BJR

■ SYSTEMATIC REVIEW

Inflammatory response in postmenopausal osteoporotic fracture healing

S. K-H. Chow,
Y-N. Chim,
J-Y. Wang,
R. M-Y. Wong,
V. M-H. Choy,
W-H. Cheung

From Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, Hong Kong

A balanced inflammatory response is important for successful fracture healing. The response of osteoporotic fracture healing is deranged and an altered inflammatory response can be one underlying cause. The objectives of this review were to compare the inflammatory responses between normal and osteoporotic fractures and to examine the potential effects on different healing outcomes. A systematic literature search was conducted with relevant keywords in PubMed, Embase, and Web of Science independently. Original preclinical studies and clinical studies involving the investigation of inflammatory response in fracture healing in ovariectomized (OVX) animals or osteoporotic/elderly patients with available full text and written in English were included. In total, 14 articles were selected. Various inflammatory factors were reported; of those tumour necrosis factor-α (TNF-α) and interleukin (IL)-6 are two commonly studied markers. Preclinical studies showed that OVX animals generally demonstrated higher systemic inflammatory response and poorer healing outcomes compared to normal controls (SHAM). However, it is inconclusive if the local inflammatory response is higher or lower in OVX animals. As for clinical studies, they mainly examine the temporal changes of the inflammatory stage or perform comparison between osteoporotic/fragility fracture patients and normal subjects without fracture. Our review of these studies emphasizes the lack of understanding that inflammation plays in the altered fracture healing response of osteoporotic/elderly patients. Taken together, it is clear that additional studies, preclinical and clinical, are required to dissect the regulatory role of inflammatory response in osteoporotic fracture healing.

Article focus
- The early inflammatory response in osteoporotic/aged fracture healing is poorly understood.
- This systematic review aimed to analyze the difference in inflammatory responses in normal and osteoporotic fractures.

Key messages
- The inflammatory response in osteoporotic/aged fracture healing is altered and this could imply poorer healing in ovariectomized (OVX) subjects or post-menopausal osteoporotic or aged individuals with fragility fracture.
- Further studies should be performed to explore the role of inflammatory response in the healing cascade for potential

immunomodulation to enhance osteoporotic fracture healing.

Strengths and limitations
- This study systematically reviewed both the local and systemic inflammatory responses in ovariectomy-induced osteoporotic fractures or fragility fractures and the potential effects of inflammatory response on differential healing outcomes.
- There is no clinical study investigating the difference in the inflammatory response between osteoporotic and non-osteoporotic patients.

Introduction
The process of fracture healing is highly complex. The success of fracture healing

Correspondence should be sent to Simon K-H Chow; email: skhchow@cuhk.edu.hk

relies on the well-coordinated stages of inflammation, callus formation, and remodelling. When a bone fracture occurs, an inflammatory (innate immune) response immediately occurs to trigger and coordinate all the subsequent healing processes such as recruitment of reparative mesenchymal stem cells (MSCs),[1,2] neoangiogenesis at the callus,[3,4] endochondral ossification,[5] and callus remodelling.[6,7] Different immune cells infiltrate the fracture site and multiple inflammatory factors are secreted. This intense inflammatory interaction has been shown to initiate the repair phase[8] and the cytokines, signalling molecules, receptors, and transcription factors involved enabling a wide range of dynamic cross-talk between cells of monocyte-macrophage-osteoclast and MSC-osteoblast lineages, which are essential to bone remodelling.[9,10] Thus, the inflammatory stage is important to ensure normal fracture healing through angiogenesis, repair of injured tissues, and eventually remodelling.[11,12] Any disruption of this inflammatory response could result in impaired or delayed fracture healing.[13-16]

Previous studies reported that ageing could affect the inflammatory response during fracture healing in terms of ageing of the immune response known as immunosenescence[17,18] and increased levels of circulating proinflammatory cytokines.[19] Some important cells in fracture healing such as macrophages,[20,21] T cells,[22] and MSCs[23,24] have all been demonstrated to undergo intrinsic age-related changes that could have a negative impact on fracture healing. In addition, bone healing in aged individuals has a higher risk of failure, resulting in an increased incidence of fracture nonunions.[25]

Postmenopausal osteoporosis has been considered as a chronic inflammatory disease.[26] Postmenopausal osteoporotic women have been shown to exhibit loss of bone-protective role of oestrogen and increased levels of proinflammatory cytokines.[27-30] Oestrogen has been shown to have both immunosuppressive and proinflammatory effects.[31,32] Tumour necrosis factor-α (TNF-α) and interleukin (IL)-6 are both mediated by the level of oestrogen indirectly through Jun or nuclear factor kappa B (NF-κB) pathways, respectively.[27] Moreover, oestrogen was reported to modulate inflammatory responses through oestrogen receptors (ERs), and ERs were found to demonstrate different expression patterns in osteoporotic fractures.[7] Following fracture injury in wild-type rats, ER expression increased during the earliest stage of repair. This response was abrogated in ovariectomized (OVX) animals, suggesting that oestrogen may function in coordination with inflammation for the altered repair response in these animals. The local inflammatory response to injury was lowered in OVX animal models mimicking oestrogen decline after menopause,[33] while there are other studies demonstrating that oestrogen deficiency increased the early inflammatory response after injury as shown by increased numbers of neutrophils and expression of proinflammatory cytokines, midkine, and IL-6 in the fracture callus in oestrogen-deficient mice.[34,35] Taken together, these studies provide evidence that oestrogen deficiency, such as in post-menopausal women, alters the inflammatory response following fracture injury, but the mechanisms of this response are still vaguely understood.

Despite some evidence on the altered inflammatory response observed in postmenopausal osteoporotic patients or OVX animals after injury, still little is known about the early inflammatory response in fragility (osteoporotic) fracture healing, which could be a potential target stage for accelerating osteoporotic fractures accompanied usually with delayed healing or even nonunions. The purpose of this systematic review was to analyze the difference in inflammatory responses in normal and osteoporotic fractures and explore the possible influence of inflammatory response on healing outcomes. This could serve as a reference for the planning of potential inflammation-related therapeutic approaches for osteoporotic fracture patients.

Methods

Search strategy. Literature search was performed on PubMed, Embase, and Web of Science (last access date on 8 January 2020). The keywords included were "osteoporo*", "inflammat*", and "fracture*". We combined the keywords as "osteoporo* AND inflammat* AND fracture*" and searched in all fields. This search strategy was used in the three databases.

Search criteria. Inclusion criteria were: 1) preclinical or clinical studies that involved osteoporotic or fragility fracture and assessments examining the inflammatory response after fracture; 2) involves the comparison between the inflammatory response in OVX-induced osteoporotic and normal control (SHAM) subjects in preclinical studies; 3) involves the examination of inflammatory response in osteoporotic or fragility fracture in clinical studies; and 4) literature published in English with full text.

Exclusion criteria were: 1) non-English papers; 2) non-fracture-related; 3) non-inflammatory response-related; 4) involves other diseases; 5) review articles; 6) reports published as conference abstracts; and 7) in vitro studies.

Selection of studies. Study selection was conducted by two reviewers (YNC and VMHC) independently. Primary screening of titles and abstracts was performed to exclude obviously irrelevant papers. Potentially relevant articles were then retrieved and reviewed according to the inclusion and exclusion criteria. Disagreements were resolved by discussion and consensus.

Data extraction. The following information was extracted by reviewers: preclinical studies including methodology, fracture models used, sample size, species, fracture location, fracture type, fixation type, inflammatory factor findings, and fracture healing outcomes; and clinical studies including subject age, sample size, fracture type, fracture site, inflammatory factor findings, and fracture healing outcomes.
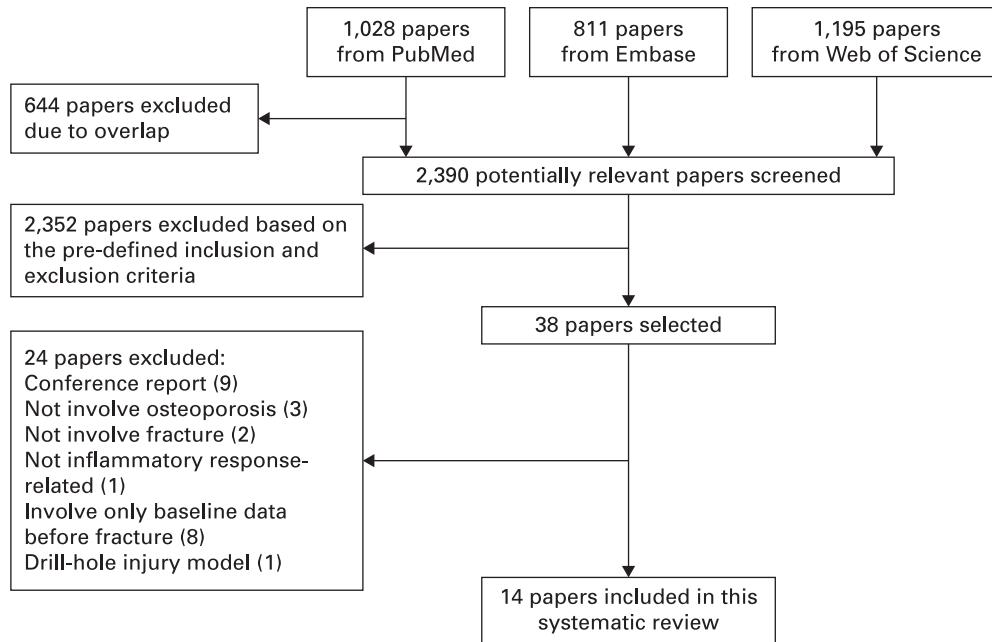
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                    │ from PubMed │  │ from Embase │  │ from Web of     │
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   │ 24 papers        │                     │
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   │ (9)              │                     │
   │ Not involve      │                     ▼
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   │ fracture (2)     │    │ this systematic review   │
   │ Not inflammatory │    └──────────────────────────┘
   │ response-related │
   │ (1)              │
   │ Involve only     │
   │ baseline data    │
   │ before fracture  │
   │ (8)              │
   │ Drill-hole injury│
   │ model (1)        │
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Fig. 1

Flow diagram showing the literature search process.

Statistical analysis. Both clinical and preclinical studies were included in this review and there was variability in terms of human subjects, animal species, and methodological and statistical heterogeneity. Outcome measures of human subjects and animal species were not comparable. Thus, only qualitative systematic review was performed.

Results

Results of the search. A total of 1,028, 811, and 1,195 studies were identified from PubMed, Embase, and Web of Science, respectively. After reviewing titles and abstracts of the 3,034 studies, 644 studies were excluded due to overlap and 2,352 studies were excluded based on the selection criteria, leaving 38 studies for further examination. After further screening, 24 more studies were excluded, out of which nine were conference reports, three did not involve osteoporosis, two did not involve fracture, and one was not inflammatory response-related. Eight studies only involved baseline data taken before fracture occurred but not data after fracture. One used a drill-hole injury model. Hence, in total, 14 studies were finally analyzed in this systematic review. The literature screening process is illustrated in the flow diagram in Figure 1.

Characteristics of the papers. The 14 studies were published between 2004 and 2019. Six of these studies were preclinical studies and seven were clinical studies. One paper included both preclinical and clinical studies.

The seven (including the one with preclinical and clinical studies) preclinical studies included four mouse studies and three rat studies. All these studies used female animals, which received OVX surgery for induction of osteoporosis. Fractures were created in the animals. Two studies performed open osteotomy with external fixator, two

studies performed open osteotomy with intramedullary pinning, and three used closed fracture with intramedullary pinning. Four studies involved fracture on the femur of the animals and three used tibia fracture model (Table I).

As for the clinical studies, all included subjects with fragility fracture, of which only two studies involved patients confirmed with osteoporosis by dual energy x-ray absorptiometry (DXA). Three included patients suffering from hip fracture and one involved patients having subcapital fracture at the intertrochanteric region of the proximal femur. One included distal radius fracture, one included long-bone fracture, and one involved femoral neck fracture. One study did not specify the fractured region of patients (Table I).

Inflammatory factors

Preclinical studies. Five out of seven preclinical studies (Table II) included TNF-α, a proinflammatory cytokine that enhances mesenchymal stromal cell (MSC) recruitment and osteogenic differentiation.[36] Chan et al[37] reported that in OVX mice, treatment with 1 ng recombinant human tumour necrosis factor (rhTNF) at the fracture site at days 0 and 1 resulted in an increased relative callus mineralization at day 14 post-fracture compared to SHAM mice. However, healing was found equivalent between the two groups at day 28. Chow et al[38] revealed that TNF-α level at the fracture callus by immunohistochemistry (IHC) was significantly lower compared to SHAM rats at week 1 post-fracture, while the serum level of TNF-α by enzyme-linked immunosorbent assay (ELISA) test was significantly higher in OVX rats at weeks 1 and 2.[38] Wang F et al[39] showed that the serum level of TNF-α by ELISA test was significantly higher in OVX mice at day 5 compared

Table 1. Summary of the study characteristics.

Included studies	Type	Study design	Species	Strain	Age	Fracture site	Fracture type	Fixation type	Timepoint	Inflammatory factor
Chan et al[37] (2015)	PC	Day 14: OVX + 1 ng TNF (n = 6); OVX + PBS (n = 6), Day 28: OVX + 1 ng TNF (n = 7); OVX + PBS (n = 7)	Mouse (Female)	C57BL/6 J	12 to 14 wks old	Tibia	Osteotomy	Intramedullary fixation pin	Days 14, 28	Local administration of rhTNF
Chow et al[38] (2019)	PC	SHAM (n = 6); OVX (n = 6)	Rat (Female)	SD	9 mths old	Femur	Closed femoral fracture	Intramedullary fixation pin	Wks 1, 2, 4, and 8	TNF, IL-6, IL-10
Fischer et al[34] (2018)	PC	SHAM & OVX (6 hrs: SHAM n = 5, OVX n = 7; Day 1: SHAM n = 8, OVX n = 6; Day 2: SHAM n = 5, OVX n = 5; Day 3: SHAM n = 6, OVX n = 5)	Mouse (Female)	C57BL/6 J	5 to 6 mths old	Femur mid-shaft	Osteotomy	External fixator with screws	6 hrs, Days 1, 2, 3	IL-6, IL-1β, IL-13, IL-4, CXCL1, MCP-1, MIP-1α
Haffner-Luntzer et al[35] (2017)	PC	SHAM & OVX	Mouse (Female)	C57BL/6 J	5 to 6 mths old	Femur mid-shaft	Osteotomy	External fixator with screws	Days 1, 3, 23	Mdk, IL-6, CCL2, CXCL1, macrophages, neutrophils, monocytes, B-lymphocytes, T-lymphocytes (cytotoxic T-lymphocytes and T-helper-lymphocytes)
Wang F et al[39] (2018)	PC	SHAM (n = 54); OVX (n = 48)	Mouse (Female)	C57BL/6 J	21 wks old	Proximal third of the right tibia diaphysis	Transverse osteotomy	Intramedullary pinning	Days 3, 5, 11	TNF-α, IL-1β
Wang J et al[41] (2018)	PC	SHAM (n = 15); OVX (n = 15)	Rat (Female)	SD	24 wks old	Femur	Closed femoral fracture	Intramedullary pinning	6 wks post fracture	TNF-α, IL-6
Zhang et al[40] (2019)	PC	SHAM (n = 10); OVX (n = 10)	Rat (Female)	Wistar	Adult	Tibia	Unilateral cross-tibial fracture	Intramedullary pinning	5 wks post fracture	TNF-α, IL-1β
Caetano-Lopes et al[63] (2011)	C	Patients suffering a hip fragility fracture (those who had surgery less than 3 days after fracture (n = 13); between 4 and 7 days (n = 33); 8 or more days (n = 10)	N/A	N/A	Mean 80 yrs old (SD 7)	Hip	Fragility fracture	N/A	Received hip arthroplasty surgery less than 3 days after fracture; 4 to 7 days; 8 or more days	TNF, IL-6, IL-1β
Fischer et al[34] (2018)	C	Patients with metaphyseal/diaphyseal fractures of long bones (Day 0: n = 26; Day 14: n = 7; Day 42: n = 4); Healthy controls (n = 20); Subgroup Day 0: Male fracture patients (n = 6); Female fracture patients after menopause (n = 13)	N/A	N/A	32 to 97 yrs, mean = 75 yrs; Subgroup Day 0: Male patients: 32 to 97 yrs, mean: 69 yrs; Female patients: 57 to 87 yrs, mean: 78 yrs	Femur, tibia, humeri, radii, ulna	Long-bone fracture	N/A	Days 0, 14, 42	Mdk

Continued

Table I. Continued

Included studies	Type	Study design	Species	Strain	Age	Fracture site	Fracture type	Fixation type	Timepoint	Inflammatory factor
Ginaldi et al[28] (2015)	C	Fractured women with osteoporosis (n = 15); unfractured women (n = 41) with osteoporosis; age-matched healthy post-menopausal women (n = 26)	N/A	N/A	65 yrs old or above	Multiple	Fragility fracture	N/A	N/A	IL-31
Lipovetzki et al[69] (2017)	C	Postmenopausal women: Group 1 - Hospitalized from Jan 2008 to Dec 2012 due to diagnosis of non-traumatic hip fracture (n = 49); Group 2 - without history of hip fracture (n = 66)	N/A	Hip	Group 1: average age: 65.1 yrs old; Group 2: average age: 78.4 yrs old	Hip	Non-traumatic hip fracture	N/A	N/A	CRP
Meyer et al[70] (2014)	C	Women (n = 18 (7 osteoporotic; 8 osteopenic by DXA; 2 no DXA data))	N/A	N/A	50 yrs old or above	Distal radii	Stable distal radii fracture	N/A	1 to 2, 3 to 4, 6 to 8, and 12 wks post fracture	hsCRP
Pesic et al[64] (2017)	C	Elderly patients with femoral neck fracture (n = 65)	N/A	N/A	Older than 65 yrs; Mean: 79.8 yrs	Femoral neck	Femoral neck fracture	N/A	12 hrs after fracture, first, third, and seventh postoperative days	TNF-α, IL-6
Tsangari et al[66] (2004)	C	Patients undergoing hip arthroplasty surgery for a subcapital fractured neck of femur) – all characterized as having fragility fracture (n = 13); Control (from postmortem) (n = 13)	N/A	N/A	70 yrs old or above	Intertrochanteric region of the proximal femur	Subcapital fractured neck of femur	N/A	Up to 6 days after fracture	IL-6
Saribal et al[65] (2019)	C	Osteoporotic patients with hip fracture (n = 40); Age-matched non-osteoporotic healthy controls (n = 40)	N/A	N/A	Patients (mean 74 yrs (SD 1)); Controls (mean 67 yrs (SD 7))	Hip	Hip fracture due to osteoporosis	N/A	Before receiving surgery; On the first and second days of postoperative period	TNF-α, IL-6

C, clinical; CCL2, chemokine (C-C motif) ligand 2; CXCL1, chemokine (C-X-C motif) ligand 1; hsCRP, high-sensitive CRP; IL, interleukin; DXA, dual energy X-ray absorptiometry; MCP-1, monocyte chemoattractant protein 1; Mdk, midkine; MIP-1α, macrophage inflammatory protein 1 alpha; N/A, not applicable; OVX, ovariectomy; PBS, phosphate-buffered saline; PC, preclinical; rhTNF, recombinant human tumour necrosis factor; SD, Sprague-Dawley; SHAM, normal control; TNF, tumour necrosis factor

Table II. Summary of inflammatory factors in the included preclinical studies. All factors were compared between ovariectomized and normal control fractured animals.

Inflammatory factor	Type	Role in fracture healing	Included studies	Type	Experiment	Systemic/local (Location)	Finding
TNF-α	Proinflammatory cytokine	Enhance MSC recruitment and proliferation and osteoblast differentiation	Chan et al[37] 2015	PC	Treatment with 1 ng rhTNF on days 0 and 1 (immediately and 24 hrs)	Local injection at the fracture site	Increased relative callus; mineralization observed in OVX* mice on day 14 compared to SHAM* mice; equivalent healing on day 28 between groups
			Chow et al[38] 2019	PC	IHC	Local (fracture callus)	Significantly lower in OVX group compared to SHAM group at week 1
					ELISA	Systemic (serum)	Significantly higher in OVX group compared to SHAM group at weeks 1 and 2
			Wang F et al[39] 2018	PC	ELISA	Systemic (serum)	Similar between SHAM† and OVX† mice on day 3; significantly higher in OVX mice on day 5
			Wang J et al[41] 2018	PC	ELISA	Systemic (serum)	Significantly higher in OVX* rats at week 6 compared to SHAM* rats
			Zhang et al[40] 2019	PC	ELISA	Systemic (serum)	Significantly higher in OVX rats at week 5 compared to normal control rats
IL-6	Proinflammatory cytokine	Stimulate angiogenesis and MSCs to differentiate towards the osteoblast lineage; modulate osteoblast and osteoclast activities	Chow et al[38] 2019	PC	IHC	Local (fracture callus)	Significantly lower in OVX group compared to SHAM at week 1
					ELISA	Systemic (serum)	Significantly lower in OVX group compared to SHAM at week 8
			Fischer et al[34] 2018	PC	Multiplex cytokine assay	Systemic (plasma)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
					ELISA	Local (fracture callus)	Significantly higher in OVX mice on day 3 compared to SHAM mice
			Haffner-Luntzer et al[35] 2017	PC	IHC	Local (fracture callus)	Increased expression in OVX mice on day 3 compared to SHAM mice
			Wang J et al[41] 2018	PC	ELISA	Systemic (serum)	Significantly higher in OVX* rats at week 6 compared to SHAM* rats
IL-1β	Proinflammatory cytokine	Stimulate osteoblast proliferation and production of mineralized bone matrix; inhibit proliferation and differentiation of chondrocytes	Fischer et al[34] 2018	PC	ELISA	Local (fracture callus)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
			Wang F et al[39] 2018	PC	ELISA	Systemic (serum)	Similar between SHAM† and OVX† mice on day 3; significantly higher in OVX mice on day 5
			Zhang et al[40] 2019	PC	ELISA	Systemic (serum)	Significantly higher in OVX rats at week 5 compared to normal control rats
IL-10	Anti-inflammatory cytokine	Suppress excessive or prolonged inflammation	Chow et al[38] 2019	PC	IHC	Local (fracture callus)	Significantly higher in OVX group at week 1 and lower at week 4 compared to SHAM

Continued

Table II.   Continued

Inflammatory factor	Type	Role in fracture healing	Included studies	Type	Experiment	Systemic/local (Location)	Finding
					ELISA	Systemic (serum)	Significantly lower in OVX group compared to SHAM at week 2
IL-13	Anti-inflammatory cytokine	Involved in osteoblast recruitment	Fischer et al[34] 2018	PC	Multiplex cytokine assay	Systemic (plasma)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
IL-4	Anti-inflammatory cytokine	Inhibit monocyte production of IL-1 and TNF-α; chemoattract osteoblasts; inhibit bone resorption	Fischer et al[34] 2018	PC	ELISA	Local (fracture callus)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
Neutrophil	Immune cell	Secrete proinflammatory and immunomodulatory cytokines and MCP-1 to attract monocytes; involved in fibrin thrombus	Haffner-Luntzer et al[35] 2017	PC	FACS	Systemic (bone marrow)	Significantly decreased on day 1 in OVX mice
					FACS	Local (fracture haematoma)	Similar between SHAM and OVX mice on day 1
					IHC	Local (fracture callus)	Significantly increased on day 3 in OVX mice
Macrophage	Immune cell	Undergo polarization and modulate the inflammatory phase; produce growth factors such as BMP-2; induce osteogenesis of mesenchymal progenitor cells	Haffner-Luntzer et al[35] 2017	PC	FACS	Systemic (bone marrow)	Similar between SHAM and OVX mice on day 1
					FACS	Local (fracture haematoma)	Similar between SHAM and OVX mice on day 1
					IHC	Local (fracture callus)	Similar between SHAM and OVX mice on day 3
Inflammatory monocyte	Immune cell	Differentiate into macrophages in tissues	Haffner-Luntzer et al[35] 2017	PC	FACS	Systemic (bone marrow)	Similar between SHAM and OVX mice on day 1
					FACS	Local (fracture haematoma)	Similar between SHAM and OVX mice on day 1
B-lymphocyte	Immune cell	Suppress proinflammatory signals; produce OPG to regulate osteoclastic differentiation and activity	Haffner-Luntzer et al[35] 2017	PC	FACS	Systemic (bone marrow)	Significantly higher on day 1 in OVX mice
					FACS	Local (fracture haematoma)	Similar between SHAM and OVX mice on day 1
					IHC	Local (fracture callus)	Similar between SHAM and OVX mice on day 3
T-lymphocyte	Immune cell	Produce RANKL to recruit, differentiate, and activate osteoclasts; secrete IL-17 to induce anti-inflammatory functions from MSCs and induce osteogenic differentiation and activity	Haffner-Luntzer et al[35] 2017	PC	FACS	Systemic (bone marrow)	Significantly reduced in OVX mice on day 1 (cytotoxic T-cells significantly reduced, whereas T-helper cells significantly increased)
					FACS	Local (fracture haematoma)	Similar between SHAM and OVX mice on day 1
					IHC	Local (fracture callus)	No difference in cytotoxic T-cells on day 3
Mdk	Proinflammatory cytokine	Chemoattract neutrophils and macrophages	Fischer et al[34] 2018	PC	Multiplex cytokine assay	Systemic (plasma)	Significantly higher in OVX mice on day 3 compared to SHAM mice
					ELISA	Local (fracture callus)	Significantly higher in OVX mice on day 3 compared to SHAM mice
			Haffner-Luntzer et al[35] 2017	PC	IHC	Local (fracture callus)	Increased expression in OVX mice on day 3 compared to SHAM mice

Continued

Table II. Continued

Inflammatory factor	Type	Role in fracture healing	Included studies	Type	Experiment	Systemic/local (Location)	Finding
CCL2	Chemokine	Regulate migration and infiltration of monocytes/macrophages	Haffner-Luntzer et al[35] 2017	PC	IHC	Local (fracture callus)	Similar between SHAM and OVX mice on day 3
MCP-1			Fischer et al[34] 2018	PC	Multiplex cytokine assay	Systemic (plasma)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
					ELISA	Local (fracture callus)	Significantly higher in OVX mice on day 3 compared to SHAM mice
CXCL1	Proinflammatory cytokine	Recruit neutrophils	Fischer et al[34] 2018	PC	Multiplex cytokine assay	Systemic (plasma)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
					ELISA	Local (fracture callus)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3
			Haffner-Luntzer et al[35] 2017	PC	IHC	Local (fracture callus)	Similar between SHAM and OVX mice on day 3
MIP-1α	Chemokine	Recruit monocytes	Fischer et al[34] 2018	PC	ELISA	Local (fracture callus)	Similar between SHAM and OVX mice at 6 hrs, day 1, 2, 3

BMP-2, bone morphogenetic protein 2; CCL2, chemokine (C-C motif) ligand 2; CXCL1, chemokine (C-X-C motif) ligand 1; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; IHC, immunohistochemistry; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; Mdk, midkine; MIP-1α, macrophage inflammatory protein 1 α; MSC, mesenchymal stromal cell; OPG, osteoprotegerin; OVX, ovariectomy; PC, preclinical; RANKL, receptor activator of nuclear factor kappa-B ligand; rhTNF, recombinant human tumour necrosis factor; SHAM, normal control; TNF-α, tumour necrosis factor-α.
*Treated with phosphate buffered saline (PBS), no other treatment added.
†Treated with physiological saline.

to SHAM mice but no significant difference was found at day 3 post-fracture. The level of TNF-α in serum by ELISA test was also found to be significantly higher in OVX rats at week 5 and week 6 by Zhang et al[40] and Wang J et al,[41] respectively.

Four out of seven preclinical studies included the proinflammatory cytokine IL-6, which stimulates angiogenesis[42] and MSCs to differentiate towards the osteoblast lineage[43] and modulates osteoblast and osteoclast activities.[44] Chow et al[38] demonstrated that IL-6 level at the fracture callus by IHC was significantly lower in OVX rats compared to SHAM rats at week 1 post-fracture. Significantly lower serum level of IL-6 was detected by ELISA test in OVX rats at week 8.[38] Fischer et al[34] showed that IL-6 level at the fracture callus by ELISA test was significantly higher compared to SHAM mice on day 3 post-fracture, while no significance was detected in plasma IL-6 level by multiplex cytokine assay at six hours and days 1, 2, and 3 after fracture. Haffner-Luntzer et al[35] reported that IL-6 expression by immunostaining was increased in periosteal callus in OVX mice compared to SHAM mice on day 3 post-fracture. Wang J et al[41] found that the serum level of IL-6 by ELISA test was significantly higher in OVX rats at week 6.

Three studies examined the proinflammatory cytokine IL-1β, which stimulates osteoblast proliferation and production of mineralized bone matrix as well as inhibiting proliferation and differentiation of chondrocytes.[45] Fischer et al[34] reported that its levels at the fracture callus by ELISA test were similar between SHAM and OVX mice

at six hours and days 1, 2, and 3 post-fracture. Wang F et al[39] showed that the serum level of IL-1β by ELISA test was significantly higher in OVX mice at day 5 compared to SHAM mice but no significant difference was detected at day 3 post-fracture. The level of IL-1β in serum by ELISA test was also found to be significantly higher in OVX rats at week 5 by Zhang et al.[40]

One study found that the anti-inflammatory cytokine IL-10, which suppresses excessive or prolonged inflammation.[46] Chow et al[38] reported that IL-10 level at the fracture callus by IHC was significantly higher at week 1 but lower at week 4 in OVX rats compared to SHAM rats. Significantly lower serum IL-10 level was detected by ELISA test in OVX rats at week 2.[38]

The anti-inflammatory cytokine IL-13 was investigated in another study, which is closely related to IL-4[47] and involved in osteoblast recruitment.[48] Fischer et al[34] reported that its levels in plasma by multiplex cytokine assay were similar between SHAM and OVX mice at six hours and days 1, 2, and 3 post-fracture.

The anti-inflammatory cytokine IL-4 was investigated in an additional study; this inhibits monocyte production of IL-1 and TNF-α,[49] chemoattracts osteoblasts,[48] and inhibits bone resorption.[50] Fischer et al[34] reported that its levels at the fracture callus by ELISA test were similar between SHAM and OVX mice at six hours and days 1, 2, and 3 post-fracture.[34]

One study investigated the immune cell neutrophils reported to secrete proinflammatory and immunomodulatory cytokines such as IL-1, IL-6, TNF-α, IL-10, and

monocyte chemoattractant protein 1 (MCP-1) to attract monocytes and be involved in fibrin thrombus.[51] Haffner-Luntzer et al[35] showed that the number of neutrophils by fluorescent-activated cell sorting (FACS) analysis significantly decreased in the bone marrow of OVX mice at day 1 post-fracture, although no significant difference was found in the fracture haematoma. Besides, OVX mice displayed significantly greater numbers of neutrophils in the periosteal callus at day 3 post-fracture.[35]

Macrophages were analyzed in another study; these undergo polarization and modulate the inflammatory phase,[52] produce growth factors such as bone morphogenetic protein 2 (BMP-2),[53] and induce osteogenesis of mesenchymal progenitor cells.[54] Haffner-Luntzer et al[35] found that the number of macrophages in the fracture haematoma and bone marrow by FACS analysis at day 1 post-fracture, and that at the marrow cavity near the fracture gap by immunostaining at day 3 post-fracture, were similar between SHAM and OVX mice.

One study involved the analysis of inflammatory monocytes that differentiate into macrophages in tissues.[52] Haffner-Luntzer et al[35] found that the number of inflammatory monocytes in the bone marrow and fracture haematoma of OVX mice was not significantly different compared to SHAM mice at day 1 post-fracture.

Another study involved the analysis of B-lymphocytes, which suppress proinflammatory signals of TNF-α and IL-2[55] and produce osteoprotegerin (OPG) to regulate osteoclastic differentiation and activity.[56] Haffner-Luntzer et al[35] demonstrated that at day 1 post-fracture, the number of B-lymphocytes by FACS analysis was significantly higher in the bone marrow of OVX mice but similar between SHAM and OVX mice in the fracture haematoma. Also, the expression of B-lymphocytes by immunostaining was not found to be significantly altered at the periosteal callus at day 3 post-fracture.[35]

One study involved the analysis of T-lymphocytes, which produce receptor activator of nuclear factor kappa-B ligand (RANKL) to recruit, differentiate, and activate osteoclasts[57] and secrete IL-17 to induce anti-inflammatory functions from MSCs[58] and induce osteogenic differentiation and activity.[59] Haffner-Luntzer et al[35] found that the number of T-lymphocytes was significantly lower by FACS analysis in the bone marrow, but no significant difference was detected in the fracture haematoma in OVX mice at day 1 post-fracture. Within the T-lymphocyte population in the bone marrow, cytotoxic T-cells were significantly reduced whereas T-helper cells were significantly increased in OVX mice. However, the number of cytotoxic T-lymphocytes at the periosteal callus by immunostaining was similar between SHAM and OVX mice at day 3 post-fracture.[35]

Two studies examined the proinflammatory cytokine midkine (Mdk) reported to chemoattract neutrophils and macrophages.[60] Fischer et al[34] found that OVX mice showed significantly higher Mdk levels in plasma by multiplex cytokine assay and at the fracture site by ELISA test compared to SHAM mice at day 3 after fracture. Haffner-Luntzer et al[35] showed that Mdk was expressed more in the fracture callus in OVX mice compared to SHAM-operated animals at day 3 post-fracture.

Two studies examined the proinflammatory cytokine chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP-1), which regulates migration and infiltration of monocytes/macrophages.[61] Fischer et al[34] found that its level at the fracture callus by ELISA test was significantly higher in OVX mice compared to SHAM mice on day 3 post-fracture, but no significance was detected in plasma MCP-1 level by multiplex cytokine assay at six hours and days 1, 2, and 3 after fracture.[34] Haffner-Luntzer et al[35] found that the protein expression of CCL2 at the fracture callus was similar between SHAM and OVX mice at day 3 post-fracture.

Two studies examined the proinflammatory cytokine chemokine (C-X-C motif) ligand 1 (CXCL1), which is found to recruit neutrophils.[62] Fischer et al[34] reported that its levels in plasma by multiplex cytokine assay and at the fracture site by ELISA test were similar between SHAM and OVX mice at six hours and days 1, 2, and 3 post-fracture. Haffner-Luntzer et al[35] found that CXCL1 protein expression at the fracture callus was similar between SHAM and OVX mice at day 3 post-fracture.

One study involved the analysis of macrophage inflammatory protein 1 α (MIP-1α), which recruits monocytes.[51] Fischer et al[34] reported that MIP-1α level at the fracture callus by ELISA test was similar between SHAM and OVX mice at six hours and days 1, 2, and 3 post-fracture.[34]

Clinical studies. Among all clinical studies included in this review (Table III), there was no clinical study investigating the difference in inflammatory response between osteoporotic and non-osteoporotic patients. All clinical data presented could, however, provide reference data for the temporal changes of common inflammatory cytokines in osteoporotic fracture patients.

Three studies examined the proinflammatory cytokine TNF, which enhances MSC recruitment and osteogenic differentiation.[36] Caetano-Lopes et al[63] found that gene expression of TNF locally at the fracture site of low energy hip arthroplasty patients were similar among all timepoints (the first three days, days 4 to 7, and days 8 or more post-fracture) without any apparent variation in pattern. Pesic et al[64] reported that the level of TNF-α in plasma detected by ELISA was similar among all timepoints (12 hours after fracture, and days 1, 3, and 7) after surgical intervention. Saribal et al[65] demonstrated plasma TNF-α levels by quantitative sandwich enzyme immunoassay were similar between healthy controls and osteoporotic hip fracture patients before and after surgery.

Five clinical studies included proinflammatory cytokine IL-6, which stimulates angiogenesis[42] and MSCs to differentiate towards the osteoblast lineage[43] and modulates osteoblast and osteoclast activities.[44] Caetano-Lopes et al[63] showed that gene expression of IL-6 locally at the fracture site was the highest during the first three days

Table III. Summary of inflammatory factors in the included clinical studies.

Inflammatory factor	Type	Role in fracture healing	Included studies	Experiment	Systemic/local (Place)	Fracture site	Finding
TNF	Proinflammatory cytokine	Enhance MSC recruitment and osteogenic differentiation	Caetano-Lopes et al[63] 2011	qPCR	Local (Bone specimen)	Hip	Similar gene expression between the first 3 days, days 4 to 7, and days 8 or more post-fracture
TNF-α			Pesic et al[64] 2017	ELISA	Systemic (Plasma)	Femoral neck	Similar between 12 hours after fracture, and days 1, 3, and 7 after surgical intervention
			Saribal et al[65] 2019	Quantitative sandwich enzyme immunoassay	Systemic (Plasma)	Hip	Similar level in fracture patients compared to healthy controls before and after surgery
IL-6	Proinflammatory cytokine	Stimulate angiogenesis and MSCs to differentiate towards the osteoblast lineage; modulate osteoblast and osteoclast activities	Caetano-Lopes et al[63] 2011	qPCR	Local (Bone specimen)	Hip	Gene expression was highest for the first 3 post-fracture days compared to days 4 to 7 and days 8 or more after fracture
			Fischer et al[34] 2018	ELISA	Systemic (Serum)	Long bone	Significantly higher in fracture patients at days 0 and 14 compared to healthy controls; similar to controls at day 42
			Pesic et al[64] 2017	ELISA	Systemic (Plasma)	Femoral neck	Significantly increased at day 1 after surgical intervention compared to 12 hours after fracture and days 3 and 7 after intervention
			Saribal et al[65] 2019	Quantitative sandwich enzyme immunoassay	Systemic (Plasma)	Hip	Similar level in fracture patients compared to healthy controls before surgery; significantly higher in fracture patients on the first and second days of postoperative period
			Tsangari et al[66] 2004	qPCR	Local (Bone specimen)	Neck of femur	Significantly higher in fracture group compared to no-fracture group; mRNA levels associated with RANKL and RANK mRNA levels
IL-1β	Proinflammatory cytokine	Stimulate osteoblast proliferation and production of mineralized bone matrix; inhibit proliferation and differentiation of chondrocytes	Caetano-Lopes et al[63] 2011	qPCR	Local (Bone specimen)	Hip	Similar gene expression between the first 3 days, days 4 to 7, and days 8 or more post-fracture
IL-31	Proinflammatory cytokine	Induce the release of the proinflammatory cytokines such as IL-1β and IL-6 and chemokines such as CCL2 and CXCL1	Ginaldi et al[28] 2015	ELISA	Systemic (Serum)	(Not specified)	Higher in fractured women with osteoporosis compared to age-matched controls, but no significant difference (p = 0.068, Mann-Whitney U test); similar between unfractured and fractured osteoporotic women (p = 0.310, Mann-Whitney U test)
Mdk	Proinflammatory cytokine	Chemoattract neutrophils and macrophages	Fischer et al[34] 2018	ELISA	Systemic (Serum)	Long bone	Significantly higher in fracture patients on days 0, 14, and 42 compared to healthy controls; significantly higher on day 14 compared to day 0 in fracture patients; significantly higher in female fracture patients after menopause on day 0 after fracture compared to male fracture patients

Table III.   Continued

Inflammatory factor	Type	Role in fracture healing	Included studies	Experiment	Systemic/local (Place)	Fracture site	Finding
CRP	Acute inflammatory protein	Act as chemoattractant for monocytes and induce tissue factor expression in macrophages such as IL-1α, IL-1β, TNF-α, and IL-6	Lipovetzki et al[69] 2017	ELISA	Systemic (Blood)	Hip	Significantly higher in postmenopausal women with fracture compared to those without
hsCRP			Meyer et al[70] 2014	Plasma protein analysis	Systemic (Serum)	Distal radii	No significant change between 1 to 2 and 3 to 4 wks post-fracture

CCL2, chemokine (C-C motif) ligand 2; CXCL1, chemokine (C-X-C motif) ligand 1; ELISA, enzyme-linked immunosorbent assay; hsCRP, high-sensitive CRP; IL, interleukin; Mdk, midkine; mRNA, messenger RNA; MSC, mesenchymal stromal cell; qPCR, quantitative polymerase chain reaction; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; TNF, tumour necrosis factor.

after fracture compared to days 4 to 7 ($p = 0.021$, analysis of variance (ANOVA)) and days 8 or more after fracture. In Fischer et al,[34] serum level of IL-6 was found to be significantly higher in fracture patients at days 0 and 14 post-fracture compared to healthy controls.[34] Pesic et al[64] demonstrated that IL-6 level in plasma was significantly higher at day 1 after surgical intervention when compared to 12 hours after fracture and days 3 and 7 after intervention. Saribal et al[65] demonstrated that plasma IL-6 levels by quantitative sandwich enzyme immunoassay were similar between healthy controls and osteoporotic hip fracture patients before surgery. Significantly higher IL-6 levels were detected in osteoporotic hip fracture patients on the first ($p = 0.005$, Mann-Whitney U test) and second ($p = 0.010$, Mann-Whitney U test) days of postoperative periods compared to controls.[65] Tsangari et al[66] reported that IL-6 messenger RNA (mRNA) level of the trabecular bone cores from the intertrochanteric region obtained from patients suffering from fragility subcapital fracture of the neck of femur was significantly higher than that of aged-matched control without fracture. IL-6 mRNA levels were found to be associated with RANKL and receptor activator of nuclear factor kappa-B (RANK) mRNA levels (Pearson's correlation of $r = 0.77$, $p < 0.001$; $r = 0.95$, $p < 0.001$, respectively) in the fracture group.[66]

One study investigated the proinflammatory cytokine IL-1β, which stimulates osteoblast proliferation and production of mineralized bone matrix as well as inhibiting proliferation and differentiation of chondrocytes.[45] Caetano-Lopes et al[63] revealed that gene expression of IL-1β at the fracture site was similar among all timepoints (the first three days, days 4 to 7, and days 8 or more post-fracture). The proinflammatory cytokine IL-31 was examined in another study; this induces the release of proinflammatory cytokines such as IL-1β and IL-6 and chemokines such as CCL2 and CXCL1.[67] Ginaldi L et al[28] found that the serum level of IL-31 by ELISA test was similar between fractured women with osteoporosis and age-matched controls. Unfractured and fractured osteoporotic women also showed similar IL-31 serum levels.

Another study involved the examination of the proinflammatory cytokine, Mdk, which is reported to chemoattract neutrophils and macrophages.[60] In Fischer et al,[34] Mdk level in serum was reported to be significantly higher in fracture patients at days 0, 14, and 42 after fracture compared to healthy controls. In fracture patients, Mdk level was significantly higher at day 14 compared to day 0 post-fracture. Also, female fracture patients after menopause showed significantly higher Mdk levels than male patients at day 0 after fracture.[34]

Two clinical studies included the acute inflammatory protein, CRP, which acts as a chemoattractant for monocytes and induces tissue factor expression in macrophages such as IL-1, TNF-α, and IL-6.[68] Lipovetzki et al[69] reported that serum level of CRP by ELISA test was significantly higher in postmenopausal women with non-traumatic hip fracture than that of postmenopausal osteoporotic women without hip fracture. Meyer et al[70] found that serum level of high-sensitive CRP (hsCRP) by plasma protein analysis showed no significant difference between one to two weeks post-fracture and three to four weeks post-fracture.

Healing outcomes

Preclinical studies. All included preclinical studies (Table IV) reported radiograph micro-CT (μCT) assessments at the fracture site, except one using DXA to measure bone mineral content (BMC) and bone mineral density (BMD) of intact femora. Significantly lower bone volume ratio (BV/TV), trabecular number (Tb.N), and increased trabecular separation (Tb.Sp) in tibial callus of OVX mice were found in Wang F et al.[39] Wang J et al[41] demonstrated significantly lower bone volume (BV), total volume (TV), BV/TV, trabecular thickness (Tb.Th), Tb.N, and cortical thickness (Ct.Th) in fracture callus of OVX rats

Table IV. Summary of healing outcomes in the included preclinical studies.

Included studies	Experiment	Parameter	Timepoint	Location	Finding
Chan et al[37] 2015	μCT analysis	Relative callus mineralization	Day 14	Callus (Tibia)	Treatment with 1 ng rhTNF significantly increased relative callus mineralization in OVX mice compared to SHAM mice
			Day 28		Healing was equivalent between groups
Chow et al[38] 2019	μCT analysis	TV, BV, BV/TV, BMD	Week 8	Fracture callus	Significantly lower BV, BV/TV, and BMD in OVX rats
	Radiography	Callus width and area	Weeks 1, 2, 3	Fracture callus	Significantly lower callus width and area in OVX rats
Haffner-Luntzer et al[35] 2017	μCT analysis	Callus development and bridging	Day 23	Fracture callus (Femur)	Disturbed bony callus development and poor cortical bridging at the fracture callus in OVX mice
Wang F et al[39] 2018	Three-point bending test	Callus stiffness	Day 35	Callus (Tibia)	Significantly lower callus stiffness in OVX mice
	μCT analysis	BV/TV, Tb.N, Tb.Sp	Day 24		Significantly decreased BV/TV and Tb.N and increased Tb.Sp in OVX mice
	H&E staining	Bone morphometry	Day 5		More fibrous callus and hematoncus (haematoma formed around the fracture site) in OVX mice
			Day 11		Chondrocyte proliferation and hypertrophic differentiation still took place in OVX mice
			Day 24		Less new bone tissue generated in OVX mice
	Real-time PCR	mRNA expression of aggrecan, Col10a, VEGF, MMP13, Col1a, OCN	Day 11	Callus tissue	In OVX mice, significantly higher levels of aggrecan and Col10a; significantly lower VEGF, Col1a, and OCN levels; no apparent change in MMP13
			Day 24		Significantly higher mRNA expression levels of aggrecan and Col10a; no apparent change in VEGF; significantly higher MMP13 level; significantly lower expression of Col1a and OCN
	ELISA	Aggrecan, MMP13, VEGF	Day 5	Serum	Significantly higher VEGF level; no significant increase in aggrecan and MMP13 levels
			Day 11		Significantly higher aggrecan, lower MMP13 and VEGF levels in OVX mice
Wang J et al,[41] 2017	μCT analysis	BV, TV, BV/TV, Tb.Th, Tb.N and Ct.Th	Week 6	Fractured femur	Significantly lower BV, TV, BV/TV, Tb.Th, Tb.N, and Ct.Th in OVX rats compared to SHAM rats
	Biomechanical measurement	Maximal fracture load		Femoral neck	Significantly lower maximal fracture load in OVX rats
	H&E staining	Callus bony area, cartilage area, callus total area		Femur callus callous area	Significantly lower callus bony area, higher cartilage area, and lower callus total area in OVX rats
	IHC	BMP-2		Fractured femur section	Significantly lower BMP-2 expression in OVX rats
	Real-time PCR	mRNA expression of BMP-2, RUNX2, ALP, OPG, RANKL		Fractured femur	Significantly lower BMP-2, RUNX2, and ALP expression in OVX mice; significantly lower OPG and higher RANKL expression in OVX rats
	Western blot	BMP-2, RUNX2, ALP		Fractured femur	Significantly lower BMP-2, RUNX2, and ALP expression in OVX rats
	TRAP staining	Number of osteoclasts		Fracture callus section	Significantly higher numbers of osteoclasts in OVX rats
Zhang et al[40] 2019	DXA	BMC, BMD	Week 5	Femur	No significance in BMC; significant reduction of BMD in OVX rats relative to normal control group
	ELISA	TRAP, ALP, CTX, OCN		Serum	Significantly higher in OVX rats compared to normal control rats

Aggrecan, chondrocyte proliferation marker; ALP, alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BMP-2, bone morphogenetic protein 2; BV, bone volume; BV/TV, bone volume ratio; Col1a, collagen type I (osteogenesis marker); Col10a, collagen type X (chondrocyte hypertrophic marker); CTX, C-terminal telopeptide of type 1 collagen; Ct.Th, cortical thickness; DXA, dual energy x-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; H&E, haematoxylin and eosin; IHC, immunohistochemistry; MMP13, matrix metallopeptidase 13 (cartilage matrix degradation marker); mRNA, messenger RNA; OCN, osteocalcin (osteogenesis marker); OPG, osteoprotegerin; OVX, ovariectomy; PCR, polymerase chain reaction; RANKL, receptor activator of nuclear factor kappa-B ligand; rhTNF, recombinant human tumour necrosis factor; RUNX2, runt-related transcription factor 2; SHAM, normal control; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TRAP, tartrate-resistant acid phosphatase; TV, tissue volume; VEGF, vascular endothelial growth factor (vascularization marker); μCT, x-ray micro-CT

compared to SHAM rats.[41] Chow et al[38] also found significantly lower BV, BV/TV, and BMD at the fracture callus of OVX rats.[38] Chan et al[37] presented significantly increased relative callus mineralization (p = 0.0099, independent-samples t-test) in mice treated with 1 ng rhTNF compared to phosphate buffered saline (PBS) controls.[37] Disturbed bony callus development and poorer cortical bridging at the fracture callus were observed in OVX mice compared to SHAM mice in Haffner-Luntzer M et al[35] Zhang et al[40] reported significantly lower BMD but similar BMC in OVX rats compared to normal control rats.

One study measured callus width and area through weekly lateral radiography. Chow et al[38] reported that OVX rats had significantly lower callus width and area than SHAM rats at weeks 1, 2, and 3 post-fracture.

Two studies included mechanical testing to evaluate the bone properties of the fracture bones, including Wang F et al[39] and Wang J et al.[41] Wang F et al[39] reported significantly lower callus stiffness by three-point bending test in fractured tibia of OVX mice. Wang J et al[41] presented significantly lower maximal fracture load of the femoral neck by loading vertical pressure on the femoral head in OVX rats.

Histomorphometric analysis was reported in two preclinical studies, including Wang F et al[39] and Wang J et al.[41] Wang F et al[39] demonstrated slower bone regeneration as shown by less new bone tissue formed to replace cartilage callus around the fracture site of OVX mice compared to controls. Wang J et al[41] also reported significantly higher osteoclast numbers in fracture callus of OVX rats compared to SHAM groups. In addition, significantly lower callus bony area (-10.7%), higher cartilage area, and lower callus total area were reported at the fracture callus of OVX rats in Wang J et al.[41]

Two studies evaluated the gene expression at the fracture site, including Wang F et al[39] and Wang J et al.[41] Wang F et al[39] found that mRNA expression of *aggrecan* and collagen type X (*Col10a*), which are related to chondrogenesis, and matrix metallopeptidase 13 (*MMP13*) were found to be significantly higher, whereas expression of collagen type I (*Col1a*) and osteocalcin (*OCN*), which are related to osteogenesis, and vascular endothelial growth factor (*VEGF*) was significantly lower in the callus tissue of OVX mice.[39] Wang J et al[41] also reported significantly lower levels of osteogenic marker, alkaline phosphatase (*ALP*), bone morphogenetic protein 2 (*BMP-2*), and runt-related transcription factor 2 (*RUNX2*) expression in fractured femora of OVX rats. Significantly lower *OPG* and higher *RANKL* expression was also shown in fractured femora of OVX rats.[41]

Three studies involved the assessments of protein expression. Wang J et al[41] performed western blot on the fractured femora, and Wang F et al[39] and Zhang et al[40] performed ELISA in serum. Wang J et al[41] found that expression of RUNX2 and ALP was significantly lower in fractured femora of OVX rats compared to the SHAM group. Significantly lower BMP-2 expression was also

reported at the fracture site of OVX rats.[41] Systematically, Wang F et al[39] demonstrated that the serum level of VEGF was significantly higher at day 5 post-fracture but lower at day 11 in OVX mice. Significantly higher aggrecan and lower MMP13 levels in serum were also found in OVX mice.[39] Zhang et al[40] presented significantly higher serum levels of TRAP, ALP, C-terminal telopeptide of type 1 collagen (CTX), and OCN in OVX rats.

Clinical studies. CT parameters were reported in a study by Meyer et al[70] This study found that total bone mineral density (D.tot), cortical bone mineral density (D.cort), trabecular bone mineral density (D.trab), and Tb.Th were significantly increased at the fracture site at three to four weeks post-fracture when compared to one to two weeks post-fracture in osteoporotic/osteopenic women, but no difference was reported in compression stiffness (S. comp), torsion stiffness (S.tors), or bending stiffness (S. bend) (Table V).[70]

Gene expression at the fracture site was evaluated in two clinical studies. Tsangari et al[66] demonstrated significantly greater ratio of RANKL/OPG and RANK mRNA expression in the fracture group compared to the no-fracture group.[66] In Caetano-Lopes et al,[63] BMP-2 expression was found to be the highest at the first three post-fracture days compared to days 4 to 7 and days 8 or more after fracture. BMP-4 and insulin-like growth factor 1 (IGF-1) expression were similar among the timepoints (the first three days, days 4 to 7, and days 8 or more post-fracture). Transforming growth factor β 1 (TGF-β1) expression decreased constantly after fracture, while fibroblast growth factor 2 (FGF-2) and platelet-derived growth factor subunit B (PDGF-β) expression was found to decrease slightly from the first three days to days 4 to 7, and drop clearly at days 8 or more post-fracture. Moreover, OPG expression was reported to decrease over time after fracture, while RANK expression showed the opposite trend. Both RANKL expression and RANKL/OPG ratio were found to be highest at days 4 to 7 post-fracture and decrease thereafter. Osteoblast-related genes were also investigated. Core-binding factor, α subunit 1/runt-related transcription factor 2 (CBFA1/RUNX2) expression was shown to be slightly higher at days 4 to 7 post-fracture and remain constant thereafter. Osterix (Osx) expression remained constant and increased after days 4 to 7 post-fracture. On the other hand, ALP expression was slightly lower at days 4 to 7 post-fracture and remained constant later. For osteocyte-related genes, sclerostin (SOST) expression was found to decrease over time after fracture. For osteoclast-related genes, expression of tartrate-resistant acid phosphatase (TRAP), cathepsin K (CTSK), and ATPase H+ transporter (ATP6V0D2) increased over time, while β3 subunit of the avb3 integrin (ITGB3) expression remained constant.[63] Protein expression in serum was investigated in three studies. Lipovetzki et al[69] presented significantly higher OPG levels in postmenopausal women with fracture compared to those without. Meyer et al[70] found no significance in carboxy-terminal

Table V. Summary of healing outcomes in the included clinical studies.

Publication	Experiment	Parameter	Source of sample	Finding
Caetano-Lopes et al[63] 2011	Real-time PCR	BMP-2, BMP-4, TGF-β1, IGF-1, FGF-2, PDGF-β	Femoral epiphysis	BMP-2: highest until 3 days post-fracture and decreased thereafter; BMP-4: remained stable over time; TGF-β1: decreased constantly over time; IGF-1: remained stable over time; FGF-2, PDGF-β: decreased slightly until 8 days after fracture; decreased clearly after 8 days after fracture
		OPG, RANK, RANKL, RANKL/OPG		OPG: Decreased over time; RANK: increased over time; RANKL, RANKL/OPG: highest at days 4 to 7 post-fracture and decreased thereafter
		CBFA1/RUNX2, Osx, ALP		CBFA1/RUNX2: slightly higher at days 4 to 7 post-fracture and remained constant later; Osx: remained constant and increased after days 4 to 7 post-fracture; ALP: slightly lower at days 4 to 7 post-fracture and remained constant later
		SOST		Decreased over time
		TRAP, CTSK, ITGB3, ATP6V0D2		TRAP: increased over time; CTSK: increased over time; ITGB3: remained constant; ATP6V0D2: increased over time
Lipovetzki et al[69] 2017	ELISA	OPG	Serum	Significantly higher in postmenopausal women with fracture compared to those without
Meyer et al[70] 2014	HR-pQCT	D.tot, D.cort, D.trab, Ct.Th, Tb.N, Tb.Th, Tb.Sp	Distal radii	Significantly increased D.tot, D.cort, D.trab, and Tb.Th at 3 to 4 wks compared to 1 to 2 wks post-fracture, similar between 1 to 2 and 3 to 4 wks post-fracture for other parameters
	μFEA	S.comp, S.tors, S.bend		No significant change between 1 to 2 and 3 to 4 wks post-fracture
	Radioimmunoassay	ICTP, PINP	Serum	No significant change between 1 to 2 and 3 to 4 wks post-fracture
Tsangari et al[66] 2004	Real-time PCR	RANKL, OPG, RANKL/OPG, RANK, CTR, OCN	Intertrochanteric trabecular bone	Significantly greater ratio of RANKL/OPG and RANK mRNA expression in fracture group compared to no-fracture group, whereas no significance for other parameters

ALP, alkaline phosphatase; ATP6V0D2, ATPase H+ transporter; BMP-2, bone morphogenetic protein 2; BMP-4, bone morphogenetic protein 4; CBFA1/RUNX2, core-binding factor, alfa subunit 1/runt-related transcription factor 2; CTR, calcitonin receptor; CTSK, cathepsin K; Ct.Th, cortical thickness; D.cort, cortical bone mineral density; D.tot, total bone mineral density; D.trab, trabecular bone mineral density; ELISA, enzyme-linked immunosorbent assay; FGF-2, fibroblast growth factor 2; HR-pQCT, high-resolution peripheral quantitative CT; ICTP, carboxy-terminal telopeptide of type I collagen; IGF-1, insulin-like growth factor 1; ITGB3, β3 subunit of the avb3 integrin; mRNA, messenger RNA; OPG, osteoprotegerin; PCR, polymerase chain reaction; PDGF-β, platelet-derived growth factor subunit B; PINP, procollagen type-I N-terminal propeptide; OCN, osteocalcin; Osx, Osterix; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; SOST, sclerostin; S. bend, bending stiffness; S.comp, compression stiffness; S.tors, torsion stiffness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TGF-β1, transforming growth factor beta 1; TRAP, tartrate-resistant acid phosphatase; μFEA, micro-finite element analysis

telopeptide of type I collagen (ICTP) and procollagen type-I N-terminal propeptide (PINP) among all timepoints (one to two and three to four weeks post-fracture).

Discussion

We reviewed both the local and systemic inflammatory responses in ovariectomy-induced osteoporotic fractures or fragility fractures, and the potential effects of inflammatory response on differential healing outcomes.

In terms of systemic inflammatory response, six out of the seven preclinical studies in this review demonstrated that OVX animals had higher systemic inflammatory response. In terms of proinflammatory cytokines, OVX animals showed significantly higher levels of TNF-α, IL-1β, and Mdk at the early healing stage.[34,38,39] Also, at later stages of fracture healing markedly increased serum levels of TNF-α, IL-1β, and IL-6 were detected in OVX animals.[38,40,41] As for immune cells, Haffner-Luntzer et al[35] reported significantly increased numbers of B-lymphocytes and T-helper cells in the bone marrow of OVX mice at day 1, with the numbers of other immune cells including macrophages and inflammatory monocytes being similar between SHAM and OVX mice. The findings in these six studies demonstrated that at both shorter and longer timepoints post-fracture, OVX groups showed systemically higher levels of proinflammatory cytokines, implying that systemic inflammation remains higher in OVX animals, which would interfere with the local inflammatory response and the subsequent regulation of the healing cascade.

We also reviewed literature that addressed the local inflammatory response following fracture injury. Fischer et al[34] reported that significantly higher levels of IL-6, Mdk, and MCP-1 were detected at the fracture callus in OVX mice at day 3 after fracture. Haffner-Luntzer et al[35] also showed that IL-6 and Mdk expression were higher in the fracture callus of OVX mice at day 3 post-fracture. In terms of immune cells at the fracture callus, only neutrophil population was found to be higher at day 3 in OVX mice, with other immune cell expression being similar between SHAM and OVX mice.[35] These two studies together reported a higher local inflammatory response
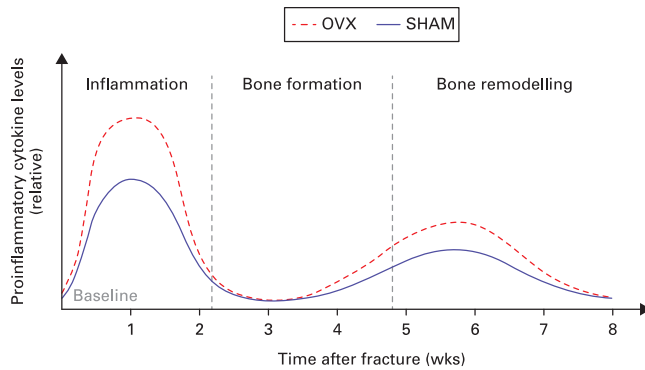
Fig. 2

Schematic diagram depicting the relative levels of systemic proinflammatory cytokines of normal and osteoporotic fracture healing. OVX, ovariectomy; SHAM, normal control.

in OVX animals. On the other hand, Chow et al[38] found that at the fracture site of OVX rats, the levels of TNF-α and IL-6 were lower while IL-10 level was higher at week 1 post-fracture. In the study by Chan et al,[37] with the local injection of 1 ng rhTNF at the fracture site immediately or day 1 after fracture, OVX mice showed increased relative callus mineralization at day 14 compared to SHAM mice. This study demonstrated that the local inflammatory response during fracture healing was reduced in OVX animals and thus healing could be augmented with the enhancement of the inflammatory response via administration of proinflammatory cytokines at the fracture site. Taking these studies together, it is inconclusive whether osteoporotic fracture has higher or lower local inflammatory response compared to normal fracture. Despite being controversial, all of these preclinical studies showed that OVX animals demonstrated inferior healing outcomes, including poorer bone formation[35,38–41] and biomechanical strength.[39,41] Besides, lower levels of osteogenic markers were detected in OVX animals.[39–41] One study also demonstrated that the number of osteoclasts at the fracture site was increased in OVX animals.[41] Thus, it can be seen that healing outcomes in OVX animal fracture models are impaired at either elevated or reduced local inflammatory response, thus indicating that an optimal level of response was disrupted under oestrogen-deprived conditions.

Controlled release of cytokines is of critical importance for normal bone regeneration. Previous studies showed that disrupted inflammatory response would exert a negative effect on fracture healing outcomes. In general, summarizing the findings from all preclinical studies, the level of systemic proinflammatory cytokines was found to be higher in OVX-induced osteoporotic fracture compared to normal fracture at the early inflammation and later bone remodelling stages (Figure 2). Indeed, inflammatory cytokines secreted by different immune cells have been shown to have close interactions with bone cells, including osteoblasts, osteoclasts, and osteocytes.[71,72] Inflammatory cytokines including TNF-α and IL-6 have been shown to

influence osteoblastogenesis or osteoclastogenesis, which would have direct impacts on callus formation, maturation, and remodelling,[36,42] suggesting that these inflammatory cytokines could be key regulators of bone formation. However, excessive expression of proinflammatory cytokines had negative impact on bone formation. In culture, continuous exposure of osteoblasts to IL-1β inhibits their functions, including inhibition of collagen and non-collagen protein synthesis, ALP expression, and cell replication.[73] The administration of a high concentration of TNF-α (10 ng/ml) in C2C12 and primary mouse calvaria cells is shown to inhibit osteoblast differentiation through enhancing the transcription of Smurf1 in an activator protein-1 (AP-1) and Runx2-dependent manner.[74]

Inflammatory cytokines have been reported to have distinct functions at different healing stages. TNF-α has been shown to stimulate MSC recruitment at the early healing stage and later enhance bone resorption activity of osteoclasts to encourage the bone remodelling process.[75] Impaired callus strength and reduced osteoclast numbers were shown in the absence of IL-6 in the early healing stage, while enhanced callus stiffness was observed at the later remodelling stage.[76] Both local and systemic inflammatory cytokines play key roles in different stages of fracture healing. However, the cell types that release these cytokines locally are under-investigated for their roles in coordinating the inflammatory cascade and subsequent healing phases. Therefore, further studies should be conducted to dissect the regulatory role of the inflammatory response in osteoporotic fracture healing. Different fracture regions such as the metaphyseal region could be examined as different fracture sites may show different inflammatory responses after injury.[77,78] In addition, parabiosis mice models could be used to investigate factors that are responsible for age-dependent tissue regeneration, as previous studies identified that a youthful circulation can rejuvenate aged-bone healing.[79,80]

All the included clinical studies reported either the temporal changes of inflammatory markers after fragility fracture or comparison of inflammatory factor levels between fragility fracture patients and normal controls without fracture. So far, there is no clinical study investigating the inflammatory response between osteoporotic fracture and normal fracture, probably due to the difficulties in recruiting such types of patients. Therefore, the differences in the inflammatory response between elderly or osteoporotic patients and normal patients cannot be known at this stage until more studies in this respect are conducted.

It is suggested that in the study design of future clinical studies, fracture patients of younger age should be recruited to serve as the control group and be compared with elderly/osteoporotic patients with fragility fracture, similar to the design of preclinical studies in which SHAM and OVX animals are compared. This way we can really find out the differences of the inflammatory response

between normal and fragility/osteoporotic fracture healing in human subjects and fill the existing knowledge gap.

All of the included clinical studies are prospective cohort studies in which human subjects with different characteristics such as age, fracture site, and fracture type were recruited. Although four studies looked at the temporal changes of inflammatory factors, the timepoints and outcome measures were different. The remaining four studies examined the differences of inflammatory factor expression between fragility fracture and controls without fracture, but their parameters were different as well. Also, the studies do not involve common treatment. Thus, it is not feasible to perform a meta-analysis due to the heterogeneity in terms of participants, interventions, and outcomes of the included clinical studies.

Taking all the studies into consideration, of all the inflammatory markers reported IL-6 and TNF-α were two major markers shown to have a close relationship with bone formation or healing-related outcomes. Currently, these two markers are being commonly studied in both animal and human studies on fracture healing. Thus, they are suggested to be included in future clinical studies by examining their levels in serum/plasma or gene expression locally at the fracture site.

In this review, three clinical studies reported the temporal changes of inflammatory cytokines in fragility fracture. All of them included the investigation of IL-6, and they all demonstrated that IL-6 increased considerably at earlier stages of fragility fracture healing and diminished over time.[34,63,64] It has been postulated that IL-6 is responsible for stimulating the early stage of fracture healing, as reported in a previous study showing that IL-6 level in trauma patients decreased six months after injury.[81] Another study also reported that IL-6 elevated to a great level in patients with head injury within hours of injury, as IL-6 and receptor complex may be capable of stimulating cells of the mesenchymal lineage and therefore may enhance the formation of osteoblastic bone.[82] Besides, two included preclinical studies (Haffner-Luntzer M et al[35] and Fischer V et al[34]) also found significantly higher IL-6 levels at the fracture callus of OVX mice compared to normal mice at day 3 post-fracture, further showing the significant increase of IL-6 at the early phase of fracture healing in osteoporotic subjects. Therefore, the expression of IL-6 or other major proinflammatory markers in osteoporotic fracture patients at the first three days as compared to non-osteoporotic fracture patients is a key knowledge gap that should be further investigated.

In addition, one preclinical study (Wang J et al[41]) found that OVX rats showed significantly higher serum IL-6 level and RANKL mRNA expression at week 6 post-fracture. One clinical study (Tsangari et al[66]) reported that the IL-6 mRNA level was shown to be positively correlated with the RANKL level. These two studies showed a positive association between levels of IL-6 and RANKL, which was shown to activate osteoclasts and promote bone resorption.[83] However, there is no other study investigating the relationships between inflammatory cytokines and fracture healing outcomes. This proposes a pressing need for further studies to provide more evidence on the effects of changes of inflammatory response on different fracture healing outcomes.

As seen from the findings of all the included studies, the inflammatory response has a great impact on the healing process; any disruption of the inflammatory reaction will negatively affect different healing outcomes. Bone healing in aged or osteoporotic individuals has higher risk of failure, usually accompanied with delayed healing or nonunions. Novel therapies to improve age-associated impairment in fracture healing are needed. So far, there have been different studies targeting bone formation-related pathways such as the BMP signalling pathway.[84,85] The tight interaction between the immune system and the skeletal system has been recognized in the emerging research field of osteoimmunology.[86] Thus, the inflammatory response could also be one potential target for immune-modulatory approaches to enhance fracture healing, as a previous study demonstrated that modulation of inflammatory reaction could enhance osteogenesis.[87] Further investigation into the roles and interaction of inflammatory factors and bone cells is necessary for a better understanding of fragility/osteoporotic fracture healing and development of treatment to enhance it.

There are several limitations in this review. Firstly, not all subjects in the clinical studies were confirmed as having osteoporosis. Secondly, meta-analysis was not feasible due to the heterogeneity of the included studies, which may weaken the conclusion. Thirdly, only studies written in English were included, which may not fully cover all the evidence available.

In conclusion, the systemic inflammatory response during osteoporotic fracture healing is generally higher compared to normal fractures as demonstrated by the expression of proinflammatory cytokines and elevation in immune cell counts in animals. As for the local inflammatory response, it is inconclusive whether OVX animals have higher or lower local inflammatory response. But the healing outcomes have been shown to be impaired. Regarding the clinical studies, there is no study investigating the difference in the inflammatory response between osteoporotic and non-osteoporotic patients. All clinical data presented could only serve as reference data for the temporal changes of common inflammatory cytokines in osteoporotic fracture patients. Taken together, a suboptimal inflammatory response could imply poorer fracture healing in OVX subjects or postmenopausal osteoporotic or aged individuals with fragility fracture, thus there is a need for further studies to better understand the role of the inflammatory response in the healing cascade for potential immunomodulation to enhance osteoporotic fracture healing.

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Author information:
- S. K-H. Chow, BSc, MSc, PhD, Research Assistant Professor
- W-H. Cheung, BSc, PhD, Associate Professor
  Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, Hong Kong; The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, China.
- Y-N. Chim, PhD, BEng, Postdoctoral Research Fellow
- J-Y. Wang, MD, PhD, Resident Doctor
- V. M-H. Choy, BSc, PhD, Postdoctoral Research Fellow
  Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, Hong Kong.
- R. M-Y. Wong, BCMPH, MBChB, MRCS(Ed), PhD, Resident Doctor, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, Hong Kong.