA and a DTH greater than 30% indicates the necessity for TFA, but that there is a "grey area" between these limits.

Discussion

It is clear from Figure 2 that there are considerable differences between the normal, TTA and TFA groups in terms of the degree of hypoxia detected in the skin along the limb. This can also been seen in Figure 4 where the percentage of values <5%, 10% and 20% SO_2 are shown for each individual within the three groups. The value of 10% SO_2 was chosen as the critical level of hypoxia (SO_{2crit}) on the basis of the fact that it was the value which, although not perfect on its own, could be used with the greatest sensitivity and specificity to discriminate between the groups. This level of hypoxia has also been used to delineate ischaemic regions of the human myocardium during coronary bypass operations (Frank *et al.*, 1989[2]).

A value of 10% SO_2 in arterial blood is equivalent a pO_2 of 1.3 kPa (10 mmHg)). In ischaemic tissue, however, decreased affinity of haemoglobin for O_2 due to increased H^+ ion activity (Harrison and Walker, 1979), may give rise to a higher tissue pO_2 of up to 2.0 kPa (15 mmHg) which is well above the level indicative of critical intracellular hypoxia (>1% of pO_2 values below 0.7 kPa (5 mmHg)) when cytochrome $a+a_3$ becomes reduced (Starlinger and Lübbers, 1973; Chance, 1988). However, Figure 4a shows that all of those patients in the TFA group and a substantial number in the TTA group had more than 10% of values along the leg of less than 5% SO_2 (equivalent to about 1 kPa (8 mmHg) pO_2 at pH 7.2) which would tend to indicate substantial critical intracellular hypoxia. Surprisingly, the value of 10%, and not 5%, SO_2 turned out in practice to be the most sensitive value for SO_{2crit} in discriminating between the TTA and TFA groups. This is probably due largely to the fact that with the catchment volume of the lightguides used (Egglishaw, 1994), the majority of the haemoglobin signal detected is emanating from parts of the vascular network other than capillaries. Hence the degree of anoxia represented in Figures 2-5 is probably substantially underestimated.

It can be seen both by comparing Figures 3 and 4b, which show the individual values for the three groups, and from Table 1, which compares the mean values from the groups, that DTH (i.e. the percentage of values below 10% SO_2) gives clearer differences, with greater degrees of significance, between the groups than the mean SO_2 value along the leg. However, it could be argued that the local oxygen supply along the line of the proposed

skin flap may be of more relevance than that along the part of the limb which is to be amputated.

Figure 5 shows a scatter diagram of the mean SO_2 values from the measurements at the two injection sites plotted against DTH in the TFA and TTA groups. As already seen in Figure 4b, all patients with a DTH of more than 30% along the leg had a TFA and all with a DTH less than 15% had a successful TTA. However, Figure 5 shows that although the primary criterion may be the DTH along the limb, an important secondary criterion may well be the local oxygen supply along the proposed line of amputation. It can be seen from the figure that all patients with mean site SO_2 values of greater than 30% had successful TTAs.

The present results thus permit the conclusion that a DTH of 15% measured along the limb prior to amputation is indicative of wound healing whereas a DTH of 30% or greater indicates an insufficient rate of oxygen supply to support tissue healing. More local measurements along the site of the proposed wound show that a minimum pre-amputation level of 30% SO_2 is required in the skin if tissue healing is to be assured. However, a successful TTA was carried out even at a mean site SO_2 value of 5% given a primary criterion of a DTH less than 15% in the lower leg.

If these combined criteria of lower leg hypoxia and mean site SO_2 are applied as the selection criteria for trans-tibial versus trans-femoral amputation, then both a selectivity and specificity of 1.0 can be applied to this experimental data. This compares with the skin blood flow "gold standard" with a predicted

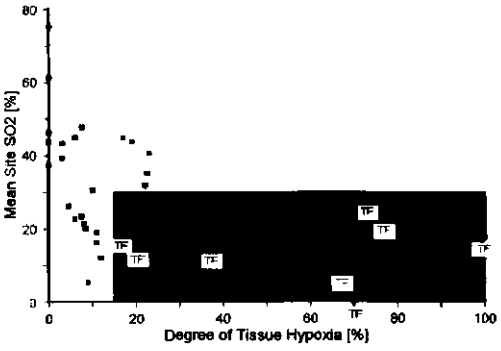

Fig. 5. Scatter plot of the mean site SO_2 (mean of the medial and lateral SO_2 values measured at the sites of SBF injection) against the degree of hypoxia along the limb. All TFA patients fall within the shaded area.

TFA for SBF < 2.5ml/100g/min and TTA for SBF > 2.5ml/100g/min which has a sensitivity of 1.0 and specificity of 0.93 in the present study.

The apparent reliability of the spectrophotometric technique, demonstrated in Figure 5 and described above, must be judged against the calibration of the system which was carried out partly *in vitro* and partly *in vivo* in human skin. The accuracy of the method is reported as $\pm 5\%$ (Harrison *et al.,* 1992), which, with close examination of Figure 5, could give rise to the mis-classification of some patients. In practice, in the present study, the pathological differences were apparently much greater than the errors involved in the technique.

This study raises one particularly interesting question. Table 1 shows that the SBF at the medial site was significantly higher than at the lateral site for the TTA group of patients. On the other hand, the SO_2 values were significantly higher at the lateral than at the medial site in the TFA group of patients. Otherwise, no significant differences were found in either parameter.

The higher blood flow at the TTA medial site is likely to be related to the presence of the saphenous and crural arteries on the medial and posterior aspects of the leg providing collateral flow (there is no equivalent on the lateral side). There may also be local regulatory mechanisms operating in order to maintain tissue oxygen supply (Harrison *et al.,* 1990). This, however, may only be possible in the presence of a continuing, albeit low, arterial supply. In the case of the TFA patients, arterial blood flow may be inadequate for the global tissue needs with the result that metabolic changes (e.g. drop in pH (Harrison and Walker, 1979)) may interfere with local regulation and produce pathological patterns of capillary blood flow (Harrison *et al.,* 1989).

Whilst the use of such a large lightguide system in the present study did not allow the detection of highly localised hypoxia (within one or two capillaries) such pathological, localised anomalies in flow patterns have been observed, using a scanning laser Doppler technique, in experimentally induced inflammation in the skin (Harrison *et al.,* 1993). The way forward for tissue spectrophotometry in the detection of local tissue ischaemia in small volumes may thus well be the application

of micro-lightguide techniques (Frank *et al.*, 1989[1]; Harrison *et al.*, 1994).

It is clear that this is a relatively small study and further evaluation of the technique is continuing. However, lightguide tissue spectrophotometry is fast: the sequence of measuerments and evaluation taking less than 10 minutes to complete. This compares favourably with the IAP technique which takes at least as long to carry out a single measurement, dependent on the skin blood flow. Unlike the IAP clearance technique, tissue spectrophotometry is completely non-invasive and patients do not have to keep their legs completely still during the period of measurement. It therefore offers considerable advantages for this area of application.

In summary, the current study demonstrates that lightguide tissue spectrophotometry can be used for predicting the outcome of amputation in critical limb ischaemia using the criteria described.

Acknowledgements

Purchase of the spectrophotometer was made possible by a grant from the Research Initiatives Fund, University of Dundee. David Newton was supported during the period of this work by grant from the Scottish home and Health Department (K/MRS/50/C1948) and Medical Research Council (G9327691PA).

REFERENCES

CHANCE B (1988). Early reduction of cytochrome c in hypoxia. *FEBS Lett* **226,** 343-346.

CUMMING JG, SPENCE VA, JAIN AS, STEWART C, WALKER WF, MURDOCH G (1987). Fate of the vascular patient after below-knee amputation. *Lancet* 12 September, 613-615.

DELPY DT, COPE MC, CADY EB, WYATT JS, HAMILTON PA, HOPE PL, WRAY S, REYNOLDS EOR (1987). Cerebral monitoring in newborn infants by magnetic resonance and near infrared spectroscopy. *Scand J Clin Invest* **47 (Suppl 188),** 9-17.

DORMANDY JA (1991). Natural history of intermittent claudication. *Hosp Update* **17,** 314-320.

EGGLISHAW C (1994). Quantification of catchment volumes of MCPD and EMPHO spectrophotometers, for visible wavelengths, using an in vitro model of the skin.- Dundee: BSc Dissertation University of Dundee

FORRESTER JC (1988). Wounds and their management. In: Essential surgical practice. /2nd edition./edited by A Cuschieri, GR Giles, AR Moossa.- London: John Wright. p 3-15

FRANK KH, KESSLER M, APPELBAUM K, DÜMMLER W (1989[1]). The Erlangen micro-lightguide spectrophotometer EMPHO I. *Phys Med Biol* **34,** 1883-1900.

FRANK KH, KESSLER M, APPELBAUM K, DÜMMLER W, ZÜNDORF J, HÖPER J, KLÖVEKORN WP, SEBENING F (1989[2]). Oxygen supply of the myocardium. *Adv Exp Med Biol* **248,** 561-566.

FRANK KH, KESSLER M (1992). Theoretical aspects of light scattering in living tissues of men and mammals - a wave mechanical analysis. In: Quantitative spectroscopy in tissue./edited by K Frank, M Kessler.- Frankfurt: PMI Verlag. p 16-46.

HARRISON DK, WALKER WF (1979). Micro-electrode measurement of skin pH in humans during ischaemia, hypoxia and local hypothermia. *J Physiol (Lond)* **291,** 339-350.

HARRISON DK, BIRKENHAKE S, HAGEN N, KESSLER M (1989). Capillary blood flow and local oxygen supply in skeletal muscle during acute haemodilution with hydroxyethyl starch. *Adv Exp Med Biol* **248,** 693-698.

HARRISON DK, KESSLER M, KNAUF SK (1990). Regulation of capillary blood flow and oxygen supply in skeletal muscle in dogs during hypoxaema. *J Physiol (Lond)* **420,** 431-446.

HARRISON DK, EVANS SD, ABBOT NC, BECK JS, MCCOLLUM PT (1992). Spectrophotometric measurements of haemoglobin saturation and concentration in skin during the tuberculin reaction in normal human subjects. *Clin Phys Physiol Meas* **13,** 349-363.

HARRISON DK, ABBOT NC, BECK JS, MCCOLLUM PT (1993). A preliminary assessment of laser Doppler perfusion imaging in human skin using the tuberculin reaction as a model. *Physiol Meas* **14,** 241-252.

HARRISON DK, DELANEY C, BROWN L, NEWTON D, MCCOLLUM PT (1994). Clinical applications of lightguide diffuse reflectance spectrophotometry in vascular disease. In: Biomed optoelectronic devices and systems; SPIE Proceedings series vol. 2084./edited by N Croitoru, R Pratesi, A Katzir.- Bellingham WA: SPIE. 195-203.

HAUSER CJ (1987). Tissue salvage by mapping of skin surface transcutaneous oxygen tension index. *Arch Surg* **122,** 1128-1130.

HEINRICH R, KRAWZAK HW, STROSCHE H (1989). Influence of prostaglandin E_1 on muscular tissue – PO_2 in patients with diabetic gangrene. *Adv Exp Med Biol* **248,** 699-704.

HICKMAN P, HARRISON DK, EVANS SD, BELCH JJF, MCCOLLUM PT (1994). Use of lightguide reflectance spectrophotometry in the assessment of peripheral arterial disease. *Adv Exp Med Biol* **345,** 885-892.

HOHN DC (1977). Leukocyte phagocytic function and dysfunction. *Surg Gynecol Obstet* **144,** 99-104.

MCCOLLUM PT, SPENCE VA, WALKER WF (1985). Circumferential skin blood flow measurements in the ischaemic limb. *Br J Surg* **72,** 310-312.

MCCOLLUM PT, SPENCE VA, WALKER WF (1988). Amputation for peripheral vascular disease: the case for level selection. *Br J Surg* **75,** 1193-1195.

NIINKOSKI J (1969). Effect of oxygen supply on wound healing and formation of experimental granulation tissue. *Acta Physiol Scand* **(suppl 1),** 1-72.

SILVER IA (1969). The measurement of oxygen tension in healing tissue. *Progr Resp Res* **3,** 124.

SILVER IA (1980). The physiology of wound healing. In: Wound healing and wound infection: theory and surgical practice./edited by TK Hunt. – New York: Appleton-Century-Crofts. p11-28.

SPENCE VA, WALKER WF (1976). Measurement of oxygen tension in human skin. *Med Biol Eng* **14,** 159-165.

SPENCE VA, MCCOLLUM PT, MCGREGOR IW, SHERWIN SJ, WALKER WF (1985). The effect of the transcutaneous electrode on the variability of dermal oxygen tension changes. *Clin Phys Physiol Meas* **6,** 139-145

STARLINGER H, LÜBBERS DW (1973). Polarographic measurements of oxygen pressure performed simultaneously with optical measurements of the redox state of the respiratory chain in suspensions of mitochondria under steady-state conditions at low oxygen tensions. *Pflüegers Arch* **341,** 15-22.

STEPHENS FO, HUNT TK (1971). Effect of changes in inspired oxygen and carbon dioxide tensions on wound tensile strength: an experimental study. *Ann Surg* **173,** 515-519.