Supplementary Table S4. Overview of results for each study.

First author year, country	Study design	Outcomes	Follow-up	Specimen	Analysis	Cytokines	Main findings
Beidler 2009 [1], USA	Cohort study	a. Rapid vs. delayed healing *(>40% vs. <40% area reduction)* b. Before vs. after compression	4 w	Biopsy	Luminex & ELISA (TGF-β1)	G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL12p40, IL-12p70, IL-13, IL-15, IL-17, TGF-β1, TNF-α	a. ↑ GM-CSF, IFN-γ, IL-1α, IL-1β, IL12p40, *before* compression in rapid healing vs. delayed. ↑ IL-1Ra *after* compression in rapid healing. b. ↑ G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-6, IL-8, IL12p40, TNF-α before compression vs. after. ↑ TGF-β1 after compression and trend for ↑IL-10 (p=0.076).
Charles 2008 [2], USA	Cross-sectional study	Healing vs. non-healing	NA	Biopsy	High throughout cDNA microarray	HB-EGF, PDGFRα, S100A7	↓ S100A7 at the edge of non-healing VLU, and HB-EGF in the ulcer bed. ↑ PDGF receptor in the bed of non-healing.
Drinkwater 2003 [3], UK	Cohort study	Healing vs. non-healing	At least 1 year, or until healed	WF, biopsy	ELISA, RT-PCR	ELISA: VEGF-165 (WF, biopsy), VEGF-R1 (WF). mRNA biopsy: VEGF-121, VEGF-165, VEGF-189, VEGF-R1, VEGF-R2	↑ VEGF-165 in WF in non-healing vs. healing, but not in biopsy. Non-healing wounds have a high level of expression of VEGF, at both the gene transcript and protein level. Results for mRNA cannot be reliably extracted
Escandon 2012 [4], USA	Clinical trial: Ultrasound	Correlation between change in cytokine level and healing (wound area change)	4 w	Biopsy	RT-PCR	IL-1α, IL-6, IL-8, IL-10, IL-11, TNF-α, VEGF	↓ IL-1α and TNF-α with healing (p<0.05, and a trend for reduction of IL-6 (p=0.0508), IL-8 (p=0.099), IL-11 (p=0.082) and VEGF (p=0.066).
Filkor 2016 [5], Hungary	Cohort study (unclear if prospective)	Responding vs. non-responding (≥*20% vs <20% area reduction)*	4 w	PBMC	QRT-PCR	IL-1α, IL-8, IL-10, TNF-α,	↑ IL-1α and IL-8 in non-responders
Fivenson 1997 [6], USA	Cohort study	Correlation to healing	8 w	WF	ELISA	IL-8, IL-10 (other cytokines cannot be reliably extracted)	↑ IL-8 with healing w 8 (p=0.09). A dynamic cytokine profile through the healing process. IL-10 increased after w 3 (11 .2% median healing), but was NS compared to baseline.
Gohel 2008 [7], UK	Cohort study	Correlation a. Between cytokine change and ulcer size change b. To baseline levels and ulcer size change	5 w	WF Serum	Sandwich ELISA	FGF-2, IL-1β, TGF-β,1 TNF-α, VEGF	a. ↑ TGF-β1 correlated to healing b. ↔
Grandi 2018 [8], Italy	Clinical trial: ALA-PDT	Correlation to wound mean volume reduction	Unclear, around 3 w	Biopsy	IHC	TGF-β, TNF-α (TNF-α reported for mastcells)	↑ TGF-β correlated to healing
Harris 1995 [9], UK	Cross-sectional	Healing vs. non-healing	NA	WF	ELISA, bioassay	FGF-2, GM-CSF, IL-1α, IL-1α bio,	↔

	study					IL-1β bio, IL-6 bio, PDGF-AB,	
He 1997 [10], UK	Experimental study (reperfusion injury)	Correlation to healing	3 h 20 min	Serum from leg and arm	Sandwich enzyme immunoassay	IL-1β, IL-1RA, IL-6, TNF-α	↔
Hodde 2020 [11], USA	Clinical trial: SIS wound matrix	Healed vs. non-healed: w 12	Up to 12 w	WF	Luminex ELISA: TGF-β1	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12(p70), IL-13, TGF-β1, TNF-α	↓ IL-8 and TNF-α from baseline in healed. IL-1β showed a trend towards reduction in healed but increase in non-healed. ↑ TGF-β1 from baseline in healed vs. non-healed.
Krejner 2017 [12], Poland	Retrospective cohort study	Healing per week. Poor: <5%, Moderate: 5-10%, Good: 11-15%, Fast: >15%	-4w	Serum	ELISA	IL-6, IL-8, TNF	↔
Lagattolla 1995 [13], UK	Cohort study*	a. Healed vs. non-healed b. Correlation to time to healing	6 mo	Biopsy	ELISA	FGF-2, PDGF-AB, TGF-β1	a. ↑ FGF-2 at the ulcer edge in healed vs. non-healed, but not sign. for ulcer bed. b. Increased TGF-β1 at the wound edge correlated to rapid healing (sign. trend).
Ligi 2016 [14], Italy	Cross-sectional and cohort study	Inflammatory vs. granulating VLU	During admission	WF	Luminex	FGF-2, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, PDGF-bb, TNF-α, VEGF	↑ GM-CSF, IL-1β, IL-12p70, IL-10, IL-8, VEGF in inflammatory vs. granulating VLU. ↑PDGF-bb in granulating
Ligi 2017 [15], Italy	Cross-sectional study and cohort study*	Inflammatory vs. granulating VLU	NA	WF	Luminex	TGF-β1, TGF-β2, TGF-β3	↑ TGF-β3 in inflammatory vs granulating VLU
McQuilling 2021 [16], USA	Clinical trial: Amniotic membrane	a. Healing vs. non-healing (>85% vs. < 85% closure at 12 w) b. Correlation between cytokine level and percentage area change	12 w	WF	Multiplex MAP arrays	EGF, FGF-2, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, PDGF-AA, PDGF-BB, TNF-α, TNF-β, TGF-α, TGF-1β, TGF-β2, TGF-β3, VEGF	a. ↑ IL-1ra, IL-1α, IL-2, IL-3, IL-9, TNF-β in non-healing, evaluated at all time-points. b. ↓ IL-1ra, IL-7 and IL-8, and ↑EGF correlated to size reduction.
Murphy 2002 [17], Ireland	Cohort study	Healing vs. non-healing (before & after compression)	12 w	Serum, from ulcer leg	ELISA	TNF-α, VEGF	↑ VEGF and TNF-***α*** in non-healing. Reduction in the levels to below control values with compression, correlating with healing.
Mwaura 2006 [18], Ireland	Cohort study	Healing vs. non-healing:	8 w	WF, biopsy	ELISA, IHC	PDGF-AA	↑ PDGF-AA in WF and biopsy of healing VLU. PDGF-AA

		<20% vs. >20% decrease in ulcer area					activity is associated with ulcer healing.
Pukstad 2010 [19], UK	Cohort study	Healing vs. non-healing	8 w, or healing	WF	Antibody glass array, TNF-α bioassay	40 cytokines analyzed. Results presented on IL-1α, IL-1β, IL-6R, IL-8, sTNFR-I, sTNFR-II,. TNF-α from monocytes	No test of significance. Healing associated with reduction in IL-1α and IL-1β. Increased IL-8 was seen in healing wounds, suggesting that increasing levels of IL-8 in chronic wound fluids is associated with healing. In non-healing wounds IL-8 decreased with time.
Sadler 2012 [20], Australia	Clinical trial: High vs. low dose doxycycline	Association between cytokine level w 4 and percentage reduction in ulcer area	4 w	WF	ELISA	TNF-α	↔
Senet 2003 [21], France	RCT: Platelets vs. placebo	Healing vs. non-healing	16 w	WF	ELISA	IL-8, KGF, VEGF	↑ IL-8 in non-healing at the end of the study, but not at the beginning. Healing is associated with a decrease in IL-8.
Serra 2013 [22], Italy	RCT: Minocycline vs. control	High-healing vs. slow-healing (≥1 vs. <1 cm^2/w)	Median: 13 mo	WF, plasm	ELISA	VEGF	↑ VEGF in slow-healing ulcers, in plasma and WF for patients receiving minocycline. Unclear if this only refers to the experimental arm or also the control.
Serra 2015 [23], Italy	RCT: Doxycycline vs. control	High-healing vs. slow-healing (≥1 vs. <1 cm^2/w)	Median: 13 mo	WF, plasm	ELISA	VEGF	↑ VEGF in slow-healing ulcers in plasma and WF for pt receiving doxycyline. Unclear if this only refers to the experimental arm or also the control.
Stacey 2019 [24], Australia	Cohort study	Healing vs. non-healing *(Area change over 3 w to determine the healing status each week)*	Up to 13 w	WF	Multiplex ELISA	G-CSF, GM-CSF, IFN-γ ("TFN-g"), IL-1α, IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, M-CSF, PDGF-BB, TNF-α, TNF-β, TNF-RI, TNF-RII	↑ GM-CSF and IL-16, and ↓ IL-6 and PDGF-BB, in non-healing by univariable analysis. Multivariable logistic regression: ↑ GM-CSF only remaining significant, OR 126.5, ROC: 0.92. GM-CSF is a predictive biomarker of nonhealing, and have cutoffs which can be used to differentiate between healing and non-healing VLU. Predictive factor for healing.
Tian 2003 [25], Australia	Cohort study	Healing vs. non-healing	2 w	Biopsy	IHC	EGF, FGF-2, IL-1α, IL-6, PDGF-A, TGF-β1, TNF-α, TNF-RI, VEGF	↑ All cytokines in keratinocytes in the adjacent intact skin in non-healing. ↑ VEGF and PDGF-A in keratinocytes at the ulcer edge in non-healing, but not for other cytokines.
Trengove 2000 [26], Australia	Cohort study	a. Healing vs. non-healing b. Ulcer size change	2 w	WF	ELISA & bioassay	EGF, FGF-2, IL-1α, IL-1β, IL-1 bio, IL-6, IL-6 bio, PDGF, TNF-α, TGF-β1	a.↑ IL-1α, IL-1β, IL-1 bio, IL-6 bio and TNF-α in non-healing. b. ↔ No relationship between change in ulcer size and cytokine levels

Wallace 1998 [27], Australia	Cohort study	Healing vs. non-healing	2 w	WF	ELISA & bioassay	TNF-α, TNF-α bio, sTNF-α-R p75, sTNF-α-R p55	↑ TNF-α and sTNF-α-R p75 in non-healing
Wiegand 2017 [28], USA	RCT: Ultrasound vs. control	Correlation to a. Wound size reduction b. Responder vs. non-responder (>40% vs. ≤40% area reduction w 4)	4 w	WF Biopsy	Luminex TGF-β: IHC	IL-1β, IL-6, IL-8, IL-10, TNF-α, TGF-β (the last, biopsy)	a. ↓ IL-6 correlated to percentage wound reduction (prediction of healing). IL-8 showed a similar trend. In contrast, increases of TNF-α and IL-1β was linked to higher percentage area reduction (significance unclear). b. Data challenging to extract, test of significance unclear. Cytokine levels appears different between the treatment groups, and changes with time during w 2 and w 4, e.g. IL-8 declined in responders, but increased in non-responders over time.

↑ Increased, ↓ decreased, ↔ no difference, *assumed. Abbreviations: ALA-PDT = Aminolevulinic acid photodynamic therapy; cDNA = Complementary DNA; CFU = Colony forming unit(s); ELISA = Enzyme-linked immuno sorbent assay; FAP = Frozen allogenic plasma; IHC = Immunohistochemistry; Luminex = Multiplex Assay; Mo = Months; NA = Not applicable; NLFU = Noncontact low-frequency ultrasound; NR = Not reported; PBMC = Peripheral blood mononuclear cells; Pt = Patients; QRT-PCR = Quantitative reverse transcriptase polymerase chain reaction; RT-PCR = Reverse transcriptase polymerase chain reaction; VAS = Visual analogue scale; VLU = Venous leg ulcer(s), WF = Wound fluid

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