Int J Burn Trauma 2021;11(4):275-288
www.IJBT.org /ISSN:2160-2026/IJBT0134761

Review Article

The role of matrix metalloproteinase-9 and its inhibitor TIMP-1 in burn injury: a systematic review

Chinmay Sharma[1], Geoffrey P Dobson[1], Lisa M Davenport[2], Jodie L Morris[1], Hayley L Letson[1]

[1]Heart, Trauma and Sepsis Research Laboratory, College of Medicine and Dentistry, James Cook University, Queensland 4811, Australia; [2]Townsville University Hospital, 100 Angus Smith Drive, Douglas, Queensland 4814, Australia

Received April 28, 2021; Accepted June 25, 2021; Epub August 15, 2021; Published August 30, 2021

Abstract: Matrix metalloproteinase-9 (MMP-9) and its endogenous inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), are key mediators of acute inflammation and regulators of the wound healing process. The aim of this systematic review was to determine the local and systemic involvement of the MMP-9/TIMP-1 system following burn injury. Two databases (Scopus and MEDLINE) were searched for all studies reporting MMP-9 and/or TIMP-1 after burn injury. Based on our eligibility criteria, we reviewed 24 studies involving 508 burns patients in 11 clinical studies and 367 animals in 13 preclinical studies. Local, systemic, and peripheral gene expression, protein levels and activity of MMP-9 and TIMP-1 were assessed. Increased MMP-9 was reported at the site of injury early after burn trauma in all studies, and remained elevated in non-healing wounds. Increased TIMP-1 expression in burn wounds occurred later than MMP-9, and was persistent in hypertrophic burn scars. Similar to local expression, systemic MMP-9 and TIMP-1 concentrations were significantly elevated after burn injury in response to upregulation of proinflammatory cytokines. While no association was found between systemic MMP-9 concentration and extent of injury or outcome, serum or plasma TIMP-1 showed good correlation with survival and burn severity. This review also found evidence of the MMP-9/TIMP-1 system contributing to secondary tissue damage distant from the burn site, including burn-associated musculoskeletal damage and acute lung injury. In addition, increased MMP-9 synthesis and activity in the brain after peripheral burn may lead to blood-brain barrier dysfunction and cerebral edema, a significant contributor to mortality. This systematic review provides an overview of the available evidence of the role of MMP-9 and TIMP-1 in burn injury pathophysiology and finds that TIMP-1 may be a promising biomarker in outcome prognostication of burns patients. Large-scale studies of both pediatric and adult burns patients with increased female representation and repeated sampling are recommended to validate the reliability of TIMP-1 as a prognostic marker following burn injury.

Keywords: MMP-9, gelatinase, wound, thermal, extracellular matrix, total body surface area

Introduction

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-endopeptidases that cleave extracellular matrix (ECM) proteins, and thereby play a central role in burn wound healing and remodeling [1-3]. Matrix metalloproteinase-9, or gelatinase B, has been identified as an important signaling protease able to modulate the inflammatory response and a key contributor to the wound healing process [4, 5]. Expression of MMP-9 is upregulated locally and systemically after injury in epithelial cells, endothelial cells and immune cells, including monocytes, macrophages, lymphocytes and dendritic cells [4, 6]. Organized wound healing and homeostasis is dependent on regulation of MMP-9 activity by its endogenous inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1) [7-10].

Dysregulation of the dynamic balance between MMP-9 and TIMP-1 leads to prolonged inflammation and delayed wound healing, and might contribute to burn-associated secondary tissue damage and mortality [4, 8, 10, 11]. Burn injury results in upregulation of proinflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-8, which stimulate MMP-9 synthesis [5, 12]. In turn, MMP-9 modu-

lates the inflammatory response by cleaving IL-1β and IL-8, increasing their biologic activity and potency [12, 13]. TIMP-1 also has a broad range of downstream signaling effects including preservation of endothelial cells and platelets [7]. Through these effects, MMP-9 and its inhibitor TIMP-1 may contribute to microvascular hyperpermeability and subsequent burn-associated edema [10]. A more thorough understanding of these pathophysiologic responses is required to project patient outcomes and develop new therapeutic strategies. In response to the exponential increase in MMP-9 research in the past few decades [4], we conducted a systematic review to determine the role of the MMP-9/TIMP-1 system in burn injury. This review provides an overview of the available evidence in both preclinical and clinical studies, with recommendations for future research.

Materials and methods

This systematic review was conducted and is reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [14].

Eligibility criteria

All studies reporting MMP-9 and/or TIMP-1 after burn injury were included. The search strategy included the following terms: "*matrix metalloproteinase*" or "*collagenase*" or "*matrix metalloproteinase 9*" or "*gelatinase*" or "*metalloprotease*" or "*metalloendopeptidase*" or "*tissue inhibitor of metalloproteinase*" or "*tissue inhibitor of metalloproteinase-1*" and "*burns*" or "*burn injury*". Studies on corneal burns and non-thermal burns, and studies of therapeutic interventions without an untreated burn injury group for analysis were excluded. There were no restrictions placed on Level of Evidence, study size or date of publication. Review articles, conference abstracts, and non-English studies were excluded from this review.

Information sources

The literature search was conducted on publications available up to March 21, 2021. Two databases were searched: Scopus (1996-present) and MEDLINE (1946-present). Reference lists of studies retrieved in full text were also hand searched to identify additional studies.

Study selection

After duplicates were removed, two investigators screened the titles and abstracts of all retrieved citations to identify eligible studies. Relevant studies were retrieved in full text and further reviewed by the same two investigators against the inclusion criteria. The full text of any studies for which a definite decision could not be made from the title and abstract alone was also retrieved. Where there was disagreement regarding study eligibility, a third investigator was consulted.

Data extraction and analysis

Two investigators extracted the necessary information from identified papers using a standard form developed specifically for this review. Data were extracted for: General characteristics (authors, year, title, journal), Study characteristics (study type, level of evidence, sample size), Subject characteristics (species, age, gender), Clinical characteristics (total body surface area [TBSA], burn degree/depth), and Outcome data (parameter [MMP-9, TIMP-1], sample type, detection method, timepoints). Following data extraction, the included studies were too heterogeneous with regards to species, burn severity and degree, sample type, detection method and time from burn injury, and therefore a meta-analysis was not conducted.

Risk of bias assessment

Two tools were used for Risk of Bias (RoB) Assessment depending on study type. Animal studies were assessed with The Office of Health Assessment and Translation (OHAT) RoB tool developed by the National Toxicology Program to evaluate use of randomization, blinding, and outcome assessment and reporting. The Scottish Intercollegiate Guidelines Network (SIGN) checklist was used for cohort, case-control, and cross-sectional studies to assess internal validity and risk of selection, performance, attrition and detection bias. Each study was assessed as having low, moderate, or high risk of bias.

Results

The initial databases search produced 916 articles, leaving 761 unique articles when dupli-

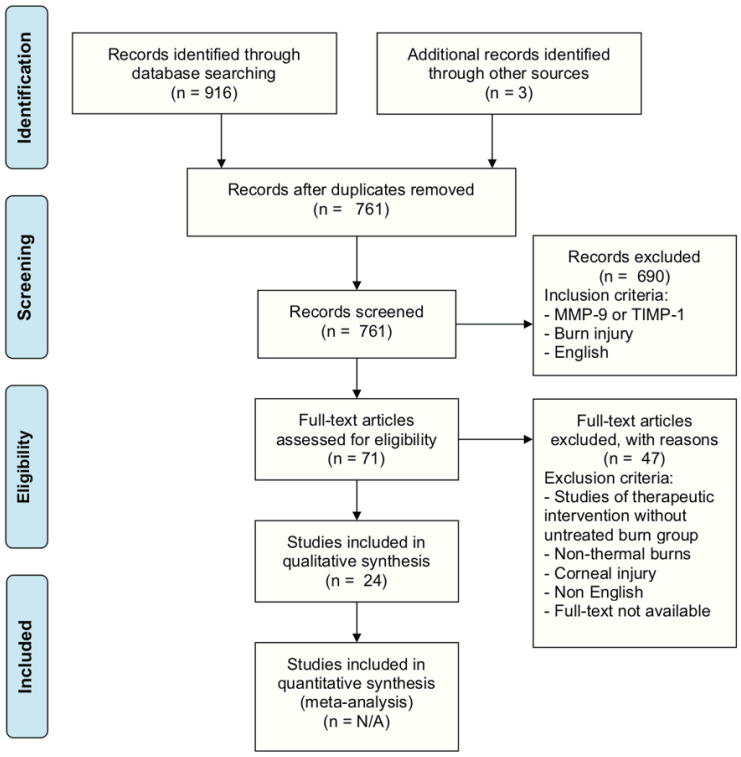

Figure 1. PRISMA flow-chart. A total of 761 studies were evaluated for reporting of MMP-9 and/or TIMP-1 after burn injury. Titles and abstracts were assessed, and 71 full-text articles were eligible for evaluation. 47 articles were excluded, and 24 articles remained for the systematic review.

cates were removed (**Figure 1**). Three additional articles were obtained through reference list searching. In total, 71 articles were examined in full text, after titles and abstracts were screened. Based on the eligibility criteria, 24 studies were included in this systematic review involving 508 burns patients in 11 clinical studies [5, 12, 15-23] and 367 animals in 13 preclinical studies (**Table 1**). Of the preclinical studies, 11 were rodent studies [3, 6, 10, 11, 24-30] and two were porcine studies [31, 32].

Quality and risk of bias assessment

Risk of bias (RoB) assessment showed nine low risk, 14 medium risk, and one medium-high risk study (**Table 2**). Five of the preclinical studies were assessed as low risk [3, 24, 26, 28, 32] and eight were considered medium risk due to lack of randomization and/or blinding [6, 10, 11, 25, 27, 29-31]. Three of the medium risk preclinical studies did not confirm extent of burn injury [10, 11, 27]. Ten of the human studies were Level IV case-control or cohort studies, with one Level III cross-sectional study (**Table 1**). Four of the Level IV studies scored as low risk using the SIGN checklist (**Table 2**) [5, 12, 16, 17]. The Level III cross-sectional study of Nong and colleagues was medium risk because it was not blinded and the patient population was insufficiently described to assess representativeness [15]. None of the studies was classified as high risk, however the cohort study of Young and Grinnell had no control group and was medium-high risk [22]. The remaining five clinical studies were medium risk due to possible selection bias [18, 20], or because the control group was not well-defined [19, 21, 23] (**Table 2**).

General information of study population

The extent of burn injury was highly variable across the studies, ranging from 1 to 80% TBSA burns (**Table 1**). Eight studies included only full-thickness (FT) burns [6, 24-30], three covered partial thickness (PT) burns only [3, 31, 32], six included a mix of FT and PT burns [17, 18, 20-23], while one also incorporated superficial burns [12]. The remaining six studies did not report the degree or depth of burn [5, 10, 11, 15, 16, 19] (**Table 1**). Of the preclinical studies, six investigated juvenile animals [3, 10, 24, 25, 28, 31] and five investigated adults [6, 11, 27, 29, 30], while Simonetti and colleagues compared young (7 to 10 months) and old (19 to 28 months) rats [26]. The majority of the clinical studies evaluated adult patients, with only pediatric patients in the Dasu *et al.* and Neely *et al.* patient cohorts [19, 21]. Only ten of the 24 studies reported the gender of the burn subjects (**Table 1**). Male animals were exclusively used in the preclinical studies, with males also more prevalent in the clinical studies making up 58 to 77% of the patient cohort.

Outcome measurements

Eleven studies reported measurements of both MMP-9 and TIMP-1, with MMP-9 only

Table 1. Summary of study characteristics

Authors	Year	Study type	Level of evidence	TBSA	Degree/ Depth	Burn Type	Sample size	Species	Age	Gender (M/F)
Brightwell et al. [24]	2020	Animal study	Foundational evidence	30%	FT	Thermal	n = 13 Burn n = 4 Control	C57BL/6-129 mouse	Juvenile (8 weeks)	NR
Hernandez et al. [11]	2018	Animal study	Foundational evidence	40%	NR	Thermal	40 n = 11 Control	SD rat	Adult	All male
Yu et al. [3]	2017	Animal study	Foundational evidence	NR	PT	Thermal	54	Nude Mouse	Juvenile (6 weeks)	NR
Nong et al. [15]	2016	Cross sectional study	Level III	>20%	NR	All	n = 244 Burn n = 35 Control	Human	NR	NR
Wiggins-Dohlvik et al. [10]	2016	Animal study	Foundational evidence	30%	NR	Thermal	n = 4 Burn n = 4 Sham	SD rat	Juvenile (5.5-9 weeks)	All male
Hastbacka et al. [12]	2015	Cohort study	Level IV	<20% and >20%	S = 2 PT = 27 FT = 33	All	n = 30 TBSA>20% n = 19 TBSA<20% n = 6 Control	Human	Adult (>18 years)	35/14
Nagy et al. [5]	2015	Retrospective cohort study	Level IV	15-80%	NR	Inhalation/Blast/ Flame/Scald	31	Human	Adult (30-74 years)	NR
Kubo et al. [25]	2014	Animal study	Foundational evidence	NR	FT	Thermal	n = 40 Burn n = 4 Control	BALB/c mouse	Juvenile (8-9 weeks)	All male
Simonetti et al. [26]	2013	Animal study	Foundational evidence	NR	FT	Thermal	n = 24 Burn n = 16 Control	Wistar rat	Young (7-10 months) Old (19-28 months)	All male
Stagg et al. [27]	2013	Animal study	Foundational evidence	30%	FT	Thermal	n = 5 Burn n = 5 Sham	SD Rat	Adult	All male
Wang et al. [16]	2013	Case-control study	Level IV	NR	NR	NR	101 n = 55 Burn	Human	Adult (17-97 years)	77/24
Sio et al. [28]	2010	Animal study	Foundational evidence	30%	FT	Thermal	36	BALB/c mouse	Juvenile (6-8 weeks)	All male
Ulrich et al. [17]	2010	Case-control study	Level IV	>5% (21-12%)	Dermal/FT	NR	69 n = 19 Burn	Human	Adult (48.2 ± 19 years)	11/8 Burn 12/39 Other
Reiss et al. [18]	2009	Cohort study	Level IV	1.5-17%	PT/FT	NR	20	Human	Pediatric & Adult (5-75 years)	NR
Reyes et al. [29]	2009	Animal study	Foundational evidence	60-70%	FT	Thermal	n = 32 Burn n = 8 Control	SD rat	Adult	All male
Berger et al. [6]	2007	Animal study	Foundational evidence	70%	FT	Thermal	35	SD rat	Adult	NR
Swann et al. [30]	2007	Animal study	Foundational evidence	60-70%	FT	Thermal	72	SD rat	Adult	All male
Dasu et al. [19]	2003	Cohort study	Level IV	>40%	NR	NR	12	Human	Pediatric (7.9 ± 2.5 years)	NR
Ulrich et al. [20]	2003	Cohort study	Level IV	>5% (38-19%)	PT/FT	NR	n = 22 Burn n = 20 Control	Human	Adult (49.32 ± 24.2 years)	14/8 Burn 8/12 Control
Neely et al. [21]	1997	Case-control study	Level IV	50%	Nearly all FT	NR	20	Human	Pediatric (17 days-15.8 years)	NR

Schaffer et al. [31]	1997	Animal study	Foundational evidence	NR	PT	Thermal/Laser	8	Domestic pig	Juvenile	NR
Stricklin et al. [32]	1994	Animal study	Foundational evidence	NR	PT	Thermal	4	Pig	NR	NR
Young & Grinnell [22]	1994	Cohort study	Level IV	10-43%	FT = 2 PT-FT = 1	NR	3	Human	Adult (42-56 years)	2/1
Stricklin et al. [23]	1993	Cohort study	Level IV	1-76%	PT/FT	Thermal/ Electricity	n = 33 Burn n = 3 Control	Human	Pediatric & Adult (1-72 years)	24/7

NR = Not Reported; TBSA = total body surface area; S = superficial; PT = partial thickness; FT = full thickness; SD = Sprague-Dawley; M = male; F = female.

Table 2. Risk assessment

Authors	Year	Study type	RoB tool	Risk assessment	Details
Brightwell et al. [24]	2020	Animal study	OHAT	Low	
Hernandez et al. [11]	2018	Animal study	OHAT	Medium	No blinding; No confirmation of injury
Yu et al. [3]	2017	Animal study	OHAT	Low	
Nong et al. [15]	2016	Cross-sectional study	SIGN	Medium	No blinding; No population details
Wiggins-Dohlvik et al. [10]	2016	Animal study	OHAT	Medium	No randomization, blinding, or confirmation of injury
Hastbacka et al. [12]	2015	Cohort study	SIGN	Low	
Nagy et al. [5]	2015	Cohort study	SIGN	Low	
Kubo et al. [25]	2014	Animal study	OHAT	Medium	No randomization or blinding
Simonetti et al. [26]	2013	Animal study	OHAT	Low	
Stagg et al. [27]	2013	Animal study	OHAT	Medium	No randomization; No confirmation of injury
Wang et al. [16]	2013	Case-control study	SIGN	Low	
Sio et al. [28]	2010	Animal study	OHAT	Low	
Ulrich et al. [17]	2010	Case-control study	SIGN	Low	
Reiss et al. [18]	2009	Cohort study	SIGN	Medium	No blinding; Possible selection bias
Reyes et al. [29]	2009	Animal study	OHAT	Medium	No randomization or blinding
Berger et al. [6]	2007	Animal study	OHAT	Medium	No randomization or blinding
Swann et al. [30]	2007	Animal study	OHAT	Medium	No randomization or blinding
Dasu et al. [19]	2003	Cohort study	SIGN	Medium	Control not well defined
Ulrich et al. [20]	2003	Cohort study	SIGN	Medium	Possible selection bias
Neely et al. [21]	1997	Case-control study	SIGN	Medium	Control not well identified
Schaffer et al. [31]	1997	Animal study	OHAT	Medium	No randomization or blinding
Stricklin et al. [32]	1994	Animal study	OHAT	Low	
Young & Grinnell [22]	1994	Cohort study	SIGN	Medium-High	No control
Stricklin et al. [23]	1993	Cohort study	SIGN	Medium	Control not well defined

RoB = risk of bias; OHAT = Office of Health Assessment and Translation; SIGN = The Scottish Intercollegiate Guidelines Network.

reported in a further 11 studies (**Table 3**). The two studies of Stricklin and colleagues measured the inhibitor alone in porcine and human skin [23, 32]. The levels of MMP-9 and TIMP-1 were measured in burned skin samples in seven studies [3, 17, 18, 21, 25, 26, 31], while Hastbacka *et al.*, Reiss *et al.*, and Young and Grinnell analyzed burn blister fluids [12, 18, 22]. Circulating levels of MMP-9 and TIMP-1 in serum or plasma were measured in ten studies [5, 12, 15, 17, 19, 20, 22, 27, 28, 30] (**Table 3**). MMP-9 expression was analyzed in lung tissue by Wiggins-Dohlvik and colleagues [10], in Achilles tendon by Hernandez and colleagues [11], and in skeletal muscle by Brightwell and group [24], who also reported TIMP-1 expression. Brain expression of MMP-9 was a primary outcome for one case-control study [16] and three rat studies [6, 29, 30]. Measurements were taken at multiple time-points in 17 out of 24 studies ranging from 30 minutes after injury to 6 months post-burn (**Table 3**). Outcome measures were (i) mRNA (gene expression) using microarray analysis, quantitative reverse transcriptase polymerase chain reaction (RT-qPCR), or in situ hybridization (ISH); (ii) protein measured using immunohistochemistry (IHC), western blot (WB), or enzyme linked immunosorbent assay (ELISA); or (iii) MMP-9 activity using gelatin zymography or Sensolyte assay.

Local expression and activity of MMP-9 and TIMP-1 after burn injury

Nine studies included in this systematic review reported local gene expression or protein levels of MMP-9 in burned skin samples [3, 17, 18, 21, 25, 26, 31] and/or blister fluids [12, 18, 22] (**Table 4**). All studies reported significant increases in MMP-9 mRNA or protein compared to unburned controls, except Ulrich *et al.* who showed no difference more than four months post-injury [17]. MMP-9 was detectable in blister fluids on the day of injury [12, 22], with significantly elevated levels in skin and blisters ranging from 6 hours to 15 days post-burn depending on the study [3, 21, 22, 25, 31]. Increased MMP-9 was reported in both pediatric and adult animals and patients, however Simonetti and colleagues demonstrated significantly higher MMP-9-positive epithelial cells in wounded old skin compared with wounded

Table 3. Study samples, measurements and timepoints

Authors	Year	Parameter	Sample	Timepoints	Detection Method
Brightwell et al. [24]	2020	MMP-9 + TIMP-1	Skeletal muscle	7, 14, 21 days	Protein (WB)
Hernandez et al. [11]	2018	MMP-9	Tendon	1, 3, 7, 14 days	Protein (WB)
Yu et al. [3]	2017	MMP-9 + TIMP-1	Skin	2, 4, 6, 8 weeks	Protein (IHC)
Nong et al. [15]	2016	MMP-9 + TIMP-1	Serum	Within 96 hours after injury	Gene expression (Microarray analysis)
Wiggins-Dohlvik et al. [10]	2016	MMP-9	Lung	3 hours after injury	Protein (WB) Activity (Sensolyte assay)
Hastbacka et al. [12]	2015	MMP-9 + TIMP-1	Blister fluid + plasma	On admission; every 3 hours to 24 hours; day 1, 2, 3, 7, 14, 21	Protein (ELISA)
Nagy et al. [5]	2015	MMP-9 + TIMP-1	Plasma	From admission to day 6	Protein (ELISA)
Kubo et al. [25]	2014	MMP-9	Skin	3, 6, 9, 12 hours; day 1, 2, 3, 5, 7, 14	Protein (IHC)
Simonetti et al. [26]	2013	MMP-9	Skin	7, 14, 21 days	Protein (IHC)
Stagg et al. [27]	2013	MMP-9	Serum	3 hours after injury	Protein (WB) Activity (zymography)
Wang et al. [16]	2013	MMP-9	Brain	Within 48 hours post-mortem (21 hours median)	Gene expression (RT-qPCR)
Sio et al. [28]	2010	MMP-9	Plasma	8 hours after injury	Protein (ELISA + WB + IHC)
Ulrich et al. [17]	2010	MMP-9 + TIMP-1	Skin + serum	>4 months after injury	Gene expression (RT-qPCR) Protein (ELISA)
Reiss et al. [18]	2009	MMP-9 + TIMP-1	Blister fluid + skin	5-32 days after injury	Protein (WB) Activity (zymography)
Reyes et al. [29]	2009	MMP-9	Brain	3, 7, 24 hours after injury	Protein (WB)
Berger et al. [6]	2007	MMP-9	Brain	3, 7 hours after injury	Gene expression (RT-qPCR)
Swann et al. [30]	2007	MMP-9	Brain + serum	7, 24, 72 hours after injury	Gene expression (RT-qPCR) Protein (ELISA) Activity (zymography)
Dasu et al. [19]	2003	MMP-9 + TIMP-1	Serum	30 min; 3, 7, 21 days	Protein (ELISA)
Ulrich et al. [20]	2003	MMP-9 + TIMP-1	Serum	Within 2 hours of injury; 1, 3, 7, 14 days; 1, 3, 6 months	Protein (ELISA)
Neely et al. [21]	1997	MMP-9 + TIMP-1	Skin	3-5 days after injury	Gene expression (RT-qPCR) Activity (zymography)
Schaffer et al. [31]	1997	MMP-9 + TIMP-1	Skin	5, 10, 15 days after injury	Gene expression (ISH)
Stricklin et al. [32]	1994	TIMP-1	Skin	6 days after injury	Gene expression (ISH)
Young & Grinnell [22]	1994	MMP-9	Blister fluid + plasma	4-8 hours after injury; then every 48-72 hours until day 8-13	Protein (IHC) Activity (zymography)
Stricklin et al. [23]	1993	TIMP-1	Skin	2-34 days after injury	Gene expression (ISH)

MMP-9 = matrix metalloproteinase-9;TIMP-1 = tissue inhibitor of metalloproteinase-1; WB = Western blot; IHC = immunohistochemistry; ELISA = enzyme-linked immunosorbent assay; RT-qPCR = quantitative reverse transcriptase polymerase chain reaction; ISH = in situ hybridization.

young skin in a rat model (60.6 ± 2.5% *vs* 30.0 ± 3.2%; $P<0.0001$) [26].

With regards to MMP-9 enzyme activity, Neely *et al.* reported a significant 20 to 30-fold increase in burned skin from pediatric patients when compared with unburned skin [21]. MMP-9 activity assays of blister fluids from human patients with both partial and full-thickness burns conducted by Reiss *et al.* [18] and Young and Grinnell [22] showed differences related to the severity of injury, and between non-healing wounds and healing or epithelializing wounds (**Table 4**). MMP-9 activity was increased in full-thickness and non-healing burns, whereas active MMP-9 was reduced or absent from less severe and healed wounds [18, 22].

TIMP-1 expression at the site of injury increased later than MMP-9 in both animals and humans, with weak or irregularly distributed TIMP-1 on days 2 to 3 post-burn [3, 23] before significant increases from about day 5 to 14 [3, 21, 32]. Schaffer *et al.* [31] and Stricklin *et al.* [23] both reported a decrease in skin TIMP-1 levels as burn wound repair progressed (**Table 4**). TIMP-1 expression at a later stage of wound healing (>4 months post-burn) was shown by Ulrich and colleagues [17] to be significantly increased in extended hypertrophic burn scars. Analysis of burn blister fluids showed detect-

Table 4. Local expression and activity of MMP-9 and TIMP-1 following burn injury

Authors	Year	Parameter	Major Findings
Yu et al. [3]	2017	MMP-9 + TIMP-1	• MMP-9 low on day 3, from day 7 to day 14 (P<0.05 *vs* control) • TIMP-1 irregularly distributed on day 3, from day 10 to 14 (P<0.001 *vs* control)
Hastbacka et al. [12]	2015	MMP-9 + TIMP-1	• MMP-9 (53.6 ng/ml) and TIMP-1 (81.7 ng/ml) detectable in burn blister fluids on burn center admission
Kubo et al. [25]	2014	MMP-9	• MMP-9 significantly from 6 hours to 14 days post-injury
Simonetti et al. [26]	2013	MMP-9	• MMP-9 in wounded skin *vs* unwounded skin in both young and old animals (P<0.0001)
Ulrich et al. [17]	2010	MMP-9 + TIMP-1	• No difference in MMP-9 expression in burn scar tissue *vs* controls • TIMP-1 in hypertrophic scars after burn (P<0.05)
Reiss et al. [18]	2009	MMP-9 + TIMP-1	• MMP-9 in injured tissue; not expressed in uninjured skin • Active MMP-9 high in full-thickness and non-healing wounds • Inactive MMP-9 higher in less severe and healed wounds • TIMP-1 only expressed in 1/20 patients
Neely et al. [21]	1997	MMP-9 + TIMP-1	• MMP-9 expression in burn samples • MMP-9 activity in burn *vs* unburned skin (20-30-fold; P<0.05) • TIMP-1 mRNA expressed in burn and normal samples, with expression in burn wounds
Schaffer et al. [31]	1997	MMP-9 + TIMP-1	• MMP-9 present in dermis and epidermis 5-15 days post-injury • TIMP-1 levels initially, as wound repair progresses
Stricklin et al. [32]	1994	TIMP-1	• TIMP-1 detected throughout wound 6 days post-burn; not present in uninjured skin
Young & Grinnell [22]	1994	MMP-9	• ProMMP-9 & MMP-9 complex observed in burn fluid on day 0 • Higher MMP-9 detected day 2, to a peak on day 4, then • Activated MMP-9 in non-epithelializing burn wounds and in epithelializing burn wounds
Stricklin et al. [23]	1993	TIMP-1	• TIMP-1 highly expressed throughout wound but not in necrotic or nonwounded areas • Weak TIMP-1 signals at day 2 and from 22-34 days post-injury

MMP-9 = matrix metalloproteinase-9; TIMP-1 = tissue inhibitor of metalloproteinase-1.

able levels of TIMP-1 protein on hospital admission in the Hastbacka *et al.* study [12], which contrasted with Reiss *et al.*'s findings of TIMP-1 in only one of 20 patients in samples collected 5 to 32 days after injury [18].

Systemic expression of MMP-9 and TIMP-1 after burn injury

Early increases in circulating levels of MMP-9 on the day of burn injury were reported by Hastbacka *et al.* [12], Nagy *et al.* [5], and Stagg *et al.* [27] (**Table 5**). The temporal expression and/or activity of serum or plasma MMP-9 differed across studies with Swann *et al.* [30] and Hastbacka *et al.* [12] showing a fall to control levels by 7 to 12 hours and 12 to 24 hours respectively. Nagy *et al.* [5] also reported decreasing levels after admission, including significantly lower MMP-9 on day 4 to 6 compared to unburned controls. In contrast, Ulrich and colleagues showed increased serum MMP-9 between day 3 and 14 post-burn [20], while systemic MMP-9 remained unchanged in a study of pediatric burn patients until day 21 [19].

All studies that reported circulating levels of TIMP-1 showed significant increases compared to pre-burn levels and/or unburned controls [5, 12, 15, 17, 19, 20] (**Table 5**). Increases were reported as early as day 1 post-burn, with higher levels maintained up to 6 months after injury [20]. Similar to findings in skin samples, serum TIMP-1 >4 months post-burn was significantly increased in patients with extended hypertrophic burn scars [17]. Hastbacka *et al.* reported significantly higher plasma levels of TIMP-1 in patients with >20% TBSA burns compared with burns covering <20% TBSA (P<0.002) [12] (**Table 5**).

Peripheral expression and activity of MMP-9 and TIMP-1 after burn injury

Two rodent studies investigated MMP-9 and TIMP-1 expression in the musculoskeletal system after 30-40% TBSA burn injury [11, 24] (**Table 6**). Brightwell *et al.* showed no change in MMP-9 levels in skeletal muscle dorsal to the burn site, but significantly increased TIMP-1 on days 7 and 14 post-burn [24]. In contrast, Hernandez and colleagues reported two significant increases in MMP-9 in Achilles tendon after burn; the first on day 3, followed by a >20-fold increase on day 14 [11]. In another rodent study, Wiggins-Dohlvik *et al.* found a non-significant elevation in MMP-9 protein together with a significant 40% increase in MMP-9 activity

Table 5. Systemic expression of MMP-9 and TIMP-1 following burn injury

Authors	Year	Parameter	Major Findings
Nong et al. [15]	2016	MMP-9 + TIMP-1	● MMP-9 and TIMP-1 upregulated *vs* non-burn controls (P<0.05)
Hastbacka et al. [12]	2015	MMP-9 + TIMP-1	● MMP-9 early (P = 0.016 *vs* controls), to control values by 12-24 hours ● TIMP-1 *vs* controls (P<0.001) and higher in >20% *vs* <20% TBSA (P<0.002) ● TIMP-1 later than MMP-9 (12 hours) and remained high in >20% TBSA burn patients
Nagy et al. [5]	2015	MMP-9 + TIMP-1	● MMP-9 elevated on admission (P<0.001) and thereafter ● MMP-9 lower on days 4-6 *vs* non-burned controls (P<0.05) ● TIMP-1 at day 2 (P<0.001) and elevated to day 5 ● MMP-9/TIMP-1 ratio lower on days 3-5 *vs* controls (P<0.01)
Stagg et al. [27]	2013	MMP-9	● MMP-9 present in burn but not sham serum 3 hours after injury ● MMP-9 activity 3 hours after burn (P<0.01)
Ulrich et al. [17]	2010	MMP-9 + TIMP-1	● No difference in serum MMP-9 in patients with extended hypertrophic scars *vs* controls ● TIMP-1 in patients with extended hypertrophic scars *vs* control patients (P<0.05)
Swann et al. [30]	2007	MMP-9	● No difference in serum MMP-9 *vs* controls at 7 hours (P>0.05) ● MMP-9 activity at 7 hours (P<0.05), returning to control levels between 7 and 12 hours
Dasu et al. [19]	2003	MMP-9 + TIMP-1	● MMP-9 levels at day 21 *vs* controls (P<0.05) ● TIMP-1 levels did not change with time after burn but were higher than controls from day 3 to day 21 (P<0.05)
Ulrich et al. [20]	2003	MMP-9 + TIMP-1	● MMP-9 between day 3 and day 14 *vs* non-burn controls (P<0.05) ● TIMP-1 at day 3 (P<0.05) and remained significantly elevated 6 months after injury ● TIMP-1 *vs* control group from day 1 to 6 months (P<0.05)
Young & Grinnell [22]	1994	MMP-9	● Little gelatinase activity detected in plasma

MMP-9 = matrix metalloproteinase-9; TIMP-1 = tissue inhibitor of metalloproteinase-1; TBSA = total body surface area.

Table 6. Peripheral expression and activity of MMP-9 and TIMP-1 following burn injury

Authors	Year	Tissue	Parameter	Major Findings
Brightwell et al. [24]	2020	Skeletal muscle	MMP-9 + TIMP-1	● No change in MMP-9 after burn ● TIMP-1 at day 7 (P = 0.048) and day 14 (P = 0.0001)
Hernandez et al. [11]	2018	Tendon	MMP-9	● MMP-9 after injury (P<0.05 *vs* control at day 3) ● Second at day 14 (>20-fold *vs* control; P<0.05)
Wiggins-Dohlvik et al. [10]	2016	Lung tissue	MMP-9	● MMP-9 protein higher in burn animals *vs* shams (P = 0.15) ● MMP-9 activity ~40% in burn animals (P = 0.02)
Wang et al. [16]	2013	Brain	MMP-9	● MMP-9 in deaths due to severe burns (P<0.05)
Reyes et al. [29]	2009	Brain	MMP-9	● MMP-9 expression and activity 7 hours post-burn (P<0.01)
Berger et al. [6]	2007	Brain	MMP-9	● MMP-9 3 hours after thermal injury (P<0.05) and remained high at 7 hours (P<0.05 *vs* unburned controls)
Swann et al. [30]	2007	Brain	MMP-9	● MMP-9 post-burn (P<0.01 at 3 hours and 7 hours), returning to control levels at 24 hours

MMP-9 = matrix metalloproteinase-9; TIMP-1 = tissue inhibitor of metalloproteinase-1.

within lung tissue, 3 hours after 30% TBSA burn injury [10].

MMP-9 expression in brain after severe full-thickness burns (60-70% TBSA) in Sprague-Dawley rats was described in three studies included in this systematic review [6, 29, 30] (**Table 6**). Brain MMP-9 mRNA was shown to be significantly increased at 3 and 7 hours after burn [6, 30], with a return to control levels by 24 hours [30]. Increased MMP-9 protein and activity were first reported at 7 hours post-injury [29]. A review of forensic autopsy cases also reported significantly elevated MMP-9 mRNA in brains of severe burns patients who died >30 minutes after injury [16].

Correlation between MMP-9 and TIMP-1 and burn injury severity

Of three cohort studies that assessed correlations between circulating MMP-9 and burn injury severity, only Nagy *et al.* showed a weak positive correlation between MMP-9 and TBSA [5, 12, 20] (**Table 7**). Reiss *et al.* reported a positive correlation between MMP-9 protein and activity in burned skin and burn depth [18]. No study found an association between MMP-9 and survival or other outcome measures (**Table 7**).

In direct contrast to MMP-9, circulating TIMP-1 was associated with survival [5, 12], with

Table 7. Correlation of MMP-9 and TIMP-1 with injury severity and outcomes

Authors	Year	Parameter	Major Findings
Hastbacka et al. [12]	2015	MMP-9 + TIMP-1	● MMP-9 at 24-48 hours did not correlate with TBSA or outcome ● Plasma TIMP-1 showed high positive correlation with TBSA ● TIMP-1 correlated with SOFA (r = 0.592, *P* = 0.002), fluid (r = 0.63, *P*<0.001) and noradrenaline (r = 0.753, *P*<0.001) ● TIMP-1 independently associated with mortality (*P* = 0.03) ● TIMP-1 at 24-48 hours predicted 90-day survival (AUC 0.846 [95% CI 0.603-0.989]; *P* = 0.002)
Nagy et al. [5]	2015	MMP-9 + TIMP-1	● Admission plasma MMP-9 showed weak correlation with burn extent (r = 0.437; *P* = 0.014) but no association with survival ● Plasma TIMP-1 did not correlate with extent of burns, but showed weak significant correlation with survival (Day 5: r = 0.432, *P* = 0.028; Day 6: r = 0.444, *P* = 0.014)
Reiss et al. [18]	2009	MMP-9 + TIMP-1	● Expression and activation of MMP-9 in injured tissue correlated with degree of injury and location relative to wound
Ulrich et al. [20]	2003	MMP-9 + TIMP-1	● No correlation between serum MMP-9 and TBSA ● Serum TIMP-1 on day 3 and day 7 correlated with TBSA (r = 0.46 and r = 0.53, respectively; *P*<0.05) ● Serum TIMP-1 at 6 months correlated with Burn Scar Index (r = 0.65, *P*<0.05)

MMP-9 = matrix metalloproteinase-9; TIMP-1 = tissue inhibitor of metalloproteinase-1; TBSA = total body surface area; SOFA = Sequential Organ Failure Assessment; AUC = area under the curve; CI = confidence interval. The Burn Scar index, often called the Vancouver Scar Scale, scores burn scars on four parameters: (1) pigmentation, (2) vascularity, (3) pliability, and (4) height.

Hastbacka and colleagues showing plasma levels at 24 to 48 hours predicted 90 day survival when controlling for age and injury severity [12] (**Table 7**). Plasma TIMP-1 also correlated with Sequential Organ Failure Assessment (SOFA), as well as noradrenaline and fluid volume administered [12]. One study reported a correlation between serum TIMP-1 and TBSA on days 3 and 7 after burn [20], but no correlation was found by Nagy *et al.* [5].

Discussion

MMP-9 and its endogenous inhibitor TIMP-1 are key regulators of inflammation and wound healing, that are are significantly upregulated both locally and systemically in burns patients. Given their pivotal role in the acute inflammatory response following burn injury, perturbations in the dynamic balance of expression and activity of MMP-9 and TIMP-1 might also contribute to secondary tissue damage and mortality. We conducted a systematic review of studies reporting local, systemic and peripheral MMP-9 and/or TIMP-1 expression after burn trauma and found a potential role for TIMP-1 as a prognostic blood biomarker. The main findings will now be discussed.

Increased MMP-9 at the site of injury and systemically

Increased MMP-9 was reported at the site of injury in all studies early after burn trauma, consistent with its known role in the early inflammatory phase of wound healing [3, 33] (**Table 4**). However in excess, MMP-9 can delay wound healing, with excessive MMP-9 activity in non-healing, non-epithelializing wounds a finding of two studies in this review [18, 22]. Critical to MMP-9 regulation is its endogenous inhibitor TIMP-1, whose expression is upregulated by fibroblasts ~2 to 3 days after burn, resulting in inactivation of MMP-9 and prevention of excessive ECM degradation [3]. Reduced TIMP-1 production has been associated with delayed wound healing in elderly patients [18], which is consistent with the higher levels of MMP-9 reported in wounded old skin compared with wounded young skin in animal models [26]. Conversely, excessive local production of TIMP-1 may increase collagen synthesis and deposition, leading to severe fibrosis, and pathologic scar formation as was found by Ulrich *et al.* [17]. Serum TIMP-1 was also significantly elevated in burns patients with hypertrophic scarring [17, 20], suggesting it may be a useful biomarker. Early identification of at-risk patients with elevated serum TIMP-1 may facilitate targeted treatment to reduce hypertrophic scarring, which requires multiple surgeries and causes significant disability.

Our analysis also found significant increases of MMP-9 in the circulation in multiple studies early after burn injury [5, 12, 15, 20, 27] (**Table 5**). This is consistent with early increases in proinflammatory cytokines after burns which stimulate MMP-9 synthesis [5, 12]. However, there was one outlier. The study of Dasu *et al.* found no systemic MMP-9 increase until day 21

after burn [19]. This may be an age-associated effect since this study included only pediatric patients (7.9 2.5 years) (**Table 1**) [19]. Differences may also be related to the extent and severity of burns. For example, earlier and higher levels of circulating MMP-9 were reported in the studies of Hastbacka *et al.* [12] and Nagy *et al.* [34] compared with Ulrich *et al.* [20], which are likely related to the severity of injury with TBSA >20%, 15-80%, and >5% for the three studies, respectively (**Table 1**). There is a positive correlation between the degree of the burn-induced inflammatory response and [35, 36], resulting in increased cytokine release in more severely injured patients, and therefore increased MMP-9. TIMP-1 synthesis is also stimulated by inflammatory cytokines [12], with multiple studies reviewed reporting elevated serum or plasma TIMP-1 after injury, and higher levels in more severely injured patients (**Table 5**) [5, 12, 19, 20]. Age-associated differences in MMP-9 and TIMP-1, as well as differences related to burn severity, may be important variables following burn trauma and need further investigation.

MMP-9/TIMP-1 influences inflammation and injury at distant sites

The present analysis found evidence of the MMP-9/TIMP-1 system contributing to inflammation and tissue injury distant from the burn site (**Table 6**). An example is burn-associated musculoskeletal damage including collagen breakdown in skeletal muscle and tendon remodeling [11, 24]. Two organs of importance are the lungs, the site of the most prevalent organ dysfunction after severe burns [37], and brain, given burn encephalopathy is highly correlated with mortality [29]. In the lungs, MMP-9 secreted by activated polymorphonuclear (PMN) leukocytes may be involved in mediating acute lung injury (ALI), which is also associated with mortality [13, 28]. Wiggins-Dohlvik *et al.* reported a non-significant elevation in MMP-9 protein in lung, but a significant 40% increase in activity [10]. In contrast to the soluble form, MMP-9 bound to PMN surfaces is resistant to TIMP-1 inhibition [13], such that the increased activity may be due to reduced inhibition rather than increased synthesis [10]. Furthermore, TIMP-1 may also be localized on the surface of activated PMNs, where it plays an opposite role by anchoring soluble MMP-9, and thereby promoting MMP-9 proteolytic activity rather than inhibiting it [38]. MMP-9 synthesis and regulation may therefore be tissue-specific, with further investigation regarding the modulation of the MMP-9-TIMP-1 system in different tissue types required to develop targeted treatments.

MMP-9 increases in the brain after burn injury

MMPs are critical for maintaining integrity of the blood-brain barrier (BBB) as well as neuronal network remodeling [1, 6]. All studies examining MMP-9 expression and activity in the brain showed significant increases following burn injury [6, 16, 29, 30], consistent with findings following other traumatic stimuli such as brain injury and stroke [39]. This increase may result from (i) neuronal expression in response to upregulated cytokines such as TNF-α and IL-1β in the brain early after peripheral burn injury [29, 30], or (ii) as a result of leukocyte infiltration, if the BBB is compromised [39]. MMP-9 itself digests the endothelial basal lamina of the BBB, leading to increased permeability and burn-associated cerebral edema [16, 29, 39]. Increased brain MMP-9 is unlikely to be caused by systemic expression given that in the study of Swann and colleagues, brain expression increased by 3 hours after injury, while serum MMP-9 levels did not [30]. Further understanding of the underlying mechanisms of MMP-9 synthesis and regulation in the brain after burns is vital for the development of therapeutic interventions to reduce or correct BBB dysfunction and cerebral complications that are highly correlated with mortality [6, 16, 29].

TIMP-1 as a prognostic marker of burn severity and outcome

Changes in plasma levels of MMP-9 and TIMP-1 are increasingly being evaluated as prognostic biomarkers in critically ill patients. While lower levels of circulating MMP-9 have been associated with improved outcomes including survival in sepsis [12], this review found no evidence of a correlation between serum or plasma MMP-9 and extent of burn injury or outcome measures (**Table 7**) [5, 12, 20]. Circulating TIMP-1, on the other hand, showed good correlation with TBSA [12, 20], survival [5, 12], and other surrogate markers of burn injury severity and outcome [12, 20], and may be a promising biomarker in outcome prognostication of burns patients.

Limitations

These conclusions are limited by the heterogeneity of the reviewed studies which included burns covering 1 to 80% TBSA and a mix of superficial, partial and full-thickness burns (**Table 1**). In addition, the studies were all monocentric and ~40% had sample sizes less than 20 subjects, including Young and Grinnell which reported on only three patients [22]. Comparisons are also limited by the range of sampling times and time between measurements, which has a strong influence on results as shown by the temporal changes in expression reported in different studies (**Tables 3**-**5**). Furthermore, burns injury is often complicated by sepsis, which has also been shown to upregulate MMPs [40]. It was not possible to assess the contribution of sepsis as a confounding variable since Hastbacka *et al.* was the only study to report suspected or verified sepsis in their patient cohort [12].

Another important consideration when evaluating these findings is the detection method used to quantitate levels of MMP-9 and TIMP-1. Measurement is complicated by the fact that MMPs exist in three forms, as inactive pro-MMPs, active MMPs, and TIMP-complexed MMPs [8]. As shown in **Table 3**, different studies included in this systematic review employed different detection methods all of which have their own limitations. Seven studies reported gene expression only [6, 15, 16, 23, 30-32], and while there is normally reasonable correlation between mRNA and protein levels [41], mRNA cannot measure the activity of proteinases. Similarly the use of Western blotting, immunohistochemistry, or ELISAs to detect MMP-9 protein in circulation or tissue does not reflect its biological activity. The antibodies used in these assays may immunoreact with pro-MMP-9, active MMP-9, and TIMP-complexed MMP-9, as well as other degradation products [4, 8]. Gelatin substrate zymography analysis which is the most common technique to measure gelatinase activity, and was reported in four studies [18, 21, 22, 27], also detects some inactive MMP proforms reducing its sensitivity and reliability [8].

Future research

The results of this systematic review suggest a promising role for TIMP-1 as a prognostic marker in burns patients. Large-scale multi-center prospective cohort studies, in both pediatric and adult patients, including equal male-female representation, with repeated sampling of blood and burn wounds from the time of injury until 6 to 12 months, would help validate the reliability of TIMP-1 as a prognostic outcome marker. In addition, the following knowledge gaps require further research: (1) Circulating MMP-9 and MMP-9: TIMP-1 ratio as predictors of morbidity including delayed wound healing in burns patients. A blood-based biomarker would be beneficial for risk stratification and personalized treatment. (2) Age-specific pathophysiological differences in MMP-9 and TIMP-1 expression following burn injury. Impaired wound healing in aged burn patients remains a therapeutic challenge [42] that will continue to grow with an aging population. Improved understanding of MMP-9/TIMP-1 system perturbations in age-associated wound healing differences may identify novel therapeutic strategies. This review also revealed a potential difference in the MMP-9 systemic response between pediatric and adult patients which requires further investigation. (3) Sex-specific pathophysiological differences in tissue and circulating MMP-9 and TIMP-1 expression following burn injury. Female patients have worse outcomes following burn injury compared to their male counterparts, though the mechanisms underlying this discrepancy are currently unknown [43]. No study has assessed sex differences in MMP-9/TIMP-1 expression following burn injury, with all preclinical studies using only male animals, and males also comprising the majority of the clinical patient cohorts (**Table 1**). Future preclinical studies should include and separately analyze male and female animals, with greater female representation also required in clinical studies. (4) Correlation between circulating MMP-9 and TIMP-1 and early development of acute lung injury and cerebral complications in burns patients to optimize delivery of care and improve outcomes.

Conclusion

TIMP-1 may be a useful serum biomarker in major burns patients. Further evaluation of the MMP-9-TIMP-1 system might aid in the development of improved prognostic guidelines and targeted therapeutic strategies for wound healing, as well as correction of hyperpermeability

in the lungs and brain following major burn injury.

Acknowledgements

The authors would like to acknowledge the College of Medicine and Dentistry, James Cook University, for their support of this work.

Disclosure of conflict of interest

None.

Address correspondence to: Hayley L Letson, Heart, Trauma and Sepsis Research Laboratory, College of Medicine and Dentistry, James Cook University, 1 James Cook Drive, Townsville, Queensland 4811, Australia. Tel: 61-7-47 81 5042; E-mail: hayley.letson@jcu.edu.au

References

[1] Rempe RG, Hartz AMS and Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab 2016; 36: 1481-1507.

[2] Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. J Pathol 2003; 200: 448-464.

[3] Yu G, Li Y, Ye L, Wang X, Zhang J, Dong Z and Jiang D. Exogenous peripheral blood mononuclear cells affect the healing process of deep degree burns. Mol Med Rep 2017; 16: 8110-8122.

[4] Vandooren J, Van den Steen PE and Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. Crit Rev Biochem Mol Biol 2013; 48: 222-272.

[5] Nagy B, Szelig L, Rendeki S, Loibl C, Rezman B, Lantos J, Bogar L and Csontos C. Dynamic changes of matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 after burn injury. J Crit Care 2015; 30: 162-166.

[6] Berger J, Sprague SM, Wu Y, Davis WW, Jimenez DF, Barone CM and Ding Y. Peripheral thermal injury causes early blood-brain barrier dysfunction and matrix metalloproteinase expression in rat. Neurol Res 2007; 29: 610-614.

[7] Grunwald B, Schoeps B and Kruger A. Recognizing the molecular multifunctionality and Interactome of TIMP-1. Trends Cell Biol 2019; 29: 6-19.

[8] Nguyen TT, Mobashery S and Chang M. Roles of matrix metalloproteinases in cutaneous wound healing. Wound healing - new insights into ancient challenges. 2016. pp. 2874.

[9] Nagase H, Visse R and Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006; 69: 562-573.

[10] Wiggins-Dohlvik K, Oakley RP, Han MS, Stagg HW, Alluri H, Shaji CA, Davis ML and Tharakan B. Tissue inhibitor of metalloproteinase-2 inhibits burn-induced derangements and hyperpermeability in microvascular endothelial cells. Am J Surg 2016; 211: 197-205.

[11] Hernandez P, Buller D, Mitchell T, Wright J, Liang H, Manchanda K, Welch T, Huebinger RM, Carlson DL, Wolf SE and Song J. Severe burn-induced inflammation and remodeling of Achilles tendon in a rat model. Shock 2018; 50: 346-350.

[12] Hastbacka J, Freden F, Hult M, Bergquist M, Wilkman E, Vuola J, Sorsa T, Tervahartiala T and Huss F. Matrix metalloproteinases -8 and -9 and tissue inhibitor of metalloproteinase-1 in burn patients. A prospective observational study. PLoS One 2015; 10: e0125918.

[13] Owen CA, Hu Z, Barrick B and Shapiro SD. Inducible expression of tissue inhibitor of metalloproteinases-resistant matrix metalloproteinase-9 on the cell surface of neutrophils. Am J Respir Cell Mol Biol 2003; 29: 283-294.

[14] Moher D, Liberati A, Tetzlaff J and Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009; 151: 264-269.

[15] Nong Q, Li S and Liu D. A comprehensive bioinformatics method to screen key genes for severe burn. Eur J Inflamm 2015; 14: 18-26.

[16] Wang Q, Ishikawa T, Michiue T, Zhu BL, Guan DW and Maeda H. Molecular pathology of brain edema after severe burns in forensic autopsy cases with special regard to the importance of reference gene selection. Int J Legal Med 2013; 127: 881-889.

[17] Ulrich D, Ulrich F, Unglaub F, Piatkowski A and Pallua N. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with different types of scars and keloids. J Plast Reconstr Aesthet Surg 2010; 63: 1015-1021.

[18] Reiss MJ, Han YP and Garner WL. Alpha1-antichymotrypsin activity correlates with and may modulate matrix metalloproteinase-9 in human acute wounds. Wound Repair Regen 2009; 17: 418-426.

[19] Dasu MR, Spies M, Barrow RE and Herndon DN. Matrix metalloproteinases and their tissue inhibitors in severely burned children. Wound Repair Regen 2003; 11: 177-180.

[20] Ulrich D, Noah EM, von Heimburg D and Pallua N. TIMP-1, MMP-2, MMP-9, and PIIINP as serum markers for skin fibrosis in patients following severe burn trauma. Plast Reconstr Surg 2003; 111: 1423-1431.

[21] Neely AN, Brown RL, Clendening CE, Orloff MM, Gardner J and Greenhalgh DG. Proteolytic activity in human burn wounds. Wound Repair Regen 1997; 5: 302-309.
[22] Young PK and Grinnell F. Metalloproteinase activation cascade after burn injury: a longitudinal analysis of the human wound environment. J Invest Dermatol 1994; 103: 660-664.
[23] Stricklin GP, Li L, Jancic V, Wenczak BA and Nanney LB. Localization of mRNAs representing collagenase and TIMP in sections of healing human burn wounds. Am J Pathol 1993; 143: 1657-1666.
[24] Brightwell CR, Hanson ME, El Ayadi A, Prasai A, Wang Y, Finnerty CC and Fry CS. Thermal injury initiates pervasive fibrogenesis in skeletal muscle. Am J Physiol Cell Physiol 2020; 319: C277-C287.
[25] Kubo H, Hayashi T, Ago K, Ago M, Kanekura T and Ogata M. Temporal expression of wound healing-related genes in skin burn injury. Leg Med (Tokyo) 2014; 16: 8-13.
[26] Simonetti O, Lucarini G, Cirioni O, Zizzi A, Orlando F, Provinciali M, Di Primio R, Giacometti A and Offidani A. Delayed wound healing in aged skin rat models after thermal injury is associated with an increased MMP-9, K6 and CD44 expression. Burns 2013; 39: 776-787.
[27] Stagg HW, Whaley JG, Tharakan B, Hunter FA, Jupiter D, Little DC, Davis ML, Smythe WR and Childs EW. Doxycycline attenuates burn-induced microvascular hyperpermeability. J Trauma Acute Care Surg 2013; 75: 1040-1046.
[28] Sio SW, Moochhala S, Lu J and Bhatia M. Early protection from burn-induced acute lung injury by deletion of preprotachykinin-A gene. Am J Respir Crit Care Med 2010; 181: 36-46.
[29] Reyes R, Guo M, Swann K, Shetgeri SU, Sprague SM, Jimenez DF, Barone CM and Ding Y. Role of tumor necrosis factor-alpha and matrix metalloproteinase-9 in blood-brain barrier disruption after peripheral thermal injury in rats. J Neurosurg 2009; 110: 1218-1226.
[30] Swann K, Berger J, Sprague SM, Wu Y, Lai Q, Jimenez DF, Barone CM and Ding Y. Peripheral thermal injury causes blood-brain barrier dysfunction and matrix metalloproteinase (MMP) expression in rat. Brain Res 2007; 1129: 26-33.
[31] Schaffer CJ, Reinisch L, Polis SL and Stricklin GP. Comparisons of wound healing among excisional, laser-created, and standard thermal burns in porcine wounds of equal depth. Wound Repair Regen 1997; 5: 52-61.
[32] Stricklin GP, Li L and Nanney LB. Localization of mRNAs representing inertstitial collagenase, 72-kDa gelatinase, and TIMP in healing porcine burn wounds. J Invest Dermatol 1994; 103: 352-358.
[33] Kyriakides TR, Wulsin D, Skokos EA, Fleckman P, Pirrone A, Shipley JM, Senior RM and Bornstein P. Mice that lack matrix metalloproteinase-9 display delayed wound healing associated with delayed reepithelization and disordered collagen fibrillogenesis. Matrix Biol 2009; 28: 65-73.
[34] Czobel M, Kaszaki J, Molnar G, Nagy S and Boros M. Nonspecific inhibition of nitric oxide synthesis evokes endothelin-dependent increases in myocardial contractility. Nitric Oxide 2009; 21: 201-209.
[35] Barber RC, Maass DL, White DJ and Horton JW. Increasing percent burn is correlated with increasing inflammation in an adult rodent model. Shock 2008; 30: 388-393.
[36] Abdel-Hafez NM, Saleh Hassan Y and El-Metwally TH. A study on biomarkers, cytokines, and growth factors in children with burn injuries. Ann Burns Fire Disasters 2007; 20: 89-100.
[37] Kraft R, Herndon DN, Finnerty CC, Shahrokhi S and Jeschke MG. Occurrence of multiorgan dysfunction in pediatric burn patients: incidence and clinical outcome. Ann Surg 2014; 259: 381-387.
[38] Wang X, Rojas-Quintero J, Wilder J, Tesfaigzi Y, Zhang D and Owen CA. Tissue inhibitor of metalloproteinase-1 promotes polymorphonuclear neutrophil (PMN) pericellular proteolysis by anchoring matrix metalloproteinase-8 and -9 to PMN Surfaces. J Immunol 2019; 202: 3267-3281.
[39] Vafadari B, Salamian A and Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem 2016; 139 Suppl 2: 91-114.
[40] Bergquist M, Hastbacka J, Glaumann C, Freden F, Huss F and Lipcsey M. The time-course of the inflammatory response to major burn injury and its relation to organ failure and outcome. Burns 2019; 45: 354-363.
[41] Buccitelli C and Selbach M. mRNAs, proteins and the emerging principles of gene expression control. Nat Rev Genetics 2020; 21: 630-644.
[42] Rani M and Schwacha MG. Aging and the pathogenic response to burn. Aging Dis 2012; 3: 171-180.
[43] Knowlin L, Cairns BA and Charles AG. Sex differences in burn injury mortality: a propensity score analysis. J Am Coll Surg 2017; 225: e186-e187.