Assessment of burn depth: A prospective, blinded comparison of laser Doppler imaging and videomicroscopy

D.J. McGill*, K. Sørensen, I.R. MacKay, I. Taggart, S.B. Watson

Canniesburn Plastic Surgery Unit, Jubilee Building, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, United Kingdom

1. Introduction

Prompt excision and skin grafting is the currently accepted treatment for the management of deep dermal and full thickness burns. This practice has been shown to shorten the duration of hospital stay, reduce the incidence of infection and the need for antibiotics [1,2]. Accurate determination of burn depth is therefore extremely important to ensure that appropriate management of the injury is initiated as early as possible. However, the assessment of burn depth remains problematic. Clinical assessment is subjective [3] and several studies found that it has an accuracy of between 60 and 80%, even when carried out by experienced burns surgeons [4–8].

Many different methods of measuring burn depth have been investigated in the past [9–13]. However, none of these methods have become widely used, due, at least in part, to a lack of consistency in reported results [6]. An alternative method is histological analysis of biopsy specimens, specifically looking for evidence of microvascular damage. Following on from Jackson’s work examining changes in burn depth in the acute post-burn period [3], the importance of the dermal capillary plexus in burn wounds has been established [14–17]. However, biopsy is an invasive procedure that will leave...
scarring. In addition, it only provides a snapshot of the burn and there can be inter-observer error between those evaluating the biopsy specimens [16]. It is therefore best regarded as a research tool [15].

This understanding of the importance of the dermal capillary plexus in burn wounds led to the development of laser Doppler flowmetry [5,7,18,19] and imaging [6,8,20–23] as techniques to evaluate burn depth. Studies demonstrated that LDI is reliable, reproducible and that there is good concordance between laser Doppler findings and biopsy results [6,8,23]. Laser Doppler imaging (LDI) has the advantages of being non-invasive, non-contact and is able to scan large areas at a time to provide a map of burn depth that can be used to determine the need for surgery. Despite this, LDI is not widely used for routine clinical use [24]. One of the main reasons for this is that it is an expensive modality.

There is therefore a place for an alternative modality in the assessment of burn depth that is as reliable but more cost-effective. Since the assessment of dermal capillary integrity within burn wounds is the aim of both biopsy and LDI, an alternative solution would be to directly assess the capillary structure using transcutaneous microscopy, in the form of the videomicroscope. The use of this modality to assess capillary ectasia in capillary vascular malformations has previously described [25–27] where it has been shown to provide reliable information on the dermal capillaries, including patency. In this blinded, prospective clinical trial we compared burn depth assessment using videomicroscopy with that of LDI. Clinical assessment was also recorded. The aims of the study were: firstly, to identify whether videomicroscopy was able to reliably differentiate between superficial and deep partial thickness burns; secondly, to establish whether burn depth assessment using the videomicroscope is comparable to LDI.

2. Materials and methods

2.1. Subjects

The local ethics committee approved the study. All patients admitted to the Glasgow Royal Infirmary Burns Unit, and those seen in the outpatient burns clinic, were considered for entry into the study. Inclusion criteria to the study were: time to presentation less than 72 h; and total burn size measuring less than 15% total body surface area. Exclusion criteria were: time to presentation greater than 72 h; burn size greater than 15% total body surface area; facial burns where infection risk and discomfort would preclude wearing laser goggles; patients otherwise medically unstable (e.g. systemic sepsis, smoke

<table>
<thead>
<tr>
<th>Table 1 – Type and location of burn are displayed along with the LDI and videomicroscope depth assessment results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn wound</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>26</td>
</tr>
<tr>
<td>27</td>
</tr>
</tbody>
</table>

a The clinical outcome for each injury is also displayed.

b See Table 2.

b SSG, split skin grafting.
inhalation requiring ventilatory support, other injuries) that might affect LDI images due to movement artefact or altered skin perfusion; and patients less than 13 years old.

Twenty patients with 27 wounds were included in the study: 17 men and 3 woman; median age 38 years (range 16–73 years); and median burn area 3.5% (range 1–8%). The majority of burns examined were located on the limb and the most common cause of injury was a flash burn (Table 1). Median assessment time was 54 h following injury, range 31–72 h.

2.2. Laser Doppler imaging

A Moor Instruments LDI was used in this study, incorporating a 633 nm helium–neon Class IIIa 2 mW laser imaging beam (Moor Instruments Ltd., Axminster England, UK). The scanner was attached to a touch-screen PC mounted on a mobile stand and operated using moorLDI V3.1 software. In this study, for each patient the scanner was set between 30 and 40 cm from the burn wound with the scan speed set at 10 ms/pixel.

The function and theoretic basis for the operation of the Moor LDI has been described previously [6,8,20,23]. Essentially, it relies upon the Doppler principle that light reflected from a moving object, such as blood cells within a capillary, will undergo a frequency shift with regards to a static observer (e.g. a photodetector) whilst that reflected from static tissue will not. The LDI, using the continuous scan technique described by Essex and Byrne [28], emits 633 nm red light and detects reflected light from the tissues scanned. The incorporated Moor software then calculates the proportion of reflected light that has undergone a Doppler shift and, using a mathematical algorithm designed to filter noise and variations in scattered light intensity, produces a two-dimensional colour image. This image of skin perfusion displays the ‘flux’ values, which are proportional to the product of the average velocity of blood cells and their concentration, and is expressed as arbitrary perfusion units (PU). During analysis the software provides a mean flux reading and standard deviation for any given area of the burn wound, which can be used to determine burn depth.

For the purposes of analysing burn depth from the scans, a standard six-colour palette was used for the assessment of the ‘flux’ images, using a scale extending from 0 to 1000 PU. This scale uses red and yellow colours to denote areas with high skin blood perfusion (flux values greater than 750 PU), whilst blue is used to denote those areas with low perfusion (flux values less than 375 PU). Normal skin will appear blue using this scale, since intact epidermis will reflect large amounts of the HeNe beam without altering the light frequency. The assessor analysing the LDI images (KS) used this scale to identify areas of superficial partial thickness (red or yellow, 500–1000 PU), deep partial thickness (green or blue, 150–500 PU) and full thickness (dark blue, <150 PU). It is important to note that even full thickness burns have some detectable blood flow on the LDI, unless extending deep into the subcutaneous tissues, since the scanner will detect flow in the superficial subcutaneous vessels.

2.3. Videomicroscope

To directly visualise dermal capillary integrity, we used transcutaneous microscopy, in the form of a compact videomicroscope designed in collaboration with PW Allen (PW Allen Ltd., Tewksbury, UK). This system includes a 200× magnification Cy-scope lens and fibre-optic light source, attached to a Sony trinitron monitor and colour video-printer (Mitsubishi Colour Video Copy Processor) for the purposes of image assessment and image capture. The Cy-scope lens views an area of skin measuring 1.02 mm² at a time (manufacturer’s measurement), and requires contact with the burn surface to visualise the dermal capillary structure. To prevent the transmission of infection from the videomicroscope lens to the skin surface, we covered the lens with a sterile drape (Tube Cover, Klinidrape, Molnycke Health Care, Finland) and used sterile surgical gloves during the assessment of burn depth.

When assessing burn depth, the operator (DJM) applied the videomicroscope lens to the burn surface and look at the integrity of the dermal capillaries. Due to the optical properties of skin and the focal length of the microscope lens it is not possible to visualise all the way through the skin into the subcutaneous tissues, so only the dermal vessels are examined. However, for the purposes of assessing burn depth, this is all that is necessary. The videomicroscope can be rapidly moved across the surface of the burn, measuring burn depth by assessing the integrity of the dermal capillaries. Areas of particular interest, such as areas that clinically appear of different depth, or areas that are indicative of the overall burn depth, can then be recorded and printed out using the video-printer.

This technique relies on the fact that the depth of burn injury depends upon the integrity of the dermal capillary plexus. The dermis contains a horizontal capillary plexus located in the reticular dermis whilst vessels extending into the papillary dermis lie perpendicular to the surface. In intact skin, the tips of the capillaries in the papillary dermis can be seen as ‘blobs’ when using the videomicroscope, whereas the horizontal capillary plexus is visible as ‘rings’ of interlinking capillaries. These findings originate from both capillaryscopy work and videomicroscopic analysis of capillary vascular malformations [25–27,29–31]. Therefore, in superficial burns, some of the superficial vessels and the deeper capillary plexus are expected to be intact and visible under the videomicroscope, whereas deeper burns extend into the reticular dermis and involve damage to the capillary plexus. From this, burn depth is assessed as follows: the presence of an intact, or mostly intact, dermal capillary plexus without thermal damage denoted a superficial partial thickness injury; the presence of capillary destruction and haemoglobin deposits in the dermis denoted a deep partial thickness injury; and the absence of any capillaries denoted a full thickness injury. For ease of interpretation, the capillary destruction seen under the videomicroscope was graded (see Table 2).

2.4. Study protocol

Patients fulfilling the inclusion criteria outlined above were identified and written informed consent obtained. Clinical assessment of the burns was carried out prior to depth assessment, either by one of the burns unit consultants or by the burns and trauma fellow, and recorded on a clinical observation sheet for the purposes of the study. The patients...
were subsequently assessed using the videomicroscope, and finally a scan was taken using the LDI. The videomicroscopy and LDI assessments combined took, on average, 15–20 min to complete. This included set-up time for the LDI scanner, which was completed with the wound dressings on to minimise the wound exposure time.

The videomicroscopy results were assessed by the first author, whilst the LDI scans were assessed by the second author, both of whom were blinded to the clinical assessments, to ensure that there was no bias in interpretation of either the videomicroscopy or LDI scans.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Findings</th>
<th>Burn depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Intact capillary plexus</td>
<td>Superficial partial thickness</td>
</tr>
<tr>
<td>1</td>
<td>Minor capillary destruction</td>
<td>Deep partial thickness</td>
</tr>
<tr>
<td>2</td>
<td>Large amounts of capillary destruction and haemoglobin deposition</td>
<td>Full thickness</td>
</tr>
<tr>
<td>3</td>
<td>Complete destruction of capillary plexus with absent capillaries</td>
<td>Full thickness</td>
</tr>
</tbody>
</table>

Fig. 1 – Superficial partial thickness burn to both dorsal forearms assessed 62 h post-injury. Cause of injury was a gas canister explosion. The wound healed in less than 21 days. LDI images demonstrate high perfusion (red) consistent with a superficial partial thickness injury, whilst videomicroscopy images demonstrate intact capillary plexus on both forearms (grade 0). (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of the article.)
The patients were then prospectively followed up to one of three clinical end-points. The consultant looking after the patients decided upon the course of treatment in each case, based upon clinical assessment alone (the consultants were blinded to the results of either of the Videomicroscopy or LDI assessments). The three end-points used in the study were: primary healing within 21 days, confirming a burn to be superficial partial thickness; theatre for tangential excision and reconstruction of the burn at which time burn depth was reassessed clinically; or failure of primary healing within 21 days of sustaining the burn. These end-points were used to identify the burn depth in each case and the videomicroscopy and LDI were compared to determine the accuracy of burn depth assessment for each method. The incidence of burn wound infections was recorded, so that any changes in burn depth occurring following assessment that were due to infection could be accounted for, to avoid confounding of the results.

Fig. 2 – Scald left lateral thigh assessed 71 h post-injury. The burn was clinically assessed as a superficial partial thickness injury and treated conservatively. The LDI image demonstrates moderate to high perfusion in the superior aspect of the image (red and yellow) consistent with a superficial partial thickness burn, which corresponds to videomicroscopy image (labelled a) demonstrating an intact capillary plexus (grades 0–1). The inferior aspect took 16 weeks to heal and is blue on the LDI (low perfusion) with corresponding videomicroscopy image (labelled b) showing capillary destruction and haemoglobin deposition, consistent with a deep partial partial thickness injury (grade 2). (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of the article.)
3. Results

Table 1 displays the burn depth assessments made by LDI and videomicroscopy and the clinical outcome in each case. In terms of clinical outcome, 14/27 (52%) burns were treated conservatively and healed within 21 days; 3/27 (11%) were treated conservatively and failed to heal within 21 days; and the remaining 10/27 (37%) had early excision and split skin grafting.

The first group of 14 injuries that demonstrated primary healing in less than 21 days were clinically consistent with superficial partial thickness (SPT) burns. In each case, LDI assessment identified high levels of skin perfusion consistent with SPT injuries: mean ± S.D. flux (in perfusion units) in this group was 764.9 ± 264.2 PU. Videomicroscopy findings in this group were of minimal capillary destruction evident (grades 0–1), also in keeping with SPT injuries (see Fig. 1).

The second group comprised three injuries that were treated conservatively but failed to heal within 21 days. The first two cases (scalds to the wrist and thigh, respectively, see Figs. 2 and 3) were clinically assessed as SPT, hence the conservative treatment. In both cases an area of the burn failed to heal primarily. LDI assessment of these injuries identified areas of decreased skin perfusion consistent with a

![Image of burn and perfusion scan](image)

**Fig. 3** – Scald to right hand and wrist, assessed 45 h after injury. A small area on ulnar aspect of the volar wrist (green area of low perfusion on the LDI scan, labelled a) failed to heal in 21 days. The corresponding videomicroscope image shows grade 3 capillary destruction. The remaining areas all healed in less than 21 days, denoted as red and yellow on LDI (high perfusion) and with a corresponding videomicroscope image (labelled b) showing intact capillaries, grades 0–1. (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of the article.)
deep partial thickness (DPT) injury in the areas on the wrist and thigh that failed to heal (417 ± 119.6 and 245.4 ± 121.5 PU, respectively), whilst demonstrating levels of skin perfusion consistent with SPT injury in the remaining areas which did heal within 21 days (665.3 ± 193.6 and 501 ± 176.1 PU, respectively). Likewise, videomicroscopy findings were grade 3 (DPT to full thickness injury) on the wrist and grade 2 on the thigh (DPT) where there was delayed healing and grades 0–1 (SPT) in the remaining areas. In the third case, a contact burn on the buttock was treated conservatively due to its small size and location, despite the clinical assessment of a DPT injury. LDI in this case demonstrated skin perfusion levels suggestive of a SPT injury (591.9 ± 89.6 PU), whilst videomicroscopy demonstrated capillary destruction (grade 2) suggestive of a DPT injury.

Fig. 4 – Burn to posterior aspect right thigh following a gas explosion. Assessed at 33 h post-injury. Treated clinically as a full thickness injury with excision and split skin grafting. LDI demonstrates an area of minimal perfusion centrally (blue) denoting a FT injury. The corresponding videomicroscopy image (labelled a), demonstrates an absence of capillaries and haemoglobin deposition (grade 3). In contrast, the videomicroscopy image b, from an area of high perfusion (red) shows an intact capillary plexus (grade 0), whilst area c is from an area of reduced perfusion (green) with the corresponding videomicroscope image showing partial loss of capillary integrity (grade 2). (For interpretation of the references to colour in this figure caption, the reader is referred to the web version of the article.)
injury. In this case the outcome was delayed healing, with the burn taking 5 weeks to heal confirming a deep partial thickness injury.

The final group of 10 burns were all clinically assessed as being either DPT (7/10) or FT (3/10) and were treated with excision and split skin grafting. In eight of these injuries, LDI found skin perfusion to be low throughout the burn wound (280.4 ± 125.1 PU), consistent with a DPT or FT injury, whilst videomicroscopy findings were graded 2–3, demonstrating destruction of the capillary plexus, which is again consistent with a DPT or FT injury (see Fig. 4). The other two cases were mixed depth burns with areas of SPT and DPT evident in each case on both LDI (areas of SPT—677.3 ± 239.4 and 731.4 ± 97.7 PU; areas of DPT—158.1 ± 87.6 and 355.4 ± 74 PU) and videomicroscopy (grades 0–1 appearance in SPT; grades 2–3 in DPT/FT).

Fig. 5 compares the mean flux readings from LDI and videomicroscope grading for each of the three clinical outcomes. Both the LDI and videomicroscopy findings demonstrated a strong correlation with clinical outcome (Kendall’s Tau correlation coefficient $r = -0.697$ and 0.751, respectively, $p < 0.001$) and also with each other ($r = -0.725$, $p < 0.001$).

Six patients had a documented episode of infection. One patient was admitted with an infected deep partial thickness burn and two patients with deep partial thickness burns developed clinical signs of infection (confirmed on wound culture) and were commenced on antibiotics prior to assessment with the LDI and videomicroscope. In each case, the burn was successfully excised and skin grafted. Only one patient developed infection within a superficial partial thickness injury, again prior to depth assessment, and this healed up without complication following a course of antibiotics. The remaining two infections occurred after measurement had been taken, in a deep partial thickness and a full thickness burn, respectively. Both were treated by excision and split skin grafting as planned. Overall, there was no suggestion that any of the burn infections resulted in a change in burn depth that would have altered the results of the study.

4. Discussion

Previous studies found LDI to be reproducible and reliable, with a sensitivity ranging from 90 to 100% and a specificity ranging from 92 to 96% [7,8,21,22]. This compares extremely well with the 60–80% accuracy reported for clinical assessment [4–8]. The LDI results from our study demonstrate that the sensitivity at detecting superficial partial thickness injuries was 100%. It is difficult to calculate an accurate level of specificity since the burn depth in those burns treated by excision and skin grafting relied upon clinical assessment and was not independently verified, for example by biopsy. Nevertheless, the LDI results for those patients either having delayed healing or going on to have excision and skin grafting correlated with the clinical outcome in 12 out of 13 injuries, and overall the LDI had a highly statistically significant correlation with clinical outcome.

The videomicroscope directly visualises the dermal capillary structure and provides evidence of capillary integrity. Previous work by Jackson [3], and more recent histological studies [14–17], have demonstrated that capillary integrity strongly correlates with the depth of burn. The findings of this study appear to support this, since the amount of capillary destruction visualised by the videomicroscope not only strongly correlates with the clinical outcome, but also with the findings of LDI: the sensitivity of videomicroscopy at detecting superficial partial thickness burns was also 100%. In addition, in the case where there was discordance between LDI findings and clinical outcome (delayed healing of a contact burn to the buttock, where LDI suggested a superficial partial thickness injury) videomicroscopy correctly identified the burn as a deep partial thickness injury. Videomicroscopy therefore appears to be both highly sensitive and reliable. However, since the videomicroscopy findings were all assessed by a single operator (DJM), it is not possible to commentate upon the reproducibility of this technique.

Learning how to use the videomicroscope and distinguish between different burn depths is relatively simple and has a rapid learning curve. Once acquired, assessment of burn wounds can be rapidly undertaken, even if large surface areas are involved, by moving the lens over the surface of the wound and concentrating on areas of interest within the burn (e.g. areas that clinically appear deeper, or areas that appear different on videomicroscopy than initially suspected). The main disadvantage of this technique is obviously that
contact with the burn surface is required, which is often uncomfortable for the patient and involves the possibility of transmitting infection. To prevent infection we covered the microscope lens with a sterile cover and used sterile gloves during assessment, and minimised contact time with the burn. In this study, one patient did ask for videomicroscopy assessment to be stopped, however that was after the main areas of interest had already been mapped, so did not affect the results. All of the other patients were able to tolerate the investigation for the length to time required to achieve an accurate burn depth measurement. It seems unlikely that videomicroscopy would be well tolerated by children, potentially reducing its usefulness in this group. However, it could be used to assess burn depth prior to surgery, whilst a child is under general anaesthesia, to identify if any areas could be spared excision.

The timing of assessment is likely to be important. Previous studies using LDI tended to measure depth between 36 and 72 h after injury, since this appears to be the most reliable time to carry out assessment, possibly due to initial capillary stasis lasting around 24 h that improves after this time [6,22]. However, Jeng et al. found that the flux values of repeated scans did not change significantly from day one post-burn injury and that these initial scans were predictive of burn depth [23]. From this, it is reasonable to suppose that if the capillary blood flow is stable enough after 24 h to allow reliable LDI images, it should also allow reliable videomicroscopy assessment. In this study, the earliest assessment was 31 h after burn, with the majority between 36 and 72 h.

There are several advantages of videomicroscopy over LDI. The reliability of LDI is reduced in several situations including: excessive patient movement; high ambient light reflection from the burn surface; curvature of the scanned surface increasing beam scattering; and an inability to accurately assess flow in the presence of eschar, blisters, slough, or certain topical antimicrobials. Some of the problems relating to movement and skin curvature may be overcome by altering the resolution of the scanner and by carrying out several small scans rather than trying to assess the entire burn area in one go. This has the disadvantage of taking longer to set up and requires the patient to be compliant and have wound exposure for a longer period. Many of these difficulties are avoided when using the videomicroscope. In particular, it is not affected by patient movement, skin curvature or high ambient light reflection, although there is still difficulty in the presence of blisters (which need to be de-roofed prior to videomicroscope assessment) slough or eschar.

In addition to the advantages outlined above, and potentially more importantly, videomicroscopy is a significantly cheaper modality than LDI. The set-up we used cost approximately £5000 (€7405 or $9438), in contrast to £35000 (£51,825 or $66,064) for a Moor LDI, making it a much more cost-effective method of measuring burn depth. Videomicroscopy is also extremely portable. Although we used a TV monitor and video-printer, the microscope can be attached to a laptop via a video-cable. This means that the videomicroscope may have applications such as assessing burns in outlying hospitals or in field hospitals, which is not practicable for LDI.

5. Conclusions

The results of this study suggest that the videomicroscope is a viable alternative to LDI as a method of differentiating superficial and deep partial thickness injuries. In addition, the videomicroscope has the advantages of increased portability, reduced cost and is not affected by factors such as patient movement, skin curvature or ambient light reflection. This is offset, when compared to LDI, by the requirement for burn wound contact during assessment. However, as discussed above, the majority of patients in this study were able to undergo videomicroscopy assessment without undue discomfort.

Although these results are very encouraging, the use of clinical endpoints is a potential area where error may occur, particularly where burns are taken for early excision and skin grafting and there is no further confirmation of burn depth. In addition, it is important to establish that videomicroscopy findings are reproducible. We intend to further validate the videomicroscopy technique by comparing videomicroscopy to biopsy measurements and also comparing the assessments of more than one operator.

Conflict of interest

The authors certify that they have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

References


