BURNS 48 (2022) 1112 –1119

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/burns

Burn depth assessment using hyperspectral imaging in a prospective single center study

Torsten Schulz [*,d], Jörg Marotz [b], Sebastian Seider [c], Stefan Langer [a], Sebastian Leuschner [b], Frank Siemers [b]

[a] Department for Orthopedics, Trauma- and Plastic Surgery-University Hospital Leipzig, Liebigstraße 20, D-04103 Leipzig, Germany

[b] Department for Plastic- and Reconstructive Surgery, Burns Unit, BG Kliniken Bergmannstrost, Merseburger Straße 165, D-06120 Halle (Saale), Germany

[c] Medical Faculty of the Martin-Luther-Universität Halle-Wittenberg, Universitätsplatz 10, D-06108 Halle (Saale), Germany

[d] Department of Orthopedic, Trauma and Plastic Surgery, Leipzig University Hospital, Germany

ARTICLE INFO

Keywords:

Laser doppler imaging
Hyperspectral imaging
Thermal imaging, burn depth assessment, burn index

ABSTRACT

Background: The assessment of thermal burn depth remains challenging. Over the last decades, several optical systems were developed to determine burn depth. So far, only laser doppler imaging (LDI) has been shown to be reliable while others such as infrared thermography or spectrophotometric intracutaneous analysis have been less accurate. The aim of our study is to evaluate hyperspectral imaging (HSI) as a new optical device.

Methods: Patients suffering thermal trauma treated in a burn unit in Germany between November 2019 and September 2020 were included. Inclusion criteria were age ≥18 years, 2nd or 3rd degree thermal burns, written informed consent and presentation within 24 h after injury. Clinical assessment and hyperspectral imaging were performed 24, 48 and 72 h after the injury. Patients in whom secondary wound closure was complete within 21 days (group A) were compared to patients in whom secondary wound closure took more than 21 days or where skin grafting was indicated (group B). Demographic data and the primary parameters generated by HSI were documented. A Mann Whitney-U test was performed to compare the groups. A p-value below 0.05 was considered to be statistically significant. The data generated using HSI were combined to create the HSI burn index (BI). Using a logistic regression and receiver operating characteristics curve (ROC) sensitivity and specificity of the BI were calculated. The trial was officially registered on DRKS (registration number: DRKS00022843).

Results: Overall, 59 patients with burn wounds were eligible for inclusion. Ten patients were excluded because of a poor data quality. Group A comprised 36 patients with a mean age of 41.5 years and a mean burnt body surface area of 2.7%. In comparison, 13 patients were allocated to group B because of the need for a skin graft (n = 10) or protracted secondary wound closure lasting more than 21 days. The mean age of these patients was 46.8 years. They had a mean affected body surface area of 4.0%. 24, 48, and 72 h after trauma the BI was 1.0 ± 0.28, 1.2 ± 0.29 and 1.55 ± 0.27 in group A and 0.78 ± 0.14, 1.05 ± 0.23 and 1.23 ± 0.27 in group B. At every time point significant differences were demonstrated between the groups. At 24 h, ROC analysis demonstrated BI threshold of 0.95 (sensitivity 0.61/specificity 1.0), on

* *Corresponding author at*: Liebigstraße 20, D-04103 Leipzig, Saxon, Germany.
E-mail addresses: torsten.schulz1988@gmail.com, torsten.schulz@medizin.uni-leipzig.de (T. Schulz).

https://doi.org/10.1016/j.burns.2021.09.010

the second day of 1.17 (sensitivity 0.51/specificity 0.81) and on the third day of 1.27 (sensitivity 0.92/specificity 0.71).

Conclusion: Changes in microcirculation within the first 72 h after thermal trauma were reflected by an increasing BI in both groups. After 72 h, the BI is able to predict the need for a skin graft with a sensitivity of 92% and a specificity of 71%.

1. Background

In 2013, 2050 people suffered burns affecting 20% or more of their body surface area were treated in burn units in Germany [1]. The mortality of these patients was 12% [1]. Alongside specialized intensive care early excision and grafting of deep burns reduces the risk of hypertrophic scars, improves function and reduces sepsis related morbidity [2–4]. Therefore, correct assessment of burn depth and extent are fundamental for adequate and timely treatment [1]. In general, wounds caused by thermal trauma such as flames or hot fluids can be categorized in four different degrees. The clinical appearance, color and capillarization of the injured skin is mainly used to determine the different degrees [5]. The accuracy of clinical judgement varies between examiners and specialization and lies between 50% and 76% [5,6]. First- and third-degree burns are easy to differentiate. Second degree burns often present a mixed picture of areas of 2a and 2b degree burns [5]. Therefore, the distinction between 2a and 2b-degree burns is much harder. Moreover, the continuing tissue damage interacts with the clinical assessment of the wounds in the first 72 h (so-called "afterburn") [7]. The majority of second degree burns in Europe are treated by doctors without particular expertise in the treatment of burns [8]. In many cases the depth of burns is overestimated and surgery was performed [9].

In the last decades a variety of non-invasive optical systems were developed to make the assessments of burns more objective and less examiner dependent. Laser doppler imaging (LDI) was first described by Niazi et al. in 1993 [10]. Up to now, more than 14 further studies have shown the sensitivity of LDI to be 91% and the specificity to be 96% [11]. Other systems that have been investigated are near infrared spectroscopy [12], indocyanine green angiography [13], spectrophotometric intracutaneous analysis [14] and thermography [15]. Nevertheless, LDI has proven to be the best among the diagnostic tools because of its many advantages. LDI is non-invasive and a large area can be evaluated [16].

Hyperspectral Imaging (HSI) is a new, non-invasive, contactless, quantitative measuring method for assessing the perfusion of the underlying tissue. The uses of HSI in medicine have been extensively reviewed elsewhere [17]. HSI uses light with a wavelength between 500 and 1000 nm. The penetration, absorption and scattering of light in biological tissue depends on its spectral range and the underlying tissue components. In general, inferences about tissue perfusion can be made by the change in remission spectra caused by the presence or absence of hemoglobin [18–20]. HSI can therefore also be used to assess the perfusion of burn wounds and might support the clinical assessment of burns [21]. Up to now, HSI was able to differentiate between three discrete levels of burn injury in an animal model [22].

The aim of our study is to evaluate this method in routine clinical practice and to describe its diagnostic accuracy in relation to secondary wound closure.

2. Patients and methods

2.1. *Study design*

In our prospective single center study, all patients with thermal burns who fulfilled the inclusion criteria were included (Fig. 1). Only competent adult patients with second- and third-degree burns presenting within 24 h of injury who provided written informed consent were included. Patients with facial burns, burns of the palmar aspect of the hand and the soles of the feet, patients with infected burns as well as patients who were treated with enzymatic debridement were excluded. Before written consent, a full explanation of the study was given. The clinical treatment was not affected by the assessments and the assessments were not used to inform clinical decision making. Data acquisition took place 24, 48 and 72 h after injury. Patients with conservatively treated burn

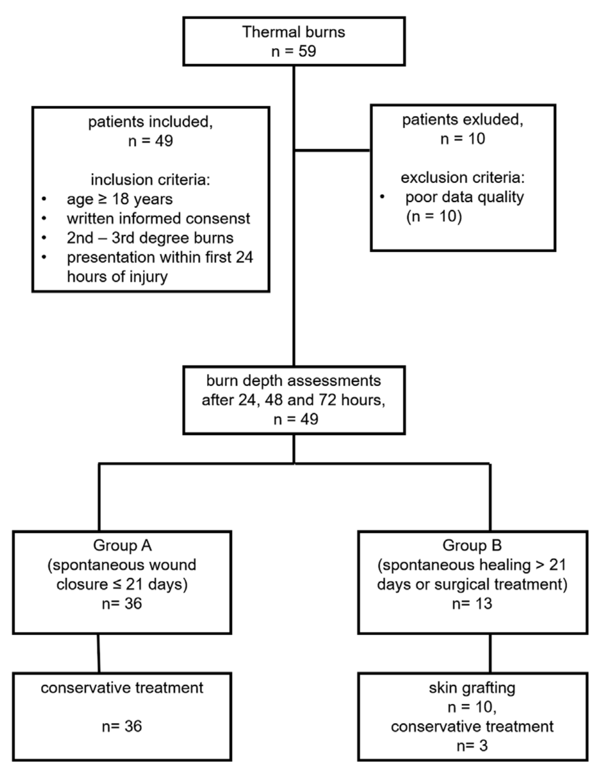

Fig. 1 – Study protocol.

wounds were followed-up two weeks after trauma and the extent of wound healing was recorded. As an objective marker of burn depth, time to spontaneous wound closure was used. All patients in whom spontaneous wound closure was complete by day 21 after injury were assigned to group A. Group B was defined by secondary wound closure after 21 days or clinical decision for burn wound excision and grafting.

2.2. *Hyperspectral imaging system*

The TIVITA© Tissue hyperspectral camera system by Diaspective Vision GmbH (Strandstrasse 15, D-182333 Am Salzhaff, Germany) was used for the present study. HSI is based on spectrometric tissue analysis. A white light source illuminates the underlying tissue. Remitted light is detected in the visible and near-infrared range at wavelengths of 500–1000 nm. The different composition of the tissue leads to different reflectance spectra. Hemoglobin, as the dominant tissue component, largely determines the individual remission rates. Oxygenated and deoxygenated hemoglobin have different reflectance spectra. Using appropriated algorithms, a three-dimensional data set is created by the remitted light [23]. Light in the near infrared range penetrates the tissue up to 4 mm. A ratio of oxygenated and deoxygenated hemoglobin is calculated using the reflectance spectra. The relative oxygen saturation and concentration of hemoglobin and its distribution in superficial (0.1 mm) and deeper tissue layers (4 mm) can be determined [19]. For further analysis of burn wounds, the software needed further development [20]. The burn wound is described using a 6-layer perfusion model. Every layer represents a histological structure of the skin [24]. In every layer the relative oxygen saturation and hemoglobin concentration are determined and shown as equally broad columns. The amount of hemoglobin in each layer was measured and referred to as vHB_1-vHB6. The oxygen saturation was also determined in each layer and referred to as O_2HB_1-O_2HB_6 (Fig. 2). To allow simple data interpretation the vHB_1-vHB6 were combined using an algorithm to calculate the BI. The O_2HB_1-O_2HB_6 was not included into the formula because of the small sample size. A multiple logistic regression was performed, which allows to check the influence of several independent variables (vHb1_6, O2Hb1_6) on a nominally scaled response variable (group A, group B). Every single layer was analyzed regarding its prognostic value. In this fashion a model with several independent variables was created. Afterwards the layers were combined. The formula for the BI is as follows:

$$P = \frac{1}{(1 + \exp(\beta_0 + \beta 1 * vHb_1 + \beta 2 * vHb2 \pm \beta x * vHbx))}$$

β represents the vector of the regression coefficients which varies at each time point. The BI ranges from 0 to 3. It describes the microcirculation of the tissue damaged by thermal burns. A low BI implies a reduced amount and/or saturation of hemoglobin which is hypothesized to reduce the probability of

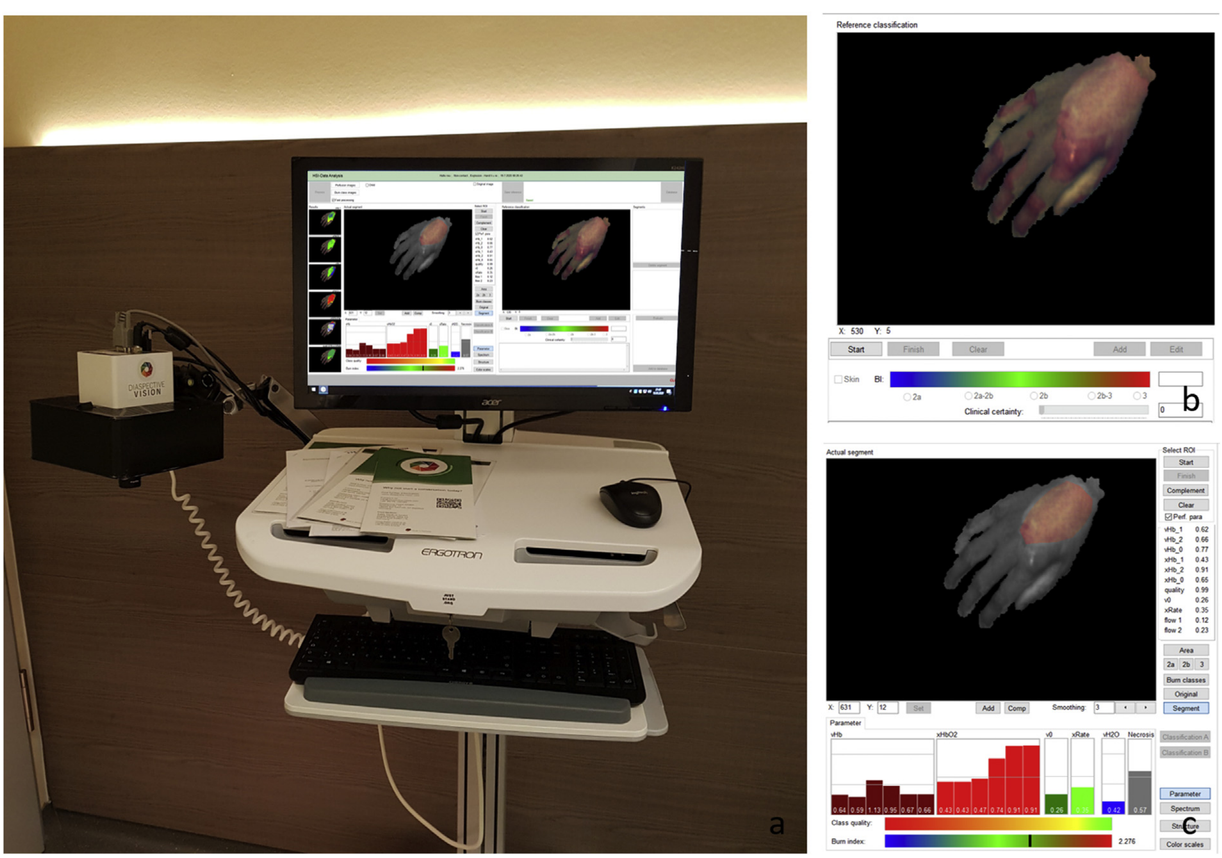

Fig. 2 – a TIVITA© Tissue hyperspectral camera. b Software system demonstrating a mixed partial thickness burns of the dorsal right hand. c After a marker is placed on the region of interest the 6-layer perfusion model including the BI is displayed.

spontaneous wound closure within 21 days. In these cases, a skin graft is recommended. A single measurement process takes 5 s. The system is easy to use, non-invasive, reliable, recordable and measures discontinuously. Therefore, it can easily be used by surgeons, nurses or students. The distance between camera and tissue is set at 50 cm. A standardized area of 30 × 30 cm is assessed with a single image capture. A complete reduction of ambient light (e.g., sunlight and room lighting) was ensured to gain optimal data quality. A green cover should be placed in the background to gain optimal data quality. HSI works on any location of the body in the same way. After a picture of the area of interest is taken it is displayed on screen. The examiner then places a marker on the region of interest. The software uses the above algorithm to calculate the mean BI within the marked area.

2.3. *Statistical analysis*

The data were analyzed using XLSTAT© version 2020.5. Demographic data like sex, age, affected body surface and the cause of the burn was recorded. vHb_1-6 and O_2HB_1-6 including the BI were recorded for both groups. Differences between both groups were deemed to be statistically significant when $p < 0.05$. Both groups were compared using Mann Whitney U tests. In a further step ROC for each time point were generated using logistic regression. Thresholds values for BI with their sensitivity, specificity, positive predictive value (ppv) and negative predictive value (npv) were calculated for each time point.

2.4. *Ethical approval*

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Ärztekammer Sachsen-Anhalt, Germany (47/19). The trial was officially registered on DRKS and is displayed on the public web site under the number: DRKS00022843.

3. Results

3.1. *Patient characteristics*

Fifty-nine patients were eligible for inclusion from November 2019 until September 2020. Ten patients were excluded because of poor data quality such as excessive ambient light, inadequate background or incorrect measuring distance. After prospective data acquisition the burn wounds were assigned to two groups as previously described (Table 1).

Group A included 36 patients with a mean age of 41.5 ± 15.2 years, a burnt body surface of 2.7 ± 1.7% and a ratio between male and female of 26:10. In total, 44 burn wounds were analyzed. Most thermal burns were caused by scalds (23), flames (10) and explosions (2). In contrast, group B included 13 patients with a mean age of 46.8 ± 21.2 years, a burnt body surface of 4.0 ± 3.9% and 19 burn wounds. In this group scalds (7) were also the most common cause. We found no statistically significant differences in the age of the patients between the groups (p = 0.48) whereas the affected body surface area was significantly greater in group B (p = 0.002).

After 24, 48 and 72 hours, volume (vHB_1-6) and oxygenation (O_2HB_1-6) of hemoglobin were recorded, the BI was calculated and the values of both groups were compared (Fig. 4). The means of vHb_1 -6 were higher in group A (range 0.99–1.63) compared to group B (0.78–1.47) at each time point. The means of o2Hb_1-6 varied from 0.37 to 0.92 in group A. In group B the means of o2Hb_1-6 were between 0.38 and 0.89 (Fig. 3). In a further step the BI was calculated for both groups at each time point. The BI was significantly higher in group A with 1.05 ± 0.28 at 24 h, 1.23 ± 0.28 at 48 h and 1.55 ± 0.27 at 72 h compared to 0.78 ± 0.14 at 24 h, 1.05 ± 0.23 at 48 h and 1.23 ± 0.27 at 72 h in group B (Fig. 4).

3.2. *Validity*

The diagnostic accuracy of the BI was evaluated using logistic regression at each time point (Fig. 5). After 24 h the optimal threshold of 0.95 had a sensitivity of 61% and a specificity of 45%. The positive predictive value was 100% and the negative predictive value was 51%. Forty-eight hours after thermal injury the threshold was set at 1.17 with a sensitivity of 51% and a specificity of 35%. The optimal threshold on the third day of 1.27 was characterized by a sensitivity of 92% and a specifity of 74%. The positive predictive value was 98% and the negative predictive value was 71% (Table 2).

4. Discussion

An accurate diagnosis of thermal burn depth is a prerequisite for appropriate treatment [25]. Currently, a

Table 1 – Patients characteristics.

	Group A n = 36	Group B n = 13	p-Value
Sex (M/F)	26:10	7:6	
Age ± SD	41.5 ± 15.2	46.8 ± 21.2	0.48
Burnt body surface area in percent ± SD	2.7 ± 1.7	4.0 ± 3.9	0.002
Number of burn wounds	44	19	
Cause of burn:			
Scalds	23	7	
Flames	10	3	
Explosions	2	3	
Electricity	1		

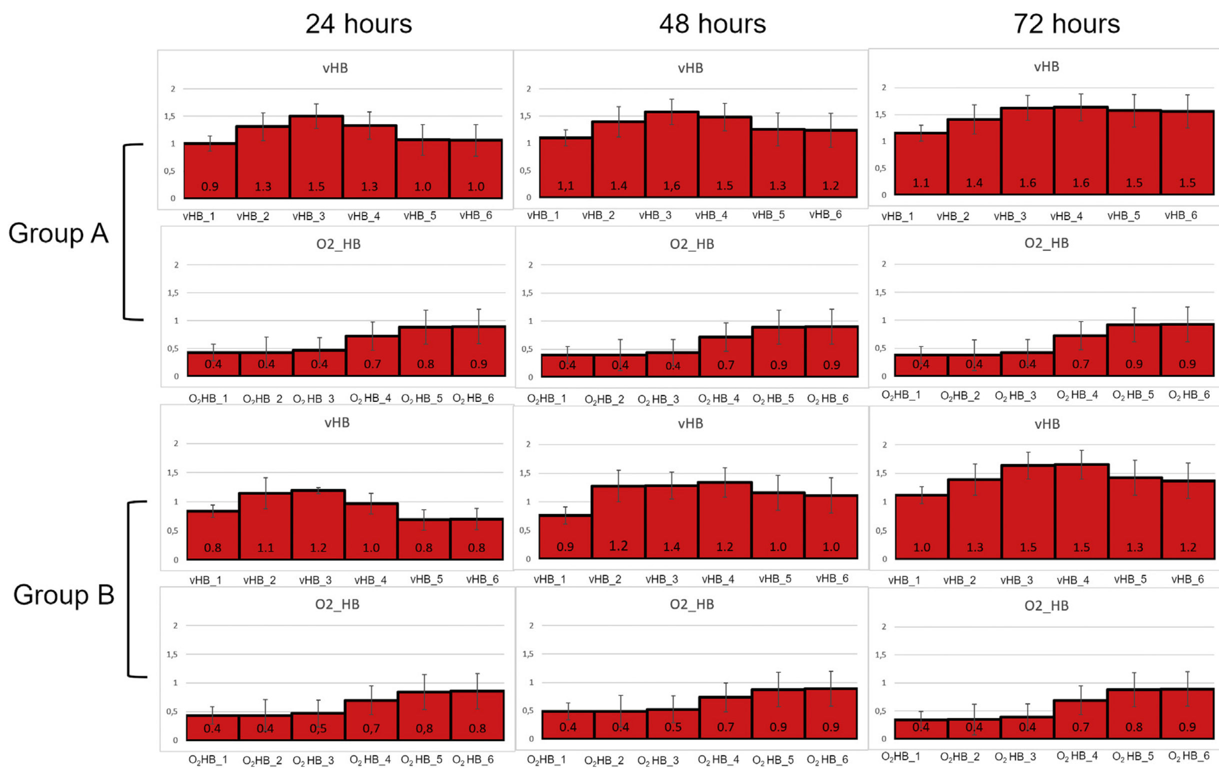

Fig. 3 – Volume (vHB_x) and oxygenation (O_2HB_x) of hemoglobin in each tissue layer at each time point in both groups after thermal burns.

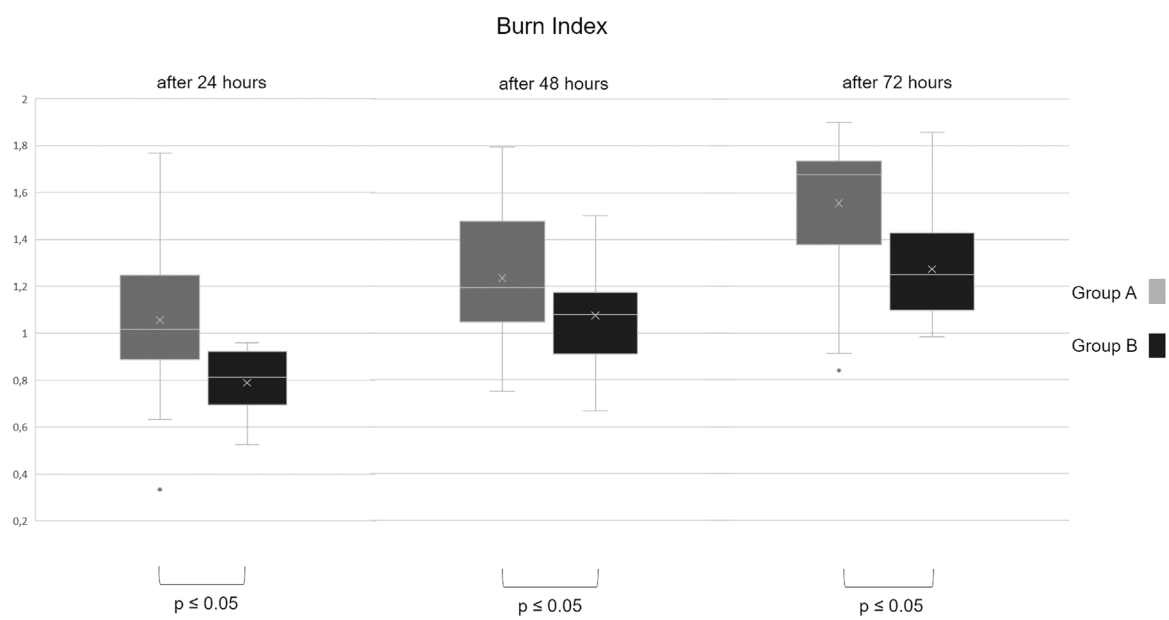

Fig. 4 – Box plots of the burn index 24, 48 and 72 hours after thermal burns for each group.

reliable diagnosis involves repeated evaluation and clinical experience [26]. Decision making is especially challenging in mixed thickness burns [27]. Histological assessments of burn depth have the highest reliability but are impractical and invasive [28]. Therefore, a variety of non-invasive diagnostic tools have been developed over the last decades. So far, LDI, HSI and thermal imaging have been assessed in clinical practice.

LDI is a well-established, non-invasive device for the determination of burn depth. [29] The sensitivity of the LDI assessment is about 80 % after 24 h and 92–95% on the third day after thermal trauma [30–32]. It is therefore recommended, to use LDI no earlier than 48 h and no later than 5 days after injury to guide clinical decision making [16,33]. The measurements take a few minutes and depend on the size of the wound [30]. The Laser Doppler Line Scanner, a further

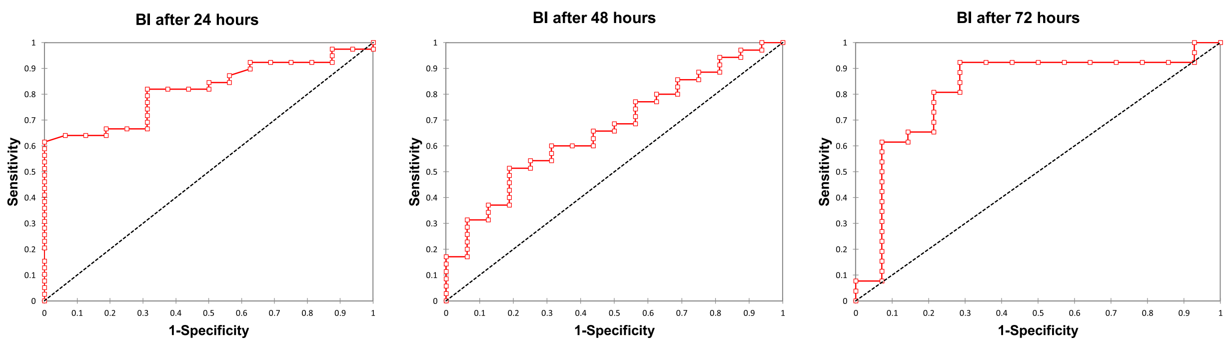

Fig. 5 – Receiver operating curve representing the diagnostic accuracy of the burn index at each time point.

Table 2 – Logistic regression a diagnostic accuracy of the BI over time.

	Threshold	Sensitivity	Specificity	PPV	NPV	AUC	p-Value
After 24 hours	0.95	0.64	0.81	1.00	0.51	0.81	<0.0001
After 48 hours	1.17	0.51	0.81	0.85	0.43	0.66	0.032
After 72 hours	1.27	0.92	0.71	0.85	0.83	0.81	<0.0001

development of the LDI, was able to reduce the time of measurement to less than one minute with the same accuracy [30]. Nevertheless, LDI is expensive and involves a learning curve [34].

Further diagnostic tools are thermal imaging and spatial frequency-domain imaging [35,36]. Thermography measures the infrared emissions from the tissue at wavelengths of 8000 −15000 nm and divides burn wounds into two or three categories like LDI. Up to now, three diagnostics studies were published which investigated the accuracy of thermal imaging for burn depth assessment. The thermal imaging device has a specificity up to 100% and but a sensitivity ranging only from 20 to 56% in paediatric patients [37]. In a direct comparison between LDI and thermal imaging, thermal imaging had a lower sensitivity ranging from 44% to 66% and specificity of 76% [32]. The assessments of burn depth with indocyanine green and spatial frequency imaging demonstrated promising result in preclinical models. However, prospective clinical studies in humans are lacking.

The first study on the use of HSI in the assessment of burn wounds on humans was published in 2015 [21]. Prior to this study, HSI has been evaluated in a preclinical model for the assessment of burn wounds [22,38]. The assessment made by HSI was incorrect in up to 7% of all cases [38]. In a further case series nine patients were evaluated using HSI and compared to LDI [39]. In order to increase the accuracy of HSI, the data processing was expanded and blood flow parameters were determined for different skin layers [24]. The volume (vHB_1-6) and oxygenation (O_2HB_1-6) of Hemoglobin in each layer reflect the blood flow in the tissue. A high blood flow indicates intact blood vessels, which are required for spontaneous wound closure. In contrast, a low volume and oxygenation of Hemoglobin is indicative of reduced blood flow and damaged blood vessels. Therefore, a low vHB_1-6 and O_2HB_1-6 are associated with a reduced probability of timely secondary wound closure. Using these parameters, the BI is calculated. The BI behaves in the same manner as its components. A high BI is associated with a high probability of timely spontaneous wound closure whereas a low BI indicates a low probability of spontaneous wound healing within 21 days. The BI assists in the assessment of burn depth making it more objective and less examiner-dependent. Like LDI, HSI demonstrated an increasing accuracy over the first 72 h (Table 3). Three days after trauma, the burn index demonstrated a diagnostic accuracy which was almost as high as LDI's accuracy. An unexpected observation in our data was that the BI increased in both groups over the 3-day study period. This suggests an improved hemoglobin volume in both groups in the early

Table 3 – Comparison of the available clinical devices.

	LDI	HSI	Thermal imaging
Devices	Moor LDI2-B1©	TIVITA Tissue©	FLIR ONE©
Healing categories	3 [43]	2	2 [40]−3 [35]
Ranges	390−750 nm	500−1000 nm	8000−15000 nm
Diagnostical studies	14 [44]	1	3 [35, 40, 45]
Time points of highest diagnostic accuracy	3^{rd} day	3^{rd} day	3^{rd} day
Costs	+++	++	+
Sensitivity	91 % [44]	92 %	44% [35]−66% [45]
Specificity	96 % [44]	71 %	76% [35, 45]

clinical course after a thermal burn. Any differences in the rate of change over this 3-day period may aid further in the distinction between superficial and deep partial thickness burns. This aspect should be addressed in future studies. Using the BI an accurate treatment decision can even be made for mixed partial thickness burns if an assessment is made 72 h after trauma. With a BI below 1.27 at 72 h the probability of spontaneous wound healing within 21 days is low and skin grafting is recommended. A BI above 1.27 is associated with a high probability of spontaneous wound closure and further conservative management is indicated. In summary, the BI estimates the perfusion of the damaged skin and predicts the probability of timely spontaneous wound closure. The current study is limited to determining BI thresholds within 72 h of a thermal burn. Due to the observed dynamics of the BI, it is questionable that the numeric thresholds provided can be generalized to later time points while vascularization occurs within the wounds. Follow-up studies should endeavour to extend the temporal window by assessing thresholds during later stages of healing. As mentioned above, the BI currently only uses data from the haemoglobin volume because of the small sample size. The algorithm for determination of the BI will likely become more accurate when more data becomes available for statistical modelling. The first diagnostical studies for LDI for instance could rely on a patient cohort three times larger than ours [40].

The limitations of our study include the small sample of patients (n = 3) whose wounds healed by secondary intention more than 21 days after injury. Patients who were clinically deemed to need a skin graft were automatically assigned to group B (n = 10). Some of these patients' wounds might have healed spontaneously within 21 days. Otherwise, wound closure at 21 days in these patients might expose them to secondary risks such as wound infections. Each one of the three patients belonging to group B refused surgical treatment. Otherwise, the current study design was purposefully not affecting usual clinical care since no previous study had established BI thresholds to use. While this design carries the risk of falsely labelling a superficial partial thickness burn as a deep partial thickness burn due to the clinical decision to excise and graft, this had to be weighed against the ethical issue of risking wound infection by delaying surgical management until after the 21st day after trauma.

Moreover, the study groups were heterogeneous with regard to sex or burnt body surface area. Some of these differences were to be expected given the nature of burns. A larger affected body surface area is indicative of a more severe noxious stimulus which in turn will also lead to deeper burns. Some of the observed differences may be due to chance in a patient cohort of this size. It seems unlikely that they had an effect on the results. While these facts introduce heterogeneity into our dataset, it may also reflect the clinical patient populations rather than a homogenous study population. Furthermore, the burnt body surface may be a predictor of secondary wound closure and could be combined with the BI. Also, the use of the O2Hb1_6 should improve the diagnostical accuracy of the BI. A bigger sample size and further studies should make this possible. This could offer a greater diagnostic potential. However, a larger dataset would be necessary to perform such calculations. Moreover, it is also highlighting that a subset of images (10/59) was unsuitable for analyses due to poor image quality.

Further clinical studies about the burn depth assessment of HSI are indicated to confirm our results. We recommend assessing the correlation between BI and burn wound histology [9] as well as a direct comparison between HSI and LDI [32] in a further step. Also, the use of the BI in children needs to be evaluated [37]. Finally, BI could be used and evaluated with the newly developed thresholds to guide treatment and assess the patient outcomes in a prospective study. We recommend the clinical use of HSI for the burn wound depth assessment for mixed partial thickness burns 72 h after injury. In clinical practice burn blisters should be removed and antiseptic dressings applied until assessment after 72 h. Burn depth assessments via HSI can aid decision making regarding skin grafting. A BI below 1.27 72 h after a burn should prompt consideration of surgical treatment.

5. Conclusion

HSI is a useful tool for burn depth assessment. It provides accurate information which correlated with spontaneous wound healing.

Disclosures

The authors declare no conflicts of interests relevant to this article.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement

All authors have made substantial contributions to the manuscript in form of the conception and design of the study, acquisition of data and analysis (1), interpretation of data (2), drafting the article or revising it critically for important intellectual content (3), final approval of the version to be submitted (4).

Torsten Schulz, 1-4; Jörg Marotz, 1-4, Sebastian Leuschner 1-4, Sebastian Seider 1-4, Stefan Langer 1-4, Frank Siemers 1-4.

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