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Wound Pain and Wound Healing Biomarkers From Wound Exudate

A Scoping Review

Taichi Goto ◆ Leorey N. Saligan

ABSTRACT

PURPOSE: Effective management of wound pain is essential for optimal wound healing. Nevertheless, the outcomes of wound pain interventions are based on subjective measures, which can prove problematic in patients with cognitive impairment. Identification of biomarkers associated with wound pain and wound healing can be used to more objectively estimate wound pain and contribute to the development of precise management options to reduce wound pain and promote wound healing. This scoping review aimed to identify wound pain and wound healing biomarkers from wound exudates and to describe different wound collection methods to identify these biomarkers.

METHODS: We searched the literature (PROSPERO database registration number: CRD42018103843) via a scoping review.

SEARCH STRATEGY: The PubMed database was searched for articles that explored relationships between cutaneous wound pain, wound healing, and biomolecules. Inclusion criteria were articles that reported original data, used adult human samples, and were published in English.

FINDINGS: Twenty-one articles were retrieved: 17 investigated molecules from wound exudate associated with wound healing status, and 4 reported molecules associated with wound pain. The most frequently observed wound pain biomarkers were proinflammatory cytokines; the most frequently observed wound healing biomarkers were proteases including those in the matrix metalloproteinase family. Six wound exudate collection methods were identified to extract potential wound pain and wound healing biomarkers from wound exudate.

IMPLICATIONS: The results can guide future wound exudate research to validate these wound pain and wound healing biomarkers and to develop therapies targeting these biomarkers to reduce wound pain and promote wound healing.

KEY WORDS: Biomarker, Wound exudate collection, Wound healing, Wound pain.

INTRODUCTION

One of the major manifestations of chronic wounds is wound pain; for example, approximately 80% of patients with chronic venous leg ulcers (VLUs, one of the most prevalent types of chronic wounds) suffer from chronic wound pain.[1-3] While acute pain serves as a warning signal informing individuals that a threat is occurring at the painful region, chronic pain reflects a more complex pathophysiologic process occurring locally and systemically.[4] Wound pain in pressure injuries (PIs) has an adverse impact on activities of daily living, mobility, and sleep,[5] and has served as a predictor of decline in health-related quality of life.[6] Furthermore, wound pain is associated with delayed wound healing.[7,8]

Pain is a subjective experience; as a result, subjective questionnaires are widely used for pain assessment in clinical settings.[9,10] Nonetheless, some patients with cognitive impairments experience difficulty responding to these scales. A more objective pain assessment tool provides an attractive alternative or complementary instrument to the use of validated subjective measures of pain.

There are several pain scales that are designed to assess pain in cognitively impaired patients using changes in facial expression, behavior, and related indirect signs and symptoms.[11-13] Nevertheless, none measure the cause of pain. Furthermore, cultural and racial differences in behavior may affect the results of pain assessment.[13,14] Skin conductance measurement has been used to evaluate surgical wound pain, but it is influenced by stressful factors other than pain.[15] Infrared thermography has also been used to assess wound pain. Research suggests that this may be used to divide VLUs into wounds with/without nociceptive pain; however, it does not capture neuropathic sources of pain.[16]

Researchers continue to investigate exudate as a marker of various wound conditions. Wound exudate can be collected noninvasively and includes abundant cellular components that can reflect several physiologic processes.[17-19] A previous study associated proinflammatory cytokines in wound exudate with postoperative pain, and the reduction in proinflammatory concentrations in the wound exudate was associated with analgesic use.[20] Another study showed that concentrations of neuropeptides in wound exudates from VLUs were associated with wound pain intensity, but the correlation coefficients were

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moderate.[21] Furthermore, while several studies have described simple methods to collect wound exudate in clinical settings, the specific cellular component or wound material that most accurately reflects wound pain remains uncertain.[19,22,23] The aims for our scoping review were to (1) identify wound pain and wound healing biomarkers from wound exudate, and (2) describe different wound collection methods used to identify biomarkers in wound exudate.

METHODS

This review was registered in the PROSPERO database (registration number CRD42018103843) and used a structured method to search the literature based on the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA).[24] The PubMed database was searched for articles using the following terms: "(wound OR ulcer) AND (pain OR healing) AND (wound exudate OR wound fluid)." Articles that explored relationships between cutaneous wound pain, wound healing, and biomolecules; analyzed adult human samples; and were published in English were included. Articles that focused on noncutaneous ulcers or injuries, were case reports, review articles, or solely on wound infection were excluded. Furthermore, study quality of the articles was evaluated based on elements of the study design and method. Data extracted from each article that included author credentials, sample characteristics such as size, age of study participants, study design, and main study focus were retrieved from the reviewed articles. We also extracted methods of pain measurement, wound healing assessment, and wound exudate collection techniques.

RESULTS

The initial search identified 733 articles. The search was refined applying the eligibility criteria, resulting in a pool of 295 articles. An additional 270 articles were excluded after a closer inspection of titles and abstracts. Each article was then read in full and an additional 4 articles were excluded that did not report original research, lacked a control group, and lacked a clear wound healing outcome. Thus, our review is based on 21 articles (Figure).

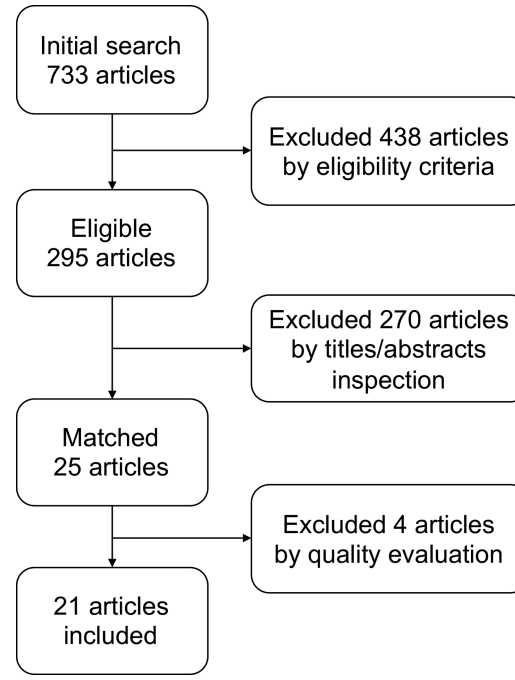

Figure. Flowchart of search and selection strategy.

The articles that focused on wound pain biomarkers are listed in Table 1 and those that focused on healing biomarkers are listed in Table 2. The most studied wound type was VLU (n = 11 of 21 elements, 52%).[21,28-32,34,35,39,40,42] Of the 21 articles, molecules from wound exudates were associated with wound healing status in 17 articles (81%) and wound pain levels in 4 articles (19%). Eight articles (38%) were cross-sectional studies, 9 (42%) used a longitudinal design, and 4 (19%) were clinical trials.

TABLE 1.
Wound Pain Biomarkers[a]

Authors	Wound Type	Sample	Pain Measurement	Biomarkers	
				Analysis Method	Name
Carvalho et al (2008)[25]	Surgical (cesarean)	N = 20 Age = 35 (mean), y Exudate = 20	Analgesic consumption	Multiplex bead array immunoassay plate	IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, TNF-α, IFN-γ, G-CSF, GM-CSF, MCP-1, MIP-1β, NGF, PG-E2, SP
Rohde et al (2010)[26]	Surgical (breast reduction)	N = 24 Age = 27-59 (range), y Exudate = 24	VAS	ELISA	IL-1β, TNF-α, VEGF, FGF-2
Rohde et al (2015)[27]	Surgical (breast reconstruction)	N = 32 Age = 51.1 (mean), y Exudate = 32	VAS	ELISA	IL-1β
Goto et al (2016)[21]	VLU	N = 13 Age = 73 (median), y Exudate = 30	NRS, SF-MPQ-2	ELISA	NGF, S100A8/A9

Abbreviations: ELISA, enzyme-linked immunosorbent assay; G-CSF, granulocyte colony–stimulating factor; GM-CSF, granulocyte-macrophage colony–stimulating factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β, NGF, nerve growth factor; NRS, numerical rating scale; PG-E2, prostaglandin E2; SF-MPQ-2, short form McGill Pain Questionnaire 2; SP, substance P; TNF, tumor necrosis factor; VAS, visual analogue scale; VEGF, vascular endothelial growth factor; VLU, venous leg ulcer.
[a]Exudate means that the number of exudate samples finally analyzed in each study.

TABLE 2.
Wound Healing Biomarkers[a]

Authors	Wound Type	Sample	Wound Healing Assessment	Biomarkers	
				Analysis Method	Name
Rasmussen et al (1992)[28]	VLU	N = 14 Age = 70 (mean), y Exudate = 14	Healing rate	RIA	PIIINP, PICP
Harris et al (1995)[29]	VLU	N = 18 Age = 52-59 (range), y Exudate = 18	Healing ulcer: re-epithelializing and forming new granulation tissue	Collagenase activity: the release of ^{3}H-labelled collagen peptides from type I collagen fibrils formed from acid-soluble rat tail tendon type I collagen. Fibronectin degradation: SDS-PAGE, western blotting Cytokines: ELISA, bioassay	Collagenase activity, fibronectin degradation, bFGF, GM-CSF, PDGF, IL-1α, IL-1β, IL-6
Hoffman et al (1999)[30]	VLU	N = 7 Age = N/A Exudate = N/A	Leg ulcer assessment chart devised by the tissue viability adviser and a Polaroid camera with a grid film	Neutrophil elastase activity: selective substrate N-methoxysuccinyl-ala-ala-pro-val-p-nitroanilide Neutrophil elastase stability: amount	Neutrophil elastase activity Neutrophil elastase stability
Drinkwater et al (2003)[31]	VLU	N = 51 Age = N/A Exudate = 32	Healing ulcer: having healed less than a year Nonhealing ulcer: failed to heal in a year	ELISA	VEGF
Mwaura et al (2006)[32]	VLU	N = 40 (healing = 20, nonhealing = 20) Age = 60 (mean), y Exudate = 40	Healing: a decrease in the size of the ulcer (>20% decrease in surface area), decrease in slough, and development of healthy granulation tissue.	ELISA, IHC	PDGF-AA, MMP-2, TIMP-2, EMMPRIN
Muller et al (2008)[33]	DFU	N = 16 (good healers = 7, poor healers = 9) Age = 61 (median), y Exudate = 16	Good healers: a reduction of at least 82% in initial wound surface at 4 wk Poor healers: reduction of <82% in wound surface at 4 wk	MMP-2, MMP-9: zymography MMP-1, MMP-8, TIMP-1: ELISA	MMP-1, MMP-2, MMP-8, MMP-9, TIMP-1
Rayment et al (2008)[34]	Multi	N = 12 (chronic wound = 9, acute wound = 3) Age = 54-88 (range), y Exudate = 12	PUSH	Zymography	MMP-9
Gohel et al (2008)[35]	VLU	N = 80 Age = 75 (median), y Exudate = 52 (assessment 1), 34 (assessment 2)	Wound area change	IL-1β, TNF-α, bFGF, VEGF, TGF-β: ELISA MMP-2, MMP-9: zymography	IL-1β, TNF-α, MMP-2, MMP-9, bFGF, VEGF, TGF-β
Iizaka et al (2010)[17]	PI	N = 28 Age = 78 (median), y Exudate = 32	DESIGN, PUSH	Albumin: bromocresol green method, total protein: Biuret method, Glucose: dehydrogenase method, zinc: atomic absorption spectrophotometry	Albumin, total protein, glucose, zinc
Wyffels et al (2010)[36]	PI	N = 34 Age = 72.3 (mean), y Exudate = 46	Wound area change	2D-PAGE	S100A9

(*continues*)

TABLE 2.
Wound Healing Biomarkers[a] (*Continued*)

Authors	Wound Type	Sample	Wound Healing Assessment	Biomarkers	
				Analysis Method	Name
Ulric et al (2011)[37]	DFU	N = 32 (treatment = 22, control = 10) Age = 61.5 (mean), y Exudate = 32	Wound area change	MMP-2: ELISA Gelatinase: Gelatinase Activity Assay Kit Elastase: spectrofluorometrically Plasmin: change in absorbance	MMP-2, Gelatinase, elastase, plasmin
Edsberg et al (2012)[38]	PI	N = 32 Age = 72.3 (mean), y Exudate = 42	Wound area change	Mass spectroscopy–based system using isotopically tagged peptides	Proteomic profile
Fernandez et al (2012)[39]	VLU	N = 29 Age = 67.3 (mean), y Exudate = 70	PUSH	Adenosine, inosine, hypoxanthine, xanthine, UA: LC/MS/MS Xanthine oxidase activity: RP-HPLC Western blotting: xanthine oxidase	Adenosine, hypoxanthine, inosine, xanthine, UA
Bernatchez et al (2013)[40]	Multi	N = 81 Age = N/A Exudate = 100	Progressing wounds: healthy appearance, robust granulation tissue, documented reductions in wound area over time Stagnating: not documented with a change in wound area over time Worsening wounds: documented with poor granulation tissue deposition and enlarging wound areas (with or without wound margin deterioration).	NOx: Doane and Horwath's method MMP-9: Fluorokine E Enzyme Activity Assay HNE: InnoZyme Human Neutrophil Elastase Immunocapture Activity Assay	NOx, MMP-9, HNE
Yao et al (2014)[41]	DFU	N = 12 (3 groups, 4 participants in each group) Age = 58 (mean) y Exudate = 12	Percent area reduction	Multiplex xMAP immunoassay	IL-6, IL-8, IL-1β, TNF-α, GM-CSF, MMP-9, VEGF, macrophages
Serra et al (2015)[42]	VLU	N = 64 (treatment = 32, control = 32) Age = 50.9 (mean), y Exudate = N/A	Means of direct ulcer tracing with computerized planimetry	ELISA	MMP-9, NGAL, VEGF
Cervero-Ferragut et al (2017)[43]	Multi	N = 47 Age = N/A Exudate = 111	Inflammatory and proliferative phase: macroscopic parameters	Immunoturbidimetric method	CRP, IgG, IgM

Abbreviations: bFGF, basic fibroblast growth factor; CRP, C-reactive protein; DFU, diabetic food ulcer; ELISA, enzyme-linked immunosorbent assay; EMMPRIN, extracellular matrix metalloproteinase inducer; GM-CSF, granulocyte-macrophage colony–stimulating factor; HNE, 4-Hydroxynonenal; IgG, immunoglobulin G; IgM, immunoglobulin M; IHC, immunohistochemistry; IL, interleukin; LC/MS/MS, liquid chromatography-tandem mass spectrometry; MMP, matrix metalloproteinase; N/A, not available; NGAL, neutrophil gelatinase-associated lipocalin; NOx, nitrogen oxide; PDGF, platelet-derived growth factor; PI, pressure injury; PICP, propeptides of collagen type I; PIIINP, propeptides of collagen type III; PUSH, Pressure Ulcer Scale for Healing; RIA, radioimmunoassay; RP-HPLC, reversed-phase high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TGF, transforming growth factor; TIMP-1, tissue inhibitor of metalloproteinases-1; TNF, tumor necrosis factor; 2D-PAGE, 2-dimensional polyacrylamide gel electrophoresis; UA, uric acid; VEGF, vascular endothelial growth factor; VLU, venous leg ulcer.
[a]Exudate means that the number of exudate samples finally analyzed in each study.

Wound Pain Biomarkers

Of the 4 articles that focused on wound pain biomarkers, 3 studied surgical wounds from cesarean delivery,[25] breast reduction,[26] and breast reconstruction surgeries,[27] while 1 article investigated chronic wounds from VLUs.[21] Carvalho and colleagues[25] investigated wound pain biomarkers from both wound exudate and serum samples and measured pain scores and analgesic consumption at 1, 6, 24, and 48 hours after elective cesarean delivery in 20 healthy women. They found no associations between cytokine concentrations in wound exudate and serum. Analgesic consumption during the first 24 hours after surgery was negatively correlated with interleukin-1β (IL-1β), IL-6, and granulocyte colony–stimulating factor concentrations in the wound exudate. Using a multiple linear regression model, they found that only IL-6 emerged as a statistically significant predictor for morphine consumption. Wound PG-E2 and various cytokines peaked early, whereas nerve growth factor showed a more delayed release. Carvalho and colleagues stated that the lack of significant correlations between wound and serum cytokine levels indicates the importance of determining site-specific release of these cytokines, if localized pathologies are to be studied. Rohde and colleagues[26] conducted a double-blind, placebo-controlled, randomized trial using pulsed electromagnetic field therapy in 24 patients undergoing breast reduction surgery. When compared to a sham device, active therapy reduced postoperative pain, narcotic use, and mean IL-1β concentration in the wound exudates during the immediate postoperative period. In a follow-up study, Rohde and coworkers[27] conducted a double-blind, placebo-controlled, randomized trial using active or sham pulsed electromagnetic field therapy in 32 patients undergoing unilateral TRAM flap breast reconstruction surgery. They found that the electromagnetic field therapy significantly reduced postoperative pain, wound exudate volume, IL-1β wound exudate concentration, and narcotic use.

Goto and colleagues[21] analyzed wound pain intensity and concentrations of nerve growth factors and S100A8/A9 (a protein produced by neutrophils and associated with prostaglandin E2 production) in a cross-sectional study of 30 wound exudates taken from 13 VLUs. They found that those 2 protein concentrations in the wound exudate were correlated with pain intensity scores.

Considered collectively, findings from these studies suggest that IL-1β and Il-6 are associated with acute surgical wound pain.[25-27] Nerve growth factors are associated with later phase of surgical wound pain and chronic wound pain.[21,25] None of these studies identified whether participants with infected wounds were included in their samples. Thus, the effect of infection on these biomarkers cannot be determined.

Venous Leg Ulcer

Eight of the 17 articles that investigated wound healing biomarkers focused on VLUs. Rasmussen and colleagues[28] examined 14 patients with VLU treated with human growth hormone. While no changes in healing rates in relation to growth hormone application were observed, they found that collagen type III increased 5 and 9 days after starting treatment and propeptides of collagen type I increased in the fourth week. The presence of these propeptides was associated with higher wound healing rates.

Harris and coworkers[29] divided the VLUs of 18 patients into healing and nonhealing wounds based on clinical criteria. They measured platelet-derived growth factor-AB (PDGF-AB), granulocyte macrophage colony–stimulating factor (GM-CSF), IL-1 α, IL-1β, IL-6, and basic fibroblast growth factor (bFGF) from wound fluid by enzyme-linked immunosorbent assay (ELISA) but found no significant differences in the levels of cytokines or collagenase between the groups. Hoffman and associates[30] conducted 2 experiments measuring neutrophil elastase in wound exudates. They also found no correlation between wound healing rate and concentration of neutrophil elastase.

Drinkwater and coworkers[31] measured vascular endothelial growth factor (VEGF) and its receptors in 35 patients with current VLU, 9 patients whose VLU had healed, and 18 patients undergoing routine operations (8 with closed suction drains and 10 with standard skin biopsies). Concentration of VEGF in wound fluid was significantly higher in nonhealing VLU than in healing venous ulcers or acute wounds. Concentration of VEGF-R1 was similar in wound fluid obtained from healing ulcers and nonhealing ulcers and acute wounds. Mwaura and colleagues[32] divided the VLUs of 40 patients into 2 groups: healing and nonhealing. High PDGF-AA in wound exudates in the healing group was found.[32]

Gohel and colleagues[35] conducted a prospective observational study of 80 participants with VLUs. They found that initial wound fluid concentrations of bFGF correlated with ulcer size and changes in wound fluid. Specifically, they reported that transforming growth factor (TGF)-β concentrations were inversely correlated with changes in ulcer size. However, wound fluid and serum cytokine concentrations were only weakly correlated. Fernandez and colleagues[39] enrolled 29 patients with VLUs; they measured uric acid and related molecules from wound exudate. They showed that the uric acid is elevated in wound fluid from chronic VLUs correlating with wound chronicity. In addition, they indicated that a corresponding depletion in uric acid precursors, including adenosine, correlated with wound severity evaluated by the Pressure Ulcer Scale for Healing (PUSH) Scale. Serra and colleagues[42] conducted an open-label, parallel-group, clinical trial of 64 patients that evaluated the effects of doxycycline on wound healing. The doxycycline treatment group showed a higher healing rate than the control group. Furthermore, in the control group, the lower healing rate was related to higher levels of matrix metalloproteinases (MMP)-9; neutrophil gelatinase-associated lipocalin and VEGF in the plasma, wound fluid, and wound tissue biopsies.

Considered collectively, findings from these studies suggest that VLU healing is associated with collagen (types I and III) propeptides, growth factors (bFGF, VEGF, TGF-β, and PDGF), and a protease (MMP-9). Three of the 8 articles identified whether infected wounds were included or excluded in their studies,[29,35,42] while the remaining 5 articles did not mention wound infection status.[28,30-32,39]

Diabetic Foot Ulcer

Of the 17 reviewed articles that investigated wound healing biomarkers, 3 enrolled participants with diabetic foot ulcers (DFUs). Muller and colleagues[33] followed 16 participants with DFUs for 12 weeks. They were divided into 2 groups: good healers and poor healers. The levels of MMP-8 and -9 in wound exudate decreased earlier in participants deemed good healers. The levels of MMP-1 rose significantly at week 2 in good healers. In addition, there was an association between a high ratio of MMP-1/tissue inhibitor of metalloproteinases (TIMP)-1 and good healing. Ulrich and coworkers[37] enrolled

32 participants with DFUs in a nonrandomized trial. Ten participants were treated with hydrocolloid dressings and 22 were treated with oxidized regenerated/collagen matrix and hydrocolloid dressings.[37] Participants treated with oxidized regenerated cellulose/collagen matrix achieved a greater reduction in wound size with a corresponding decrease in elastase, plasmin, gelatinase activities, and MMP-2 in wound exudate than those treated with hydrocolloid dressings.

Yao and associates[41] examined the effect of dose and duration of noncontact low-frequency ultrasound on 12 subjects with nonhealing DFUs. Findings indicated reduction of proinflammatory cytokines (IL-6, IL-8, IL-1β, tumor necrosis factor-α [TNF-α], and GM-CSF), MMP-9, VEGF, and macrophages in response to noncontact low-frequency ultrasound consistent with wound reduction. Differences were not statistically significant.

Considered collectively, findings from these studies suggest that DFU healing is associated with proteases including MMPs and gelatinase, growth factors including VEGF, and proinflammatory cytokines including ILs, TNF-α, and GM-CSF. Infected wounds were not observed or were excluded in 2 of the 3 articles,[33,41] while 1 article did not clarify wound infection status.[37] Thus, the effect of infection on these biomarkers cannot be determined.

Pressure Injury

Of the 17 articles that investigated wound healing biomarkers, 3 focused on biomarkers from PIs. Iizaka and coworkers[17] measured nutritional markers in a cross-sectional study of 32 full-thickness PIs. They reported that albumin levels in wound exudate and the wound fluid/serum ratio for albumin were lower during the inflammatory phase as compared to the proliferative phase of wound healing. Infected wound fluid contained less glucose than noninfected wound fluid when an intraindividual comparison of 3 cases was conducted. During the proliferative phase of wound healing, the wound fluid/serum ratio for glucose was negatively correlated with hydroxyproline levels. In contrast, zinc levels in wound fluid showed a positive correlation with hydroxyproline levels.

Wyffels and colleagues[36] tested 2-dimensional polyacrylamide gel electrophoresis in wound exudate from 9 chronic PIs at 15 time points collected over 42 days. They reported that the interior and periphery of individual wounds had different protein signatures and healed PI had an increase in protein spot numbers over time compared to unhealed ulcers. Using this technique, S100A9 was identified as a potential biomarker of wound healing. Edsberg and associates[38] applied a mass spectroscopy–based system to the wound fluid from 32 subjects with 42 PIs over 6 weeks at 15 time points. They identified 21 proteins that differed between healed and non-healing PI. The following proteins, GM-CSF, I-309, interferon- γ, IL-11, IL-12, p40, IL-15, IL-1α, IL-1β, IL-8, TIMP-1, TIMP-2, TNF RI, and TNF RII, were greater in nonhealed PI. In concentrations of intercellular adhesion molecule 1, IL-16, macrophage inflammatory protein-1 δ, MMP-10, MMP-13, MMP-3, and eotaxin-2 were higher in healed PI.

Considered collectively, findings from these studies suggest that proteinases including MMPs, S100A9, and proinflammatory cytokines including ILs are associated with wound healing in PIs. One article included 6 infected wounds of the 32 observed wounds,[17] while the remaining 2 articles did not mention wound infection status.[36,38]

Studies Enrolling Participants With Multiple Types of Wounds

Three of the 17 articles that investigated wound healing biomarkers enrolled patients with multiple types of wounds. Rayment and colleagues[34] investigated MMPs in wound exudate from chronic wounds and acute wounds, and human serum. They found excessive protease activity in chronic wound fluid as compared with human serum and acute wound fluid. Additionally, MMP-9 was identified as the predominant protease in chronic wound fluid; levels were higher in chronic wound fluid than in acute wound fluid. Furthermore, the clinical status of the ulcer was directly associated with the amounts of MMP-9 present in the wound fluid.

Bernatchez and associates[40] analyzed nitric oxide metabolites and other biomarkers in human wound fluids and correlated these markers with wound healing status. They studied 100 wound samples from 81 participants with VLUs, DFUs, PIs, burns, surgical wounds, donor sites, traumas, and drainage tube wounds. They found that nitric oxide (NOx) best discriminated deteriorating versus healing wounds. Cervero-Ferragut and colleagues'[43] group aimed to quantify blood cells and inflammatory markers involved in the healing process in exudates from wounds in different healing phases. Forty-seven patients with surgical wounds and PIs were enrolled in their study. They reported that neutrophil and platelet counts were higher, whereas the number of lymphocytes was lower in wound exudate analyzed during the inflammatory phase of healing. Wound C-reactive protein (CRP) and immunoglobulin G (IgG) levels were also higher in the inflammatory phase.

In summary, findings from these articles suggest that wound healing is associated with protease (MMP-9), NOx, CRP, and IgG. Infected wounds were not observed or were excluded in 2 of the 3 articles,[34,43] while the remaining article included several infected wounds.[40] Thus, the effect of infection on these biomarkers cannot be determined.

Wound Exudate Collection

We classified wound exudate collection methods into categories based on materials used to collect exudate (Table 3). They are dressing, immersing bandage, gauze/swab, absorbent paper, drain/negative pressure wound therapy, and needle puncture extraction. In addition, we found one study that did not describe a wound exudate collection method.[42] The most frequently used method was the dressing method, which was used in 10 of the 21 elements (48%) included in this scoping review.[17,28,29,31,32,34,35,37,39,40]

The most prevalent method is based on covering the wound with an occlusive dressing from 0.5 to 6 hours in order to allow exudate to accumulate. Wound exudate was then aspirated by a needle and a syringe from the space under the dressing. In several articles, the accumulated exudate was recovered by washing the wound with appropriate buffer solution before aspirating.[28,34,39]

The next most frequently used method was the drain/negative pressure wound therapy method; this technique was used in 5 wound types in 4 articles.[25-27,43] The drain method was specifically used for surgical wounds. For example, one article used an On-Q PainBuster Post-Op Pain Relief System (I-Flow, Lake Forest, California) that continuously delivered normal saline subcutaneously into the wound and allowed aspiration of wound exudate at specified time points.[25] Other articles used Jackson-Pratt drains that permitted the collection of wound exudates in the immediate postoperative stages of healing.[26,27] Another article used a low-pressure aspiration

TABLE 3.
Wound Exudate Collection Methods[a]

	Exudate Collection Methods						
Wound Type	**Dressing**	**Immersing Bandage**	**Gauze/Swab**	**Absorbent Paper**	**Drain/NPWT**	**Needle**	**Unclear**
VLU	6	1	1				1
PI	1		2				
DFU	1			2			
Surgical					3		
Multi	2		1		2	1	

Abbreviations: DFU, diabetic food ulcer; NPWT, negative-pressure wound therapy; PI, pressure injury; VLU, venous leg ulcer.
[a]Dressing: each wound was covered with an occlusive dressing for 0.5 to 6 hours, and then the retained exudate was collected. Immerging bandage: each bandage was immersed in appropriate buffer solution. Gauze: gauze was placed on the wound and removed 3 minutes later and soaked in appropriate buffer solution. Swab: swab was rolled over surface of the wound and soaked in appropriate buffer solution. Absorbent paper: absorbent paper was placed on the wound. Drain: several specific drainage systems were used to collect wound exudate from surgical wounds. NPWT: a negative-pressure wound therapy system was used to collect the wound exudate. Needle: the exudate was removed with needles and syringes.

drainage system that involved continuous aspiration drainage formed by polyethylene tubes connected to a vacuum.[43]

The gauze/swab method for exudate collection was used in 4 articles.[21,36,38,40] Wound exudate was absorbed by sterile gauze or polyester-tipped applicators and recovered in an appropriate buffer solution. Absorbent paper method was used in 2 articles that investigated DFUs.[33,41] Investigators applied an absorbent paper or a filter paper to the wounds for 0.5 to 5 minutes. The absorbed exudate was recovered in an appropriate buffer solution. Researchers in 2 studies reported using an immersing bandage and needle puncture extraction method.[30,34] In the needle puncture extraction method, the wound exudate was removed using 26-gauge 1.3-cm needles and syringes from naturally occurring subepidermal blisters on the feet.[34] In the immersing bandage method, each bandage that covered the wound was immersed in appropriate buffer solution.[30]

DISCUSSION

This scoping review aimed to identify wound pain and wound healing markers from wound exudate and the methods used to collect exudate. The findings from this review suggest that specific molecules in wound exudate and/or wound tissues including proteases, growth factors, and proinflammatory cytokines may be used to augment our assessment of wound pain and wound healing in multiple wound types.

The most frequently studied wound type was the VLU, which is prevalent in western countries.[44] Although wound pain is common in VLU patients, only 1 article studied the relationship of wound exudate biomarkers and wound pain levels.[21] Venous leg ulcers are sequelae of chronic venous disease resulting in elevated venous pressures in advanced stages.[45] Elevated venous pressure raises iron level in the tissue via extravasation of erythrocytes. Research suggests that elevated level of interstitial iron may activate MMPs and inhibit expression of tissue inhibitor of metalloproteinase, resulting in breakdown of the tissue and development of the wound.[46-48] The breakdown of tissue induces accumulation of immune cells including neutrophils. The neutrophils induce inflammatory response in the tissue by producing proinflammatory cytokines and growth factors.[49] For DFUs, one study that tested treatment of biogenic silver nanoparticles showed decreased expression of MMP-2 and MMP-9 in wounded granulation tissues leading to early wound healing in diabetic mice.[50] Additionally, extracellular free-growth factors are degraded rapidly by metalloproteinase (MMP)-9.[51]

Normal wound healing consists of 4 phases: homeostasis, inflammatory, proliferative, and remodeling. The phases overlap as one phase regresses and the next phase initiates.[52] Indolent or nonhealing VLU may remain in an inflammatory phase, with provisional matrix prevention movement into the proliferative and remodeling phases of healing.[45] The local environment of VLU is complex and contains a multitude of elements that influence wound healing such as inflammatory and immune cells, proinflammatory cytokines, and protease activity (especially MMP2 and 9). Current evidence suggests that multiple MMPs and growth factors are potential wound healing biomarkers for chronic wounds such as VLU and DFU.[31-33,35,37,41,42]

Pressure injury (PI) is influenced by multiple factors including tissue load and inherent tolerance, microclimate, and nutritional status.[53] Due to the complicated nature of PIs, broad proteomic or metabolomic approaches that measure multiple biomarkers were used in the studies we identified. Findings from these broad approaches demonstrate a correlation between wound healing with MMPs and ILs; these findings are broadly consistent with findings from studies of other wounds such as VLU and DFU.

Around 80% of postsurgical patients experience postsurgical pain.[54] We extracted data from 3 examined associations between various biomarkers and acute postoperative pain and found that multiple proinflammatory cytokines including ILs, TNF-α, GM-CSF, and monocyte chemoattractant protein-1 were associated with surgical wound pain. Surgical wounds heal by primary intention. In the early phase of healing of the surgical wound, neutrophils play an important role in regulating inflammatory response by producing proinflammatory cytokines that sensitize the nociceptors.[55,56] Thus, proinflammatory cytokines can be potential biomarkers for acute, surgical wound pain and healing.

Our review identified association between wound pain and selected biomarkers with wound pain; however, the magnitude of these associations (correlation coefficients) was not sufficient to use them as an objective proxy to measure pain intensity. These biomarkers act as algesic substances that sensitize the nerves in tissues causing pain.[53,57] However, pain signaling influenced by multiple factors including physical and psychological factors.[58-62] We therefore hypothesize that measurement of biomarkers reflecting pain perception, such as stress-related molecules, may be an important addition to the algesic substances identified in this review.

Additionally, although wound pain is associated with wound healing,[7,8] we found no studies that established a

causal relationship between wound pain and wound healing. Muller an colleagues[33] reported that a high ratio of MMP-1/TIMP-1 is correlated to good wound healing, but an association of this ratio with wound pain has not been established. Goto and coworkers[21] showed the relationship of S100A8/A9 and wound pain in patients with VLUs and Wyffels and associates[36] found a relationship between S100A9 and wound healing in patients with PIs. S100A9 is synthesized mainly by neutrophils and is related to prostaglandin E2 production.[63,64] While the wound types investigated were different in the 2 articles, these findings suggest that S100A9 may be a potential wound pain biomarker that also predicts wound healing.

Carvalho and colleagues'[25] and Gohel and colleagues'[35] groups compared biomarker levels between wound exudate and serum. They found no association between wound exudate and serum cytokine levels, suggesting that wound pain intensity may be linked to local inflammatory processes influencing cytokine concentration in wound exudate. These findings emphasize the need to investigate local mechanisms of wound pain and wound healing, since immune cells accumulated at the wound area locally produce proinflammatory cytokines.[49]

We identified 6 wound exudate collection methods; the dressing method being the most commonly used method. The dressing method is optimal in collecting wound exudate samples, but it takes a relatively long time to complete the procedure, at least 0.5 hours.[34] The duration of attaching the dressings to the wounds varied among the reviewed articles from 0.5 to 6 hours, which can affect the quality and quantity of molecules extracted. In addition, time-dependent protein analysis such as cytokine experiments may be affected by the variability in the dressing application. A standard duration of dressing application using this method must be established to be able to compare results between studies. On the other hand, gauze/swab and absorbent paper methods took a relatively shorter time to complete, while the duration of attaching the absorbent papers to the wounds also varied among the reviewed articles from 0.5 to 5 minutes. These methods may be appropriate to use to detect wound pain biomarkers that are time sensitive. The drain method was most appropriate in studying surgical wound pain/healing because drainage systems were commonly placed in most of surgical wounds.

Low statistical power represents a limitation of the findings reported in this scoping review. Few studies described how they determined an adequate sample size to answer the study aims. It may have been relatively hard to estimate the appropriate sample size for observing wound healing and wound pain biomarkers because the existing literature is scant in this area. The knowledge gained from this review should promote more studies with appropriate number of samples to produce more robust evidence.

CONCLUSION

We performed a scoping review of the literature and found evidence suggesting that proinflammatory cytokines are major biomarkers of wound pain. Findings also suggest that MMPs and several growth factors obtained from wound exudate are biomarkers of wound healing. Furthermore, it was suggested that S100A9 is a potential wound pain biomarker that predicts wound healing. We also found multiple options of wound exudate collection methods for wound pain/healing biomarker discovery. Using the findings from this review, more research studies with appropriate power and design to identify wound pain/healing biomarkers are warranted.

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KEY POINTS

- Interleukin-1β (IL-1β) and IL-6 are associated with acute surgical wound pain; nerve growth factor is associated with later phase of surgical wound pain and chronic wound pain; and S100A8/A9 is associated with pain in patients with venous leg ulcers.
- Wound healing rates may be associated with specific measureable components that vary by wound type and phase of wound healing, including collagen propeptides, growth factors, proteases, proinflammatory cytokines, nitric oxide, CRP, and IgG.
- Biomarker levels in wound exudate may be better correlated with pain and wound healing than serum levels.
- There are a variety of methods utilized to collect wound exudate, including dressing, immersing bandage, gauze/swab, absorbent paper, drain/negative pressure wound therapy, and needle puncture extraction.

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