Amputation level assessment using lightguide spectrophotometry


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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 (SO2). Degree of tissue hypoxia (DTH) along the leg was defined as the percentage of values along the leg less than 10% SO2. DTH and mean SO2 values were compared with skin blood flow values (I125 4-Iodoantipyrine clearance technique) and clinical outcome of trans-tibial amputation, (TTA) or trans-femoral amputation (TFA), in 41 patients. SO2 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 SO2 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.
D.K. Harrison, P.T. McCollum, D.J. Newton, P. Hickman and A.S. Jain

(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 (45 mmHg) 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
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 \(\text{SO}_2\) 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. \(\text{SO}_2\) 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 \textit{in vitro} and \textit{in vivo}
experiments to enable an accuracy of \(\pm 5\%\) to
be achieved (Harrison \textit{et al.}, 1992).

\textbf{IAP clearance}

\(\text{I}^{125}\) 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, \text{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.

\textbf{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.

\textbf{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
totally 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).

\textbf{Treatment of results}

The spectrophotometric data at each location
were analysed as described above to give \(\text{SO}_2\)
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 \(\text{SO}_2\) (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 TTA 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.

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, $p=0.05$) 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.
Spectrophotometry in amputation level assessment

1 shows that there was a significant difference (p < .01) in SO$_2$m between the TFA and TTA groups but that the difference in SO$_2$ 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
groups and the value 10% SO\textsubscript{2} was designated as being the value, SO\textsubscript{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\textsubscript{2} values less than 10% 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 < 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 be seen in Figure 4 where the percentage of values <5%, 10% and 20% SO\textsubscript{2} are shown for each individual within the three groups. The value of 10% SO\textsubscript{2} was chosen as the critical level of hypoxia (SO\textsubscript{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).

A value of 10% SO\textsubscript{2} in arterial blood is equivalent to a pO\textsubscript{2} of 1.3 kPa (10 mmHg). In ischaemic tissue, however, decreased affinity of haemoglobin for O\textsubscript{2} due to increased H\textsuperscript{+} ion activity (Harrison and Walker, 1979), may give rise to a higher tissue pO\textsubscript{2} of up to 2.0 kPa (15 mmHg) which is well above the level indicative of critical intracellular hypoxia (>1% of pO\textsubscript{2} values below 0.7 kPa (5 mmHg)) when cytochrome a+a\textsubscript{3} becomes reduced (Starlinger and Lubbers, 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\textsubscript{2} (equivalent to about 1 kPa (8 mmHg) pO\textsubscript{2} at pH 7.2) which would tend to indicate substantial critical intracellular hypoxia. Surprisingly, the value of 10%, and not 5%, SO\textsubscript{2} turned out in practice to be the most sensitive value for SO\textsubscript{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\textsubscript{2}) gives clearer differences, with greater degrees of significance, between the groups than the mean SO\textsubscript{2} value along the leg. However, it could be argued that the local oxygen supply along the line of the proposed
Spectrophotometry in amputation level assessment

A 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 transfemoral 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 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 ±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.

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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.
of micro-lightguide techniques (Frank et al., 1989; 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 measurements 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.

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Spectrophotometry in amputation level assessment


