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The difference between the healing and the non-healing diabetic foot ulcer. A review of the role of the microcirculation

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Abbreviations

CM Capillary microscopy

DM Diabetes mellitus

ES Electrical stimulation

HBO Hyperbaric oxygen therapy

HD Hyperaemia duration

HTN Hypertension

LDF Laser Doppler fluxmetry

LDI Laser Doppler imager

LD-PWA Laser Doppler pulse wave amplitude

LD-SBFV Laser Doppler skin blood flow velocity

MESH Medical subject headings

NPV Negative predictive value

pLDF Peak hyperaemic flow

PORH Post occlusive reactive hyperaemia

PPV Positive predictive value

RCT Randomised control trial

RF Resting flux

SBF Skin blood flow

SPP Skin perfusion pressure

SVR Skin vascular resistance

TBP Toe blood pressure

$TcPO_2$ Transcutaneous oxygen pressure

tpLDF Time to peak hyperaemic flow

Keywords

Diabetic foot, healing, microcirculation, ulcer,

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Abstract

Background: Diabetic foot disease carries a high morbidity and is a leading cause of lower limb amputation. This may in part be due to the effect diabetes mellitus (DM) has on the microcirculation including in the skin.

Method: We conducted a review of studies that have examined the relationship between microcirculatory function and wound healing in patients with DM. A search of the Medline, EMBASE and Web of science databases was performed coupled with a review of references for the period 1946 to March 2015.

Results: Nineteen studies of diverse methodology and cohort selection were identified. Poor function of the microcirculation was related to poor outcome. Transcutaneous oxygen pressure ($TcPO_2$) was the most commonly used method to measure the microcirculation and thresholds for poor outcome proposed ranged from 10mmHg to <34mmHg. Two studies re-examined microcirculatory function following revascularisation. Both found an increase in $TcPO_2$ however only one reached statistical significance. No significant difference in the results of microcirculation tests was found between diabetic and non-diabetic patients.

Conclusions: While it is not possible to draw firm conclusions from the evidence currently available there are clear areas that warrant research. Good microcirculation unsurprisingly appears to associate with better wound healing. The influence of DM is not clear, and neither is the degree of improvement required to achieve healing. Studies that examine a clearly

defined cohort both with and without DM are urgently required. Accurate quantitative assessment of microcirculation will aid prediction of wound healing identifying those at greatest risk of amputation.

Introduction

Currently, 3.2 million people in the United Kingdom (UK) and 29.1 million people in the United States of America (USA) are diagnosed with diabetes mellitus (DM), accounting for 6% and 9.3% of the population respectively[1,2]. It is estimated that the lifetime incidence of a foot ulcer may be as high as 25% among these patients with an associated increased risk of amputation[3]. Diabetic ulceration and associated amputation also carry a significant cost burden to society, this will continue to increase along with the rising incidence of DM[4].

DM is known to have a significant effect on the microvasculature, causing dysfunction of the arterioles and capillaries supplying the retina, kidneys and peripheral nerves[5], histological examination of capillaries has shown thickening of the basement membrane compared to non-diabetic patients[6-8]. Different methods to quantifiably examine the microcirculation and its function have been developed; these include capillary microscopy (CM), transcutaneous oxygen pressure ($TcPO_2$) and laser Doppler fluxmetry (LDF). How these measures of the microcirculation change with wound healing is not well described. The review that the International Working Group on the Diabetic Foot Guidance on prognosis is based on is a through and well performed systematic review[9]. However the focus of the review is not on methods of assessing the microcirculation and while the discussion and conclusion consider $TcPO_2$ the results that this conclusion is based on are not covered in the results. There is also no consideration of comparison to patients without DM or the role of repeated measures. The aim of this review

was to examine the current evidence available on the relationship between the microcirculation in the ulcerated diabetic foot and wound healing. Specifically the ability to predict healing, how the results for those with DM compare to those without and how the results vary when repeated measurements are taken.

Methods

A search of the Medline, EMBASE and Web of Science databases was performed. The search strategy consisted of the Medical Subject Headings (MESH) “microcirculation”, “wound healing”, “diabetic foot”, “skin ulcer”, “laser Doppler flowmetry”, “blood gas monitoring, transcutaneous”, “microscopic angioscopy”, “xenon radioisotopes”. In addition a key word search was performed. The terms used can be found in Appendix I. Non-English language and non-human studies were excluded, the date range for the search was 1946 to February 2015. Final inclusion in the review was dependant on meeting the criteria set out in Table 1, no limits were applied to length of follow up or number of patients included.

One reviewer (DL) performed the search, reviewed abstracts and selected studies for inclusion. Any areas of uncertainty were reviewed by the senior author (AT) to provide a second opinion. The original intention was to perform a meta-analysis however there were insufficient numbers of high quality studies to be able to continue this plan and so a more descriptive approach was taken to reporting the data.

Results

Two-hundred and eighty-seven articles were identified after searching the databases. Full text was obtained for all abstracts that met the inclusion criteria and all relevant data was extracted. After this assessment and review of references nineteen studies were included in the final review (Figure 1) [10-28]. The date of publication ranged from 1985 to 2014, two studies were randomised control trials (RCTs), there were three pseudo-RCTs and the rest were observational studies (Table 2). Not all studies included all the comparisons considered below and some studies used more than one method to assess the microcirculation.

Using the microcirculation to predict healing

Twelve studies out of nineteen compared the microcirculation in patients with diabetes who healed to those who did not heal[10-12,15,18-21,24,26-28]. Ten of these studies employed $TcPO_2$[11,12,15,18,20,21,24,26-28], five used LDF[11,15,20,21,28], one used laser Doppler imaging (LDI)[19] and one used isotope washout to measure skin perfusion pressure (SPP)[10]. These were all observational studies apart from one which randomised the first 14 of its participants but not the final 24[20]. For seven of the studies the participants received only standard therapy [10-12,15,26,27]. Two studies examined the effects of HBO therapy, Kalani *et al* (2002) had 2 cohorts, one of which received standard therapy and the other which received HBO. The healed and unhealed groups in this study are made up of participants from either cohort[20]. Fife *et al* performed a retrospective study of 1144 patients who received HBO

therapy[18]. Klingel *et al* reported the results of a very small pilot study (8 patients) all of whom received rheopheresis[21]. Two studies treated their participants with dermal replacement therapy (Ichioka *et al* bone marrow impregnated collagen). Newton *et al,* collagen containing glycosaminoglycans)[19,24]. Five studies only investigated patients with both diabetes and ischaemia[11,15,18,20,21], three studies excluded those with ischaemia[12,19,24], in one study it was unclear[26] and three included a mix of patients[10,27,28]. Only Yotsu *et al* divided the patients into groups depending in their aetiology (neuropathic, ischemic and neuro-ischemic)[28].

Transcutaneous oxygen pressure

Nine studies used $TcPO_2$ to predict wound healing[11,12,15,20,21,24,26-28], the results are summarised in Table 3. Five studies found that those with a higher $TcPO_2$ had a statistically significant higher chance of healing, with results ranging from 30±4mmHg to 61.11±21.16mmHg [11,15,21,26,27]. Kalani *et al* 2002 and Yotsu failed to find a significant difference between the two groups[20,28]. Pecoraro *et al* found a significant difference between those who had early healing and those who did not (56.3±2.72mmHg vs 26.9±8.26mmHg, p=0.003) however was unable to demonstrate that the difference had persisted in those that healed overall (53.67±2.99mmHg vs 37.57±11.02mmHg, p=0.126)[12].

Skin perfusion pressure

Two papers used SPP to compare the healed and unhealed groups[10,28]. Faris *et al* in 1985 used an isotope washout method on 64 patients with diabetes

and foot ulceration or gangrene. Those who healed had a mean SPP of 59 ± 16mmHg compared to those who did not heal whose mean SPP was 35 ± 11 ($p<0.001$)[10]. Yotsu *et al* in 2014 employed LDF instead of isotope washout to measure SPP on diabetic ulcers divided into the groups described above. They found that neuropathic ulcers had a higher SPP than both ischemic and neuro-ischemic ulcers, 65 ± 13.6mmHg, 27 ± 14.1mmHg and 34 ± 23.2mmHg respectively ($p<0.001$). However there was no significant difference between the healed and unhealed ulcers in each group (Table 4)[28].

Laser Doppler

Karanfilian *et al* was the only paper to use laser Doppler fluxmetry to compare between healed and unhealed patients. They demonstrated significantly higher skin blood flow velocity (LD-SBFV) and pulse wave amplitude (LD-PWA) results between those who healed and those who did not in both their study groups (Table 5)[11].

Prediction of healing

Three studies reported the accuracy of cut-off values for healing[10,15,18]. Faris and Duncan found a SPP of less than 40mmHg was an indicator of poor healing (sensitivity of 97%, specificity 80%, positive predictive value (PPV) 87% and negative predictive value (NPV) 95%)[10]. Kalani *et al* (1999) used a cut-off of 25mmHg for $TcPO_2$ and 30mmHg for toe blood pressure (TBP) using LDF. For $TcPO_2$ the sensitivity was 85%, specificity 92%, PPV 79% and NPV 94%. For TBP the sensitivity was 15%, specificity 97%, PPV 67% and NPV 77%[15]. Fife *et al* tested multiple potential cut-offs for sea level $TcPO_2$ as a predictor of failure of hyperbaric therapy. They found that 25mmHg was the

best cut-off with a 2.5 times greater chance of success. However the accuracy was still relatively poor with sensitivity of 67%, specificity 50%, PPV 35% and NPV 79%[18].

Diabetes compared to no diabetes

Two out of nineteen studies compared subjects both with DM and without DM[11,14]. Both of these papers used $TcPO_2$ to make their comparisons, in addition Karanfilian *et al* employed LDF[11]

Padberg *et al* reported the predictive accuracy for healing of $TcPO_2$ in critically ischaemic wounds. 204 wounds were stratified depending on the presence of DM, dialysis dependant chronic renal failure or neither disease. Probability of healing curves for each group were plotted and compared using multiple logistic regression. $TcPO_2$ in DM patients had a predictive accuracy, sensitivity and specificity of 81%, for chronic renal failure these figures were 77%, 73% and 82% respectively, and for neither disease 84%, 86% and 82%[14].

Only one study identified compared the mean results of microcirculatory tests for patients with diabetes and those without[11]. The patients were all men with ulceration to the foot (34 with diabetes, 22 without). One off measurements of $TcPO_2$ and LDF (LD-SBFV and LD-PWA) and follow up of at least 30 days was performed. The results are presented in Table 5. Patients without diabetes who did not heal had a lower $TcPO_2$, LD-SBFV and LD-PWA than patients with diabetes who did not heal. In the healed groups for the patients without diabetes the $TcPO_2$ was higher than the patients with

diabetes. However the LD-SBFV and LD-PWA were lower in the group without diabetes. The authors have not reported whether these differences are statistically significant[11].

Multiple measurements during observation period

Eight out of nineteen studies reported the results of more than one measurement on the same group of patients[13,16,19,21-25]. One study detected no change, two noted a decrease in reading, a further two noted an increase and three noted a pattern of increasing then decreasing. Jorneskog *et al* used LDF and capillary microscopy to examine 10 patients with diabetes who received low molecular weight heparin for a period of eight weeks. Measurements of the microcirculation (post occlusive reactive hyperaemia (PORH), structural appearance of capillaries in the forefoot and toes) were undertaken 1-2 weeks prior to receiving heparin, after 4-7 weeks of treatment and two weeks after treatment was stopped. They found that there was no significant change in any of the laser Doppler parameters during or after treatment. It was however noted that six patients who had improved healing also had an improvement in their capillary stage, three others also improved clinically but one had no change in their capillaries, one initially improved but then deteriorated again and in one it was not possible to determine their capillary stage. One patient deteriorated both clinically and on microscopic examination[13].

Petrofsky *et al* published on electronic stimulation (ES) for diabetic foot ulcers in both 2007 and 2010[22,25]. In 2007 the study groups were, ten

patients who received global heating and ES, nine who received local heating and ES and ten patients who received conventional therapy only. The measure of the microcirculation was blood flow using LDI (measured in arbitrary unit flux). The control group did not undergo LDI measurement, only wound area was measured. In 2010 the aim of the study was to examine the role that heating had compared to ES and heating. Ten patients received local heating only and a further ten local heating and ES. The treatment period for both studies was four weeks. In both studies the blood flow around and in the ulcer had decreased by the end of the study. In the 2007 study the mean blood flow at baseline was reported for 1cm from the ulcer (182.3±26.1 increasing to 245.0±28.5 with ES) and the edge of the ulcer (223.4±34.1 increasing to 301.0±29.3 with ES). The result for the centre of the ulcer is reported as being similar and is illustrated in a graph but the actual values are not stated. At four weeks only the values for the centre of the ulcer are stated (228±36.2 increasing to 256.7±46.3 with ES). The change in blood flow before and during ES at baseline and at four weeks is displayed in Table 6; there was a significant reduction in the increase at four weeks (<0.01). The results for the local heating group are illustrated in a graph and stated as being similar but of a smaller magnitude to the global group but the actual mean values are not quoted[22]. In 2010 Petrofsky found that the mean resting blood flow from all three areas and both groups had reduced by 54.5±22.3% after four weeks[25].

Lawson *et al* as described above also investigated the effect of electrical stimulation on wound healing. They measured blood flow at the centre and

outside of the ulcer using LDI at baseline, two weeks and four weeks. When looking at the outside of the ulcer the pre-stimulation results for the DM group showed larger increase in the blood flow than for the non-DM group (DM, 0-2 weeks 35%, 0-4 weeks 21%; Non-DM, 0-2 weeks 0%, 0-4 weeks 18%). However at the centre of the wound the non-DM had a greater increase (DM, 0-2 weeks 8%, 0-4 weeks 5%; Non-DM, 0-2 weeks 22%, 0-4 weeks 38%). The statistical significance of these results is not reported[23].

Koblik *et al* performed an RCT comparing optimisation of insulin therapy and injection of an antithrombotic drug (sulodexide) with optimisation of insulin therapy and placebo injections for 10 weeks. Measurements were taken at baseline and eight weeks using LDF. The parameters measured were resting flux (RF), peak hyperaemic flow (pLDF), time to peak hyperaemic flow (tpLDF) and hyperaemia duration (HD) after an occlusion of thirty seconds. These measures were repeated following a sixty-second occlusion once the readings had stabilised. In the placebo group (6 patients) there was no significant change in the RF at eight weeks (Baseline: mean flux 11.6± standard error of mean 1.3. Eight weeks 12.3±1.1. p = ns). The pLDF for both the thirty (51.7±15.2 to 147.0±16.2, $p<0.01$) and sixty second occlusion (110.5±13.0 to 164.8 ±15.4, $p<0.01$) significantly increased at eight weeks[16].

The results from two studies with small numbers are presented in graphical form in Figure 2. Newton *et al's* 7 ulcers all healed or showed improvement at eight weeks. Four measurements using LDI were performed at baseline, two, five and eight weeks. Four patients had an increase in blood flow over the first few weeks followed by a decrease to below baseline at eight weeks.

One increased throughout the measurement period. One decreased at two weeks, increased at weeks five and eight but did not return to baseline level. One decreased throughout (Figure 2a). Those that had healed at eight weeks, two increased then decreased, one increased throughout and the other decreased throughout[19]. Of Klingel's eight patients who received rheopheresis five underwent $TcPO_2$ at baseline, twelve and twenty-four weeks and three underwent $TcPO_2$ at baseline and twelve weeks (due to minor amputation in one patient and major amputation in two). Of the four patients who showed an improvement in their ulcer two had an increase in blood flow followed by a decrease, the other two increased throughout. In the patients whose ulcers were unchanged one increased $TcPO_2$ at twelve weeks, the other increased at both twelve and twenty-four weeks. Of the two patients who deteriorated one had a small increase at twelve weeks and the other had a small decrease (Figure 2b)[21].

Ichioka *et al* in their DM subgroup showed, in graphical form, a trend of increasing $TcPO_2$ in the healed group and a decrease at 4 days in the unhealed group. The mean $TcPO_2$ at 4 and14 days are not reported, however logistic regression analysis showed the results at these time point contributed significantly to the prediction of outcome ($p<0.001$ and 0.002 respectively)[24].

Discussion

Within this group of studies the most commonly used method to assess the microcirculation was $TcPO_2$ (n=12), followed by LDF (n=7), LDI (n=4), capillary

microscopy (n=2) and isotope washout (n=1). These proportions are probably representative of the current state of clinical usage of these methods with $TcPO_2$ and LDF being the most common.

Within this group of studies, a variety of methods for examining the microcirculation have been used. Some of these methods have now fallen out of favour as technology has developed less invasive methods. This includes Xe clearance and SPP using isotope washout. LDF, $TcPO_2$ and capillary microscopy remain in regular use. LDF is relatively underrepresented in this cohort, which is surprising considering that its utility in evaluating patients with critical limb ischaemia is well-documented[29-31]. One reason for this may be the relative age of many of the studies included (only three since 2000 and going back as far as 1978). $TcPO_2$ was the most commonly used method in this review, which fits with its presence in the literature on critical limb ischaemia and diabetic foot disease as a whole.

There is disagreement on how to carry out each of the methods of assessing the microcirculation, including positioning of the probes and in the case of $TcPO_2$ the skin temperature that recordings were made at. Probes were most commonly positioned on the dorsum of the foot[11,12,15,17,20,26,27], but they are also positioned peri-wound[12,18,21,24] and in one case it was not stated[14]. A possible explanation for Yotsu *et al* not detecting a significant difference is their method of measurement[28]. Multiple measurements were taken in two areas of the foot and the lowest result recorded. Of particular note, the contra-lateral foot was used if there was extensive ulceration, this may well

have skewed their results. $TcPO_2$ was most commonly measured at 44°C[12,15,18,20,21,24] but also at 45°C [11,14] or not stated[17,26-28]

Due to the variety of countries and inclusion/exclusion criteria, the cohorts differed across the studies. For example, Yang[26] and Lawson[23] excluded patients with evidence of osteomyelitis whereas most of the other studies did not.

Unsurprisingly, the overall trend from the results is that if the microcirculation is functioning poorly then wound healing is poorer and outcomes are worse. Due to the larger number of studies, $TcPO_2$ best demonstrates this. Most studies demonstrated a significantly higher $TcPO_2$ in those patients who healed. What is less clear is the threshold at which healing occurs. The $TcPO_2$ thresholds quoted for successful outcome in this review range from 10mmHg to 34mmHg. Karanfilian quotes sensitivity of 100% and specificity of 88% for healing if the $TcPO_2$ is >10mmHg[11]. Pecoraro found that a $TcPO_2$ of <20mmHg was associated with a 39 fold increased risk of early healing failure[12]. Both Kalani and Yang used the threshold of <25mmHg and quoted sensitivities and specificities of 85% Vs 92% and 88.6% Vs 82.4% respectively[15,26]. This threshold, when looking at the collated results in the healed and unhealed groups in Table 3, appear to hold true when considering the healed groups, all the mean results are above 25mmHg. However it is worth observing that the mean $TcPO_2$ is also higher than 25mmHg in six of the unhealed groups[12,24,27,28]. The current consensus among experts is that patients with a SPP ≥40mmHg, TBP ≥45mmHg or $TcPO_2$ ≥25mmHg are more likely to heal than their counterparts with poorer

perfusion and that a TBP <30mmHg or $TcPO_2$ <25mmHg is an indication for urgent vascular imaging[9,32]. This is based on a recent review examining the utility of prognostic markers in diabetic foot disease in which the authors faced similar difficulties to us in identifying studies of sufficient quality to draw conclusions from[9]. Eventually eleven studies involving 5890 patients were included however there was still significant heterogeneity and difference in the measures used. Their conclusions were based predominantly on three papers of acceptable rather than high quality (Quality in Prognosis Studies Tool)[10,15,33].

Only one study in this current review truly compared the results of testing the microcirculation in patients with DM and those without[11]. Karanfilian found that the DM patients who healed had a lower $TcPO_2$ than non-DM patients who healed. Conversely the LDF results were higher in the DM healed group. In the unhealed groups the opposite is true. The accuracy of $TcPO_2$ for predicting healing is shown to be reasonable in those with DM, slightly poorer than those without DM but better than those with CRF. It is hard to explain this pattern however cohort selection may offer an explanation as the non-DM cohort had significant PVD whereas the DM cohort was made up of a mix of patients with diabetic foot disease, with and without PVD[11].

The results from the repeated measures suggest that there is a change in the microcirculation during healing but the true trend and how it relates to healing has not yet been identified.

Conclusions

Due to the heterogeneity of the cohorts and the data presented it is not possible to draw any firm conclusions from a review of the current literature. We can, however, surmise that good microcirculation associates with better wound healing. The influence of DM and associated neuropathy is not clear, and neither is the degree of improvement required to achieve healing. Studies that examine a clearly defined cohort both with and without DM are urgently required. Accurate quantitative assessment of microcirculation will greatly aid predicting feet at risk, of predicting wound healing with and without surgery, and for identifying those at greatest risk of amputation.

Figures

Table 1: Review Inclusion criteria

Inclusion criteria

• English language article
• At least one method of assessing the microcirculation
• Patients with active tissue loss
• Wound healing as an outcome measure
• Results from patients with diabetes to be analysed separately from patients without diabetes in one of the following three formats.
  o Patients with diabetes compared to patients without diabetes
  o Patients with diabetes who healed compared to patients with diabetes who did not heal
  o Repeated measurements from the same patient during the period of active diabetic ulceration being investigated

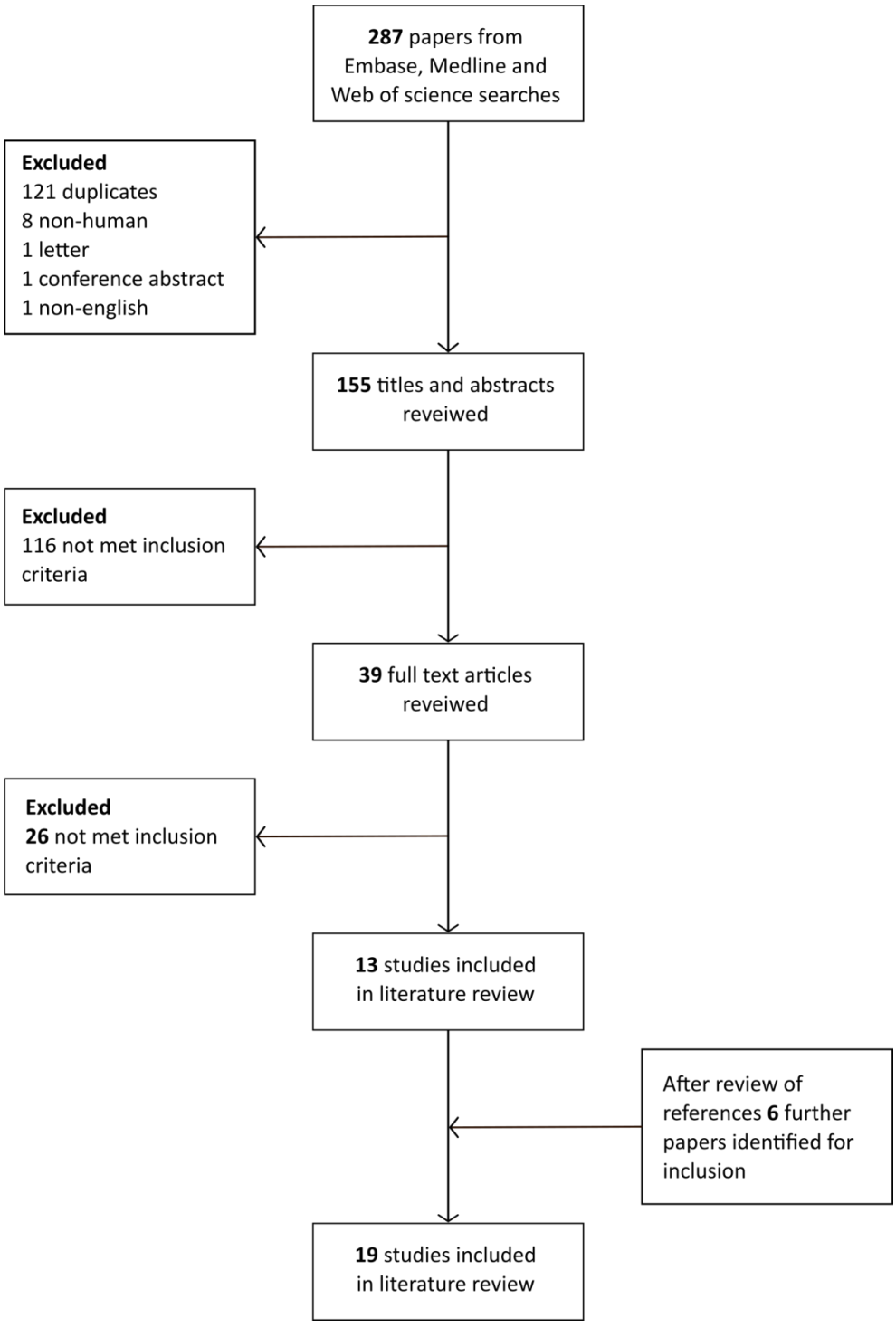

Figure 1: Flow diagram illustrating study identification process

Table 2: Characteristics of included studies

Author	Year	Country	Type of study	Microcirculation method	Number of subjects	
					DM	Non-DM
Faris, I.[10]	1985	Australia	Cross-sectional	SPP using isotope washout	64	-
Karanfilian, R.[11]	1986	USA	Cohort	1)LDF 2)$TcPO_2$	34	22
Pecoraro, R.E.[12]	1991	USA	Cross-sectional	1) $TcPO_2$ 2) $TcPCO_2$	46	-
Jorneskog, G.[13]	1993	Sweden	Cross-sectional	1)LDF-PORH 2)Capillary microscopy	10	-
Padberg, F.T[14]	1996	USA	Case control	$TcPO_2$	129	97
Kalani, M.[15]	1999	Sweden	Cross-sectional	1) $TcPO_2$ 2)TBP using LDF	50	-
Koblik, T.[16]	2001	Poland	Double blind RCT	LDF, PORH and resting flow	18	-
Zimny, S.[17]	2002	Germany	Cross-sectional	$TcPO_2$	31	-
Fife, C.E.[18]	2002	USA	Cross-sectional (retrospective)	$TcPO_2$	1144	-
Newton, D.J.[19]	2002	UK	Cross-sectional	LDI	5	
Kalani, M.[20]	2002	Sweden	Pseudo-RCT	1) $TcPO_2$ + $TcPCO_2$ during O2 inhalation 2)TBP using LDF	38	-
Klingel, R.[21]	2003	Germany	Cross-sectional	1) $TcPO_2$ 2)LDF 3)Capillary microscopy	8	-
Petrofsky, J.S.[22]	2007	USA	Pseudo-RCT	LDI	29	-
Lawson, D.[23]	2007	USA	Pseudo-RCT	LDI	10	10

Ichioka, S.[24]	2009	Japan	Case control	$TcPO_2$	31	22
Petrofsky, J.S.[25]	2010	USA	RCT	LDI	20	-
Yang, C.[26]	2013	China	Cross-sectional	$TcPO_2$	61	-
Wang, A.[27]	2014	China	Cross-sectional	$TcPO_2$	194	-
Yotsu, R.R.[28]	2014	Japan	Cohort	1)SPP using LDF 2) $TcPO_2$	73	-

Abbreviations: RCT – randomised control study, SBF – skin blood flow, SPP – skin perfusion pressure, SVR – skin vascular resistance, LDF – laser Doppler fluxmetry, $TcPO_2$ – transcutaneous oxygen pressure, $TcPCO_2$ – transcutaneous carbon dioxide pressure, PORH – post occlusive reactive hyperaemia, TBP – toe blood pressure, LDI – laser Doppler imaging.

Table 3: $TcPO_2$ results for patients who healed compared to patients who did not heal.

Author	Measurement/groups	$TcPO_2$ (mmHg)		p
		Healed (n)	Unhealed (n)	
Klingel[21]	Mean change in $TcPO_2$ week 0-12. Improved and deteriorated groups	13.23±9.57 (4)	-2.3 ± 6.65 (2)	<0.05*
Kalani (2002)[20]	Basal $TcPO_2$, dorsum of foot. All patients	26 ± 10 (23)	24 ± 10 (9)	ns
Karanfilian[1][1]	Dorsum of foot. All diabetic patients	30 ± 4 (16)	7 ± 2.5 (18)	<0.05
Yang[26]	Dorsum of foot. Group 1 (ulcers healed with intact skin), Group 3 (Ulcers that did not heal or deteriorated including requiring amputation)	32 ± 10 (36)	15 ± 12 (17)	<0.001
Ichioka[24]	Peri-wound $TcPO_2$. Diabetic subgroup (combination of treatment and conventional therapy group)	34.5 ±19.2 (32)	26.4 ±16.7 (10)	Not stated
Yotsu†[28]	Multiple measures from 2 areas on foot, lowest value recorded. Contralateral foot used if extensive ulceration. Ischaemic group	38, 12-40 (9)	30, 3-45 (11)	ns
	Neuro-ischaemic group	38, 22-51 (9)	17, 16-32 (5)	ns
	Neuropathic group	48, 40-56 (34)	44, 43-50 (5)	ns
Kalani (1999)[15]	Dorsum of foot. Healed with intact skin compared to impaired ulcer healing	50 ± 20 (20)	13 ± 14 (13)	<0.001
Pecoraro[12]	Peri-wound $TcPO_2$ overall healing	53.67±2.99 (39)	37.57±11.02 (7)	ns
	Peri-wound $TcPO_2$ early healing	56.3 ± 2.72 (38)	26.9 ± 8.26 (8)	0.003
Wang[27]	Site of measurement not stated. Healing and non-healing groups	61.11±21.16 (162)	46.5 ± 18.06 (20)	<0.01

*Wilcoxon test for matched pairs for significance of change between weeks 0-12 for each group separately. All values mean ± SD apart from †median and IQR reported

Table 4: Skin perfusion pressure results for patients who healed compared to patients who did not heal[28].

Group	SPP (mmHg)		p
	Healed (n)	Unhealed (n)	
Neuropathic	67, 57-75 (34)	65, 40-69 (5)	0.192
Ischemic	37, 17-43 (9)	20, 15-37 (11)	0.341
Neuro-ischemic	38, 22-51 (9)	17, 16-32 (5)	0.141

All values median, IQR

Table 5: $TcPO_2$ and LDF results in patients with diabetes compared to patients without diabetes[11].

	Diabetes (Mean ± SE)		No diabetes (Mean ± SE)	
	Healed (16)	**Unhealed (18)**	**Healed (15)**	**Unhealed (7)**
$TcPO_2$ (mmHg)	30 ± 4.0	7 ± 2.5*	42 ± 3.5	2 ± 1.6*
LD-SBFV (mV)	98 ± 13.0	50 ± 8.0*	88 ± 15.0	37 ± 2.0*
LD-PWA (mV)	14 ± 3.0	4 ± 0.5*	8 ± 1.4	2 ± 0.3*

* Significant difference between healed and unhealed groups ($p<0.05$)

Table 6: Change in blood flow associated with electrical stimulation at baseline and at four weeks (global heating group only)[22].

Position of measurement	Flux±SD		p-value
	Baseline (10)	Week four (10)	
1cm from ulcer	63.5±11.9	18.3±10.8	
Edge of ulcer	77.6±11.6	48.7±9.6	<0.01
Centre of ulcer	33.6±3.1	28.4±15.8	

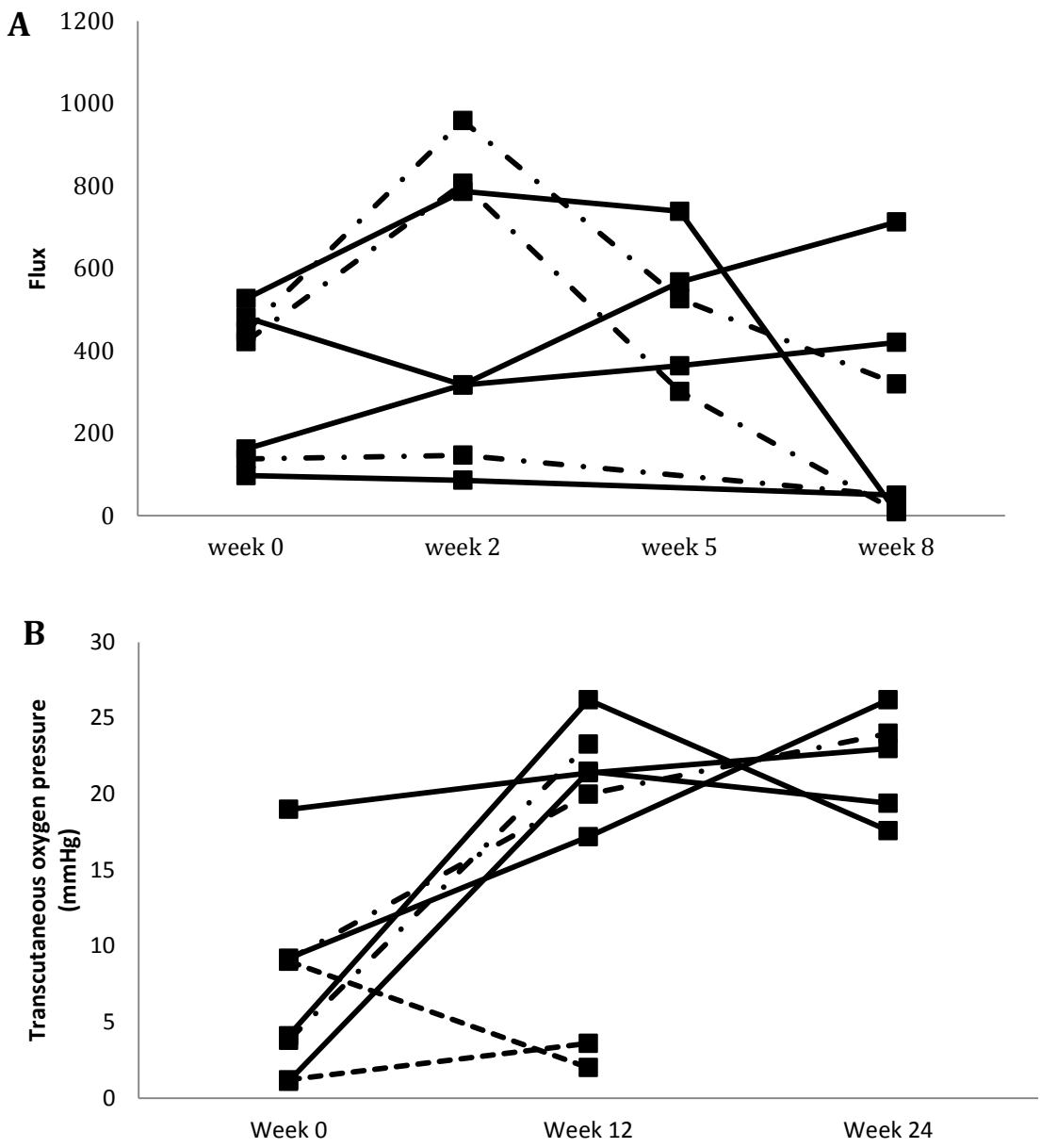

Figure 2: Trends during healing for LDI and $TcPO_2$. **A:** Adapted from Newton *et al.*[19] Solid line, ulcers healed Alternating dashed line ulcers improved. **B:** Adapted from Klingel *et al.*[21] Small dashed line ulcers deteriorated.

Appendix I: Search strategy

MESH Search

Search	Search terms
1	microcirculation
2	wound healing
3	diabetic foot
4	skin ulcer
5	Laser Doppler flowmetry
6	blood gas monitoring, transcutaneous
7	Microscopic angioscopy
8	Xenon radioisotopes
9	3 or 4
10	5 or 6 or 7 or 8
11	1 and 2 and 9 and 10
12	11 limited to English and humans

Keyword search

Search	Search terms
1	capillar* or venule* or arteriole* or small adj2 vessels or skin microcirculation or skin blood supply or skin blood flow or microangiopath* or microcircula* disturbance*
2	transcutaneous adj3 oxygen* or transcutaneous PO2 or transcutaneous oximetry or transcutaneous adj3 carbon dioxide or TcPO2 or TcPCO2
3	laser Doppler* or laser Doppler fluxmetry or laser Doppler Imaging or laser Doppler velocimetry or laser Doppler flux or LDF or LDI or Post occlusive reactive hyperaemia or PORH
4	capillary microscopy or capillary pressure or capillaroscopy
5	skin adj2 pressure or skin adj2 perfusion
6	xenon clearance or isotope clearance or haemodynamic test* or venoarteriolar response
7	2 or 3 or 4 or 5 or 6
8	wound* or ulcer* or ulcer healing or tissue loss or healing or wound complication* or non-healing or nonhealing or granulation tissue or amputat*
9	1 and 7 and 8
10	9 limited to English and humans only

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