Serum caspase-1 levels serve as a predictive biomarker for the prognosis of patients undergoing arthroscopic-assisted locking plate internal fixation in the treatment of high-energy pilon fractures

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Abstract. The present study aimed to investigate the serum levels of caspase-1 in patients with high-energy pilon fractures, and its correlation with prognosis and clinical results. In this prospective study, 136 patients with high-energy pilon fractures who were treated with a locking plate combined with ankle arthroscopy from July 2015 to July 2020 were included. The treatment efficacy was evaluated according to the Mazur ankle function score. Serum caspase-1, interleukin (IL)-6, IL-1β and C-reactive protein (CRP) levels were measured using enzyme-linked immunosorbent assay. Reverse transcription-quantitative PCR was used to measure the mRNA expression of caspase-1. Additionally, demographic data and clinical characteristics, such as sex, age, intraoperative blood loss, fracture healing time, fracture classification and complications were collected and analyzed. The study revealed that the intraoperative blood loss, proportion of Ruedi-Allgower III and the serum levels of caspase-1 in the poor prognosis group were significantly higher compared with those in the good prognosis group. Additionally, patients with high-energy pilon fractures in the poor prognosis group exhibited significantly higher levels of caspase-1 and IL-1β serum levels at all time points in contrast to those in the good prognosis group. Spearman's analysis revealed a significant association between caspase-1, IL-1β levels and Mazur scores. Furthermore, caspase-1 could serve as a potential diagnostic biomarker for poor prognosis of patients with high-energy pilon fractures. Caspase-1, IL-1β, intraoperative blood loss and Ruedi-Allgower grade were the risk factors for poor prognosis in patients with high-energy pilon fractures. In summary, this study demonstrated that serum caspase-1 levels were progressively reduced during the treatment of high-energy pilon fractures patients and prominently lowered in those with a favorable prognosis. These findings could provide novel targets and a comprehensive approach to protecting patients with high-energy pilon fractures.

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Abbreviations: IL, interleukin; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; CT, computed tomography; AUC, area under curve

Key words: high-energy pilon fracture, locking plate, pyroptosis, caspase-1, risk factors of clinical outcomes

Introduction

Pilon fractures are lower segment injuries of the tibia that usually occur due to high-energy impact and affect the inferior articular surface. These fractures may be accompanied by additional fractures to the medial, lateral or posterior malleoli (1). A distal tibial plafond fracture is also known as a pilon fracture, which describes the high-energy axial compression force of the tibia acting as a pestle, driving vertically into the talus. These fractures constitute 1-10% of the lower leg or tibial fractures and are frequently accompanied by severe bone comminution and soft-tissue compromise (2,3). These fractures may also extend to the metaphyseal extension and be associated with fibular fractures (4). At present, high-energy pilon fractures are primarily caused by traffic accidents and high falls (5).

The two most common classification systems used to describe pilon fractures are the Ruedi-Allgower classification and the Arbeitsgemeinschaftfür Osteosynthesefragen Foundation/Orthopedic Trauma Association classification. Currently, the Ruedi-Allgower classification is the most commonly used clinical classification for pilon fractures, which is mainly evaluated according to the degree of articular surface involvement and bone block displacement (6). Type II and III pilon fractures are mostly caused by high-energy traumas (5,7). When soft-tissue damage is severe and the articular surface is broken, surgical reduction and fixation become challenging, and the overall prognosis for the patient is unfavorable (8). Pilon fractures can be some of the most difficult fractures to treat; as they are often associated with high-energy trauma, both soft-tissue involvement and comminuted fracture patterns pose challenges to fixation (9). Traditional clinical

treatment for high-energy pilon fractures typically involves external fixation combined with limited internal fixation (10) and delayed open reduction and internal fixation (11-13). With advancements in instruments and the refinement of surgical techniques, arthroscopy is now also being utilized for the treatment of high-energy pilon fractures (14-17).

Fractures can result in a variety of cellular responses, including pyroptosis (18). Pyroptosis is a form of programmed cell death that occurs when cells are exposed to inflammatory signals or pathogens, and caspase-1 serves as a significant biomarker associated with pyroptosis (19). The molecular mechanism of pyroptosis involves the activation of a protein complex called the inflammasome, which leads to the cleavage and activation of caspase-1 (20). Once caspase-1 is activated, it can then cleave several downstream substrates, including gasdermin D. Cleavage of gasdermin D leads to the formation of membrane pores, which allow the release of cytoplasmic contents and ultimately result in cell death (21). Additionally, the activation of caspase-1 can also lead to the release of pro-inflammatory cytokines, such as interleukin (IL)-1β and IL-18, which can further exacerbate inflammation and tissue damage (22). The latest research has revealed the value of the NLRP3/caspase-1/gasdermin D pyroptosis pathway in fracture repair (23). However, to the best of our knowledge, no studies have addressed the clinical significance of serum caspase-1 in patients with fractures.

The present prospective observational study was conducted from July 2015 until July 2020, with 136 cases of high-energy pilon fracture being treated using an arthroscopic-assisted locking plate. The objective was to investigate serum levels of caspase-1 in patients with high-energy pilon fractures and their correlation with clinical results and prognosis.

Patients and methods

Patients. The present prospective observational study included 136 patients (mean age, 47; age range, 24-66; 52.9% female) with high-energy pilon fractures who were treated with locking plates combined with ankle arthroscopy in Ordos Central Hospital (Ordos, China) from July 2015 to July 2020. The entire study was conducted according to the Declaration of Helsinki. The study was approved by the Ethics Committee of Ordos Central Hospital (approval no. IRB2015-OCHOS-116). Written informed consent was obtained from all participants.

The inclusion criteria were: i) Meeting the diagnostic criteria of a pilon fracture, with a history of high-energy trauma with soft tissue injury; ii) admission immediately after trauma, with the time from trauma to admission <360 min; and iii) complete clinical data. The following patients were excluded: i) Bilateral pilon fracture; ii) osteofascial compartment syndrome; iii) severe injury to other organs; and iv) shock.

Rüedi and Allgower classifies pilon fractures into three types. Type I fractures involve column displacement of the articular surface. Type II fractures involve displacement of the articular surface without comminution. Type III fractures are comminuted, involving the metaphysis and articular surface (24). The present study combined type I and II fractures into type II.

Surgery. All patient treatments and surgeries were performed by the same team at Ordos Central Hospital. At admission, the affected ankle joints of all patients were reconstructed with anteroposterior and lateral axial computed tomography (CT) three-dimensional reconstruction to accurately reconstruct the fracture morphology and understand the fracture pattern. The doctor determined the operation time based on the extent of soft tissue damage of each patient. Notably, the three-dimensional reconstruction of the CT was performed prior to the selective operation to ensure that the fracture block remained unchanged after traction. The CT scan was performed after calcaneal traction in all cases that required traction.

General anesthesia was administered to all patients who were positioned supine. A lower limb tourniquet was used under the premise that pressure and time were controlled. The fibula was internally fixed to restore the anatomical length of the fibula as much as possible. Any osteochondral debris and blood clots present in the joint were removed, and the displacement of the articular fracture was observed. The fracture was reduced through prying and pulling under arthroscopy and secured with Kirschner wire. Following confirmation of a level articular surface on the distal tibia, the arthroscopy was withdrawn. A C-arm X-ray machine was used to check the reduction. The steel plate was positioned based on the structural characteristics of the fracture and the condition of the skin surrounding the ankle joint. A small incision was made locally to minimize the excessive stripping of the periosteum and protect the soft tissue as much as possible. With the assistance of the tibial tunnel locator, the contralateral fracture block was secured using the appropriate tibial multiaxial locking plate. The reduction of the articular surface was examined. If the reduction was successful, holes were drilled and nails were placed to complete tibial fixation. Microscopically, the articular cartilage defect was treated with microfracture to repair the damaged ligament structure around the ankle joint. After washing and suturing, a drainage tube was placed.

For postoperative treatment, the affected limb was fixed with a brace and then raised. The prophylactic use of antibiotics is recommended for open high-energy pilon fractures to prevent infection. The drainage tube was removed in a timely manner according to the drainage condition. The patient was instructed to conduct exercises so long as no obvious pain was experienced in the affected area to ensure a step-by-step progression during postoperative functional rehabilitation. All patients underwent postoperative radiographs with time intervals, as well as postoperative CT scans.

Blood sampling measurement. The enzyme-linked immunosorbent assay (ELISA) was utilized to measure the serum levels of caspase-1, IL-6, IL-1β and C-reactive protein (CRP). All cases had their blood samples collected from the median cubital vein (5 ml) after fasting for 8 h but within 24 h after admission for all cases. These blood samples were centrifuged at 2,000 x g and a temperature of 4˚C for 15 min, and the serum (supernatant) was analyzed by ELISA testing utilizing commercially available kits [caspase-1 (cat. no. MBS084975); IL-6 (cat. no. MBS175877); CRP (cat. no. MBS177184); IL-1β (cat. no. MBS175901); all from MyBioSource, Inc.]. Serum biomarker levels were measured at 0, 1, 2, 3, 4 weeks and 3, 6, 12 and 24 months after treatment initiation.

Reverse transcription-quantitative PCR (RT-qPCR). All 136 patients in the two groups were measured for their

Table I. Basic characteristics of all patients.

Variable	Good prognosis group (n=107)	Poor prognosis group (n=29)	P-value
Age, years	46 (24-66)	51 (29-65)	0.112
Female sex, n (%)	58 (54.2)	14 (48.2)	0.480
BMI	25.11±2.46	24.71±2.40	0.890
Intraoperative blood loss, ml	106.17±8.91	117.06±6.82	<0.001
Fracture healing time, weeks	13 (6-22)	17 (8-23)	0.037
Injury causes, n (%)			
Falling	58 (54.21)	13 (44.83)	0.663
Traffic accidents	49 (45.79)	16 (45.17)	0.663
Ruedi-Allgower, n (%)			
II	66 (61.68)	5 (17.24)	<0.001
III	41 (39.32)	24 (82.76)	<0.001
Fracture types, n (%)			
Closed	64 (59.81)	18 (62.07)	0.885
Open	43 (40.19)	11 (37.93)	0.885
Complications, n (%)			
Infection	5 (4.67)	2 (6.90)	0.767
Non-union	3 (2.80)	1 (3.45)	0.999
Other	5 (4.67)	2 (6.90)	0.767
Caspase-1, pg/ml	54.07±2.68	56.13±3.37	0.001
CRP, pg/ml	1,986.02±162.13	1,969.80±150.73	0.629
IL-1β, pg/ml	20.95±3.15	20.58±3.39	0.577
IL-6, pg/ml	23.13±3.88	22.79±4.51	0.683
Mazur score	93 (87-99)	75 (64-85)	<0.001

Continuous data with non-normal distribution were expressed by median (minimum to maximum) and analyzed by Mann-Whitney U test (age, fracture healing time and mazur score). Continuous data with normal distribution were expressed by mean ± standard deviation and analyzed using unpaired Student's t-test (BMI, intraoperative blood loss, Caspase-1, CRP, IL-1β and IL-6). Chi square test was used for rates (Injury causes, Ruedi-Allgower, fracture types and complications). BMI, body mass index; CRP, C-reactive protein; IL, Interleukin.

mRNA expression of caspase-1 using RT-qPCR. For total RNA extraction from serum samples, the present study used the RNAiso Plus kit (Takara Bio, Inc.), and subsequently transcribed it into cDNA using the Prime-Script™ one-step RT-qPCR kit (Takara Bio, Inc.) according to the manufacturer's protocols. The RT-qPCR process was performed on the ABI PRISM7300 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the SYBR Premix ExTaq (Takara Bio, Inc.). The thermocycling conditions used for qPCR were as follows: An initial activation step at 95˚C for 15 min, followed by 35 cycles of denaturation at 94˚C for 15 sec, annealing at 55˚C for 25 sec and extension at 70˚C for 30 sec. Primer sequences for caspase-1 were as follows: Forward, 5'-CACACCGCCCAGAGCACAAG-3', and reverse, 5'-TCCCACAAATGCCTTCCCGAATAC-3'. Primer sequences for GAPDH were as follows: Forward, 5'-ACACCATGTATTCCGGGTCAAT-3', and reverse, 5'-CCACCACCCTGTTGCTGTAG-3'. GAPDH was utilized as an internal control. Expression levels of caspase-1 were calculated using the $2^{-\Delta\Delta Cq}$ method (25).

Data collection and scale scoring. Demographic and clinical statistics, such as age, BMI, sex, intraoperative blood loss, fracture healing time, Ruedi-Allgower grade, injury causes (falling or traffic accidents), fracture types (closed or open) and post-surgery complications, were collected. In addition, all patients were followed up for at least 24 months, and data from the 24-month period were used with follow-up through to the end of July 2022, and patients received the last follow-up in July 2022. The Mazur ankle joint evaluation system was used to assess the effectiveness of the treatment (26). Patients with Mazur scores ≥87 were defined as having a good prognosis, while patients with Mazur scores ≤86 were defined as having a poor prognosis.

Statistical analysis. The database was established using the statistical software SPSS 25.0 (IBM Corp.). The normal distribution of data was confirmed by the Kolmogorov-Smirnov analysis. Mean ± standard deviation was used to express normal distribution data, while non-normal distribution data were expressed as median (range). The comparison between two groups was carried out using the Mann-Whitney test or unpaired Student's t-test. Rates were determined using χ^2 test, and correlation analysis was conducted using Spearman's rank correlation. Receiver operating characteristic (ROC) curve analysis was conducted to diagnose the ability of caspase-1 for predicting poor prognosis in patients with high-energy pilon

Table II. Comparison of baseline data for sex subgroup analysis.

Variable	Male (n=64)	Female (n=72)	P-value
Age, years	44 (24-64)	49 (24-66)	0.370
BMI	25.33±2.40	24.78±2.49	0.280
Intraoperative blood loss, ml	109.35±9.77	107.72±9.43	0.325
Fracture healing time, weeks	13 (6-22)	16 (6-23)	0.362
Caspase-1, pg/ml	54.75±2.92	54.30±2.98	0.376
CRP, pg/ml	1,958.18±156.90	2,004.23±159.48	0.093
IL-1β, pg/ml	20.79±3.04	20.94±3.35	0.783
IL-6, pg/ml	23.42±3.59	22.74±4.35	0.326
Mazur score	91 (64-99)	92 (64-99)	0.906
Poor prognosis, n (%)	15 (22.7)	14 (19.4)	0.603

Continuous data with non-normal distribution were expressed by median (minimum to maximum) and analyzed by Mann-Whitney U test (age, fracture healing time and mazur score). Continuous data presented normal distribution were expressed by mean ± standard deviation and analyzed by unpaired Student's t-test (BMI, intraoperative blood loss, Caspase-1, CRP, IL-1β and IL-6). Chi square test was used for rates (poor prognosis). BMI, body mass index; CRP, C-reactive protein; IL, Interleukin.

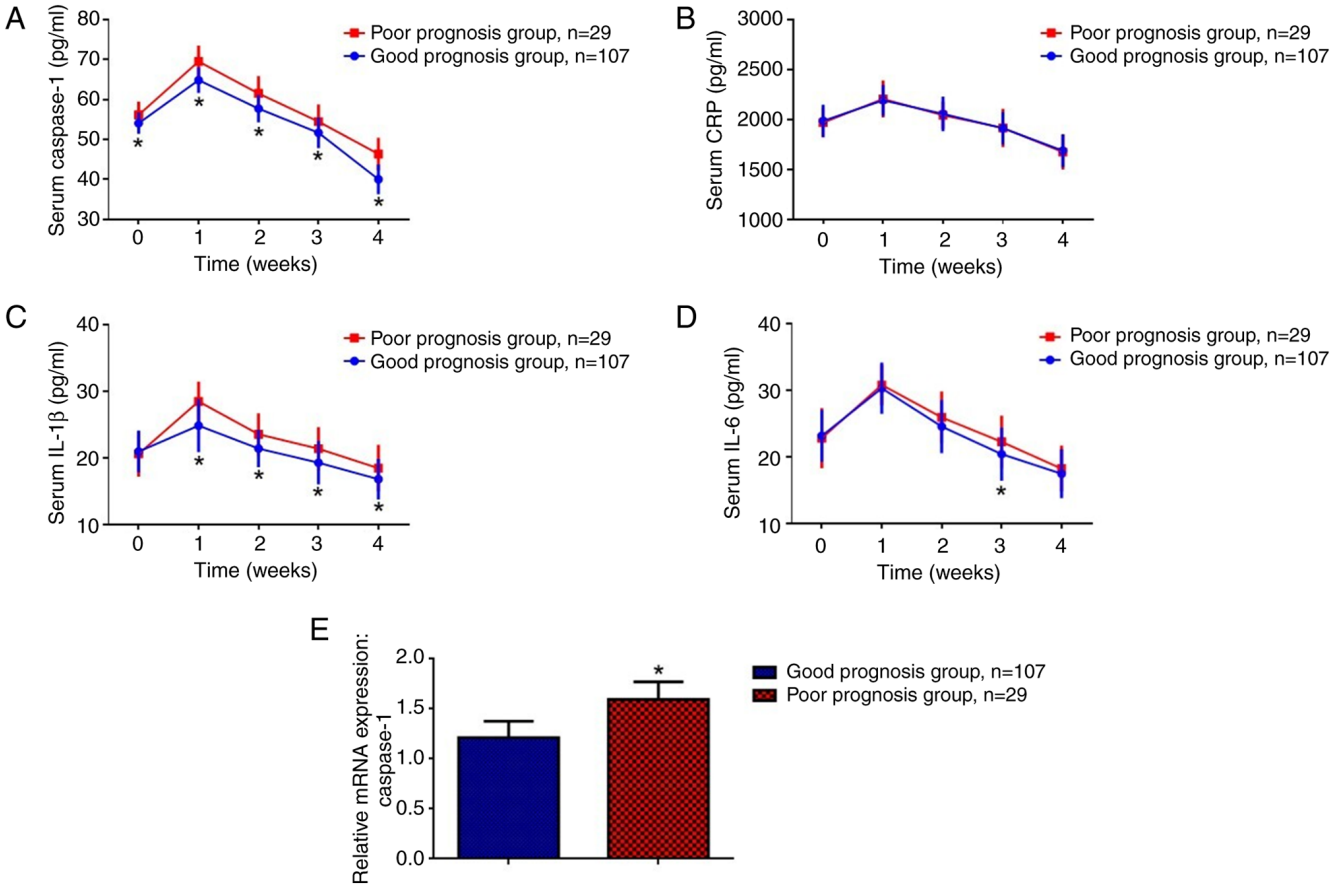

Figure 1. Changes in expression of serum (A) caspase-1, (B) CRP, (C) IL-1β and (D) IL-6 during the study time. (E) mRNA levels of caspase-1 at 1 week after surgery. Good prognosis group (n=107), poor prognosis group (n=29). *P<0.05 compared with the poor prognosis group at the same time point. CRP, C-reactive protein; IL, Interleukin.

fractures. Logistic regression was performed to identify risk factors for poor prognosis in these patients, with high-energy pilon fractures. All data were analyzed with three replicates to ensure statistical robustness. P<0.05 was considered to indicate a statistically significant difference.

Results

Basic characteristics of all patients. The present research enrolled a total of 136 patients with high-energy pilon fractures. All patients measured Mazur ankle joint evaluation system

after 24 months of surgery. The participants were categorized into two groups: The good prognosis group (Mazur score ≥87; n=107) and the bad prognosis group (Mazur score ≤86; n=29). Comparing the demographic and clinical data of two groups when the patients hospitalized, the present study observed significant differences. The poor prognosis group exhibited markedly higher levels of intraoperative blood loss, proportion of Ruedi-Allgower III and the serum levels of caspase-1 compared with the good prognosis group (P<0.05; Table I). The Mazur scores in patients with good prognoses were markedly higher than those in patients with bad prognoses (P<0.05; Table I). Additionally, the fracture healing time in patients with bad prognoses was significantly longer compared with the patients with poor prognoses (P<0.05; Table I). Subsequently, the present study conducted a subgroup analysis based on sex and observed no significant differences in intraoperative blood loss, postoperative healing time, baseline serum cytokine levels and Mazur scores between male and female patients (Table II).

Comparisons of caspase-1 and inflammatory factors between the good and bad prognosis groups. To further investigate the connection between caspase-1 and inflammation in patients with high-energy pilon fractures, the present study plotted line graphs for all participants. As the levels of these cytokines had already returned to normal levels from the 3rd month after surgery, and there was no significant difference between the two groups, the present study used serum levels of the cytokines in the first month postoperatively for analysis. These graphs depict the fluctuations of caspase-1 and inflammatory biomarkers over the course of the study. The levels of serum biomarkers (caspase-1, CRP, IL-1β and IL-6) increased in both groups within the first week, but gradually decreased during the subsequent treatment and follow-up periods (Fig. 1A-D). Furthermore, significant differences in the levels of serum caspase-1 and IL-1β levels were observed among patients with high-energy pilon fractures in the poor prognosis group compared with those in the good prognosis group at all time points (P<0.05). Conversely, no significant differences were found in serum CRP and IL-6 levels were found between the two groups (Fig. 1B and D). The serum levels of the cytokines at the 3rd, 6th, 12th and 24th months after surgery for all patients are presented in Table SI. There were no significant differences in any serum cytokine levels between the good and poor groups from 3-24 months after surgery.

As cytokines levels during the first postoperative week were the highest of all time points during the study, based on the methodology of previous studies (27-29), serum biomarkers levels in the first week after surgery were used for further analysis. To verify the high expression of caspase-1 in patients with high-energy pilon fractures who have a poor prognosis, the present study analyzed the mRNA expression of caspase-1 in the serum of all patients during the first week after surgery using PCR. The results indicated that the mRNA expression of caspase-1 was significantly higher in patients with a poor prognosis compared with those with a good prognosis (P<0.05; Fig. 1E). Spearman's analysis demonstrated a significant association between caspase-1 and IL-1β levels and Mazur scores (P<0.05; Table III). The results of the correlation analyses at the other time points are presented in Tables SII-V, and starting from the third week after surgery, serum caspase-1 levels were not significantly associated with other inflammatory factors and Mazur scores.

Table III. Correlation analysis between caspase-1 and clinical factors.

	Caspase-1	
Variables	Spearman's correlation	P-value
Intraoperative blood loss	0.111	0.200
Fracture healing time	0.130	0.132
CRP	0.117	0.175
IL-1β	0.183	0.033
IL-6	-0.015	0.876
Mazur scores	-0.294	0.001

CRP, C-reactive protein; IL, Interleukin.

Table IV. Logistic regression for risk factors of poor prognosis of patients with high-energy pilon fractures at 1 week after surgery.

Variables	Wald	Odds ratio	95% CI	P-value
Age	3.706	1.074	0.999-1.154	0.054
Sex	0.003	0.954	0.181-5.019	0.955
BMI	0.884	1.174	0.840-1.641	0.347
Intraoperative blood loss	11.056	1.249	1.069-1.424	0.001
Fracture healing time	0.441	1.061	0.891-1.262	0.506
Ruedi-Allgower	7.482	13.351	2.085-85.501	0.006
CRP	0.091	0.999	0.994-1.004	0.763
IL-1β	6.499	1.335	1.069-1.668	0.011
IL-6	0.272	0.940	0.744-1.187	0.602
Caspase-1	7.733	1.470	1.120-1.928	0.005

BMI, body mass index; CRP, C-reactive protein; IL, Interleukin.

Predictive value of caspase-1 for poor prognosis of patients with high-energy pilon fractures. The present used the serum caspase-1 levels at baseline and at 1, 2, 3 and 4 weeks after surgery to draw ROC curves to evaluate the predictive value of caspase-1 for poor prognosis of patients with high-energy pilon fractures. The results showed that caspase-1 had the greatest predictive value for poor prognosis in patients with high-energy pilon fractures during the first week after surgery (Fig. 2), the AUC for caspase-1 was 0.811, with a cutoff value of 67.45 ng/ml; the sensitivity and specificity were 72.4 and 74.8%, respectively.

Logistic regression for risk factors of poor prognosis of patients with high-energy pilon fractures. Finally, a binary regression analysis was conducted for the risk factors associated with poor

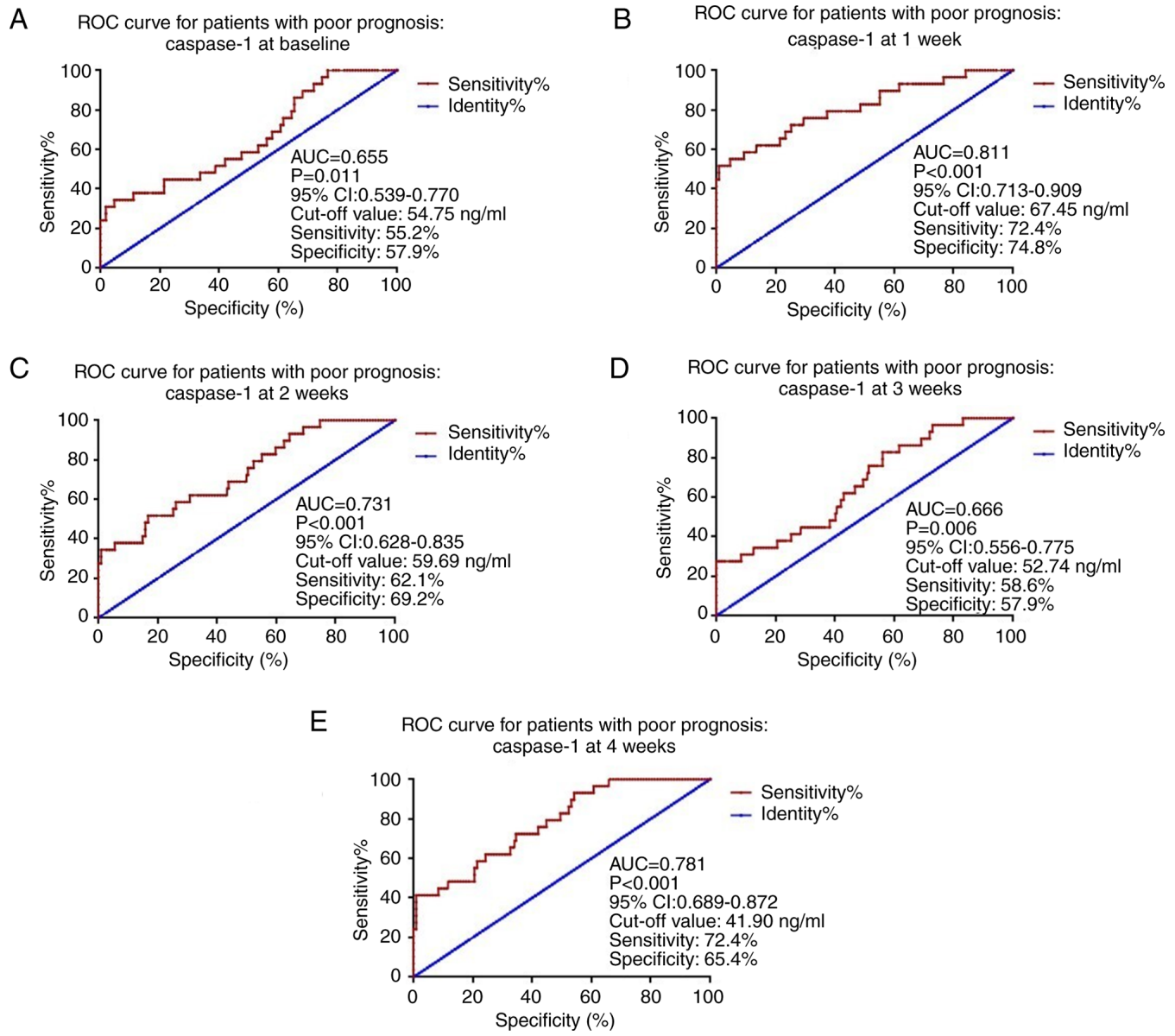

Figure 2. ROC curves for diagnostic value of caspase-1 at (A) baseline, (B) 1 week, (C) 2 weeks, (D) 3 weeks and (E) 4 weeks for patients with poor prognosis after high-energy pilon fractures. ROC, receiver operating characteristic; AUC, area under curve; CI, confidence intervals.

prognosis in patients with high-energy pilon fractures. It was revealed that caspase-1 (95% CI, 1.120-1.928; P=0.005), IL-1β (95% CI, 1.069-1.668; P=0.011), intraoperative blood loss (95% CI 1.069-1.424; P=0.001) and Ruedi-Allgower grade (95% CI 2.085-85.501; P=0.006) at 1 week after surgery were the risk factors for poor prognosis of patients with high-energy pilon fractures (Table IV). The results of the multivariate logistic regression of serum biomarkers at the other time points are presented in Tables SVI-IX, and serum caspase-1 level at other time points within 1 month of surgery was a risk factor for a poor prognosis in patients with high-energy pilon fractures.

Discussion

The levels of pilon fracture (30) can be divided into three, as follows: i) Slight fracture on the articular surface of the distal tibia; ii) obvious joint displacement with a small degree of comminution; and iii) severe comminuted fracture on the articular surface of the distal tibia. Regardless of the severity of the fracture, the patient experiences considerable physical and psychological pain, thereby impacting their quality of life (31). If the treatment method is not appropriate, it not only fails to alleviate the fracture but also exacerbates the condition, resulting in complications such as irregularity of the joint surface, arthritis, malunion and infection (32,33). Therefore, it is urgent to develop new biomarkers and comprehensive techniques to prompt diagnosis and promptly treatment of the patients who may experience a poor prognosis. The present study revealed that the serum levels of caspase-1 had the potential to serve as a diagnostic biomarker for identifying poor prognosis in patients with high-energy pilon fractures.

Combining a locking plate with ankle arthroscopy offers several advantages: Firstly, it leads to minimal trauma during the operation, which in turn reduces blood loss; secondly, since the joint remains unexposed, the probability of infection is thus significantly reduced; thirdly, with the use of fluoroscopy,

the amount of radiation from fluoroscopy is lower; fourthly, the fracture can be observed inside the joint, resulting in a more accurate treatment position. Disadvantages are threefold. Firstly, it results in a larger open fracture site, which aggravates the fracture; secondly, proper attention should be paid to the classification of the joint and the severity of fracture when reducing and fixing it; thirdly, when using arthroscopy, sodium chloride solution should be continuously injected into the joint (34,35). This can penetrate into every compartment of the leg, leading to an increase in the frequency of membranous space syndrome (36). The severity of the trauma, the extent of the initial soft tissue injury and the accuracy of the articular surface reconstructions are key to treating high-energy pilon fractures.

In the present study, according to the fracture classification, the postoperative recovery effect of Rüedi-Allgower type II fractures was significantly improved compared with that of type III fractures. Thus, a greater severity of fracture injury resulted in a lower final recovery effect. This finding may be closely related to the severe injury or displacement observed in type III fractures, complications and high incidence rates. In the present paper, the total effective rate of locking plate internal fixation combined with ankle arthroscopy intreating of high-energy pilon fracture patients was 80.15%, which is comparable to the effectiveness of other treatments (26,37,38).

In the context of fractures, pyroptosis can be triggered by the release of damage-associated molecular patterns (DAMPs) from injured cells (39). These DAMPs can activate the inflammasome and trigger pyroptosis in nearby cells, resulting in a localized inflammatory response. This response can contribute to the progression of tissue damage and impair the healing process (40). At present, several cell and animal studies have investigated the link between cell pyroptosis and fractures. Yang *et al* demonstrated that high glucose concentrations may activate pyroptosis through the caspase-1/gasdermin D/IL-1β pathway, which inhibits the proliferation and differentiation of osteoblasts in the alveolar bone (41). Zhu *et al* revealed that pyroptosis is a crucial pathway in osteomyelitis, and inhibition of the pyroptosis pathway can alleviate the bone destruction caused by *Staphylococcus aureus*-induced osteomyelitis, providing a potential therapeutic target for osteomyelitis (18). In addition, Zhang *et al* observed that inhibiting the pyroptosis pathway of NLRP3/caspase-1/gasdermin D promotes the proliferation and differentiation of osteoblasts, thereby improving osteoblast function in fracture repair (23). The present study also found that the level of the key factor of pyroptosis, caspase-1, gradually decreased during the treatment of patients with high-energy pilon fractures, and decreased more quickly in patients with a better prognosis. In addition, the serum level of caspase-1 was significant associated with the serum level of IL-1β.

The present research also had some limitations. First, the present study only included a small size of the study population. Secondly, only a small number of inflammatory factors were investigated. Thirdly, the observed correlations, although statistically significant, are weak, and further in-depth research is needed to understand the clinical significance and molecular mechanism of caspase-1 in pilon fractures.

In conclusion, the current study illustrated that the serum caspase-1 levels were gradually decreased during the treatment of patients experiencing high-energy pilon fractures, and decreased more rapidly in patients with a better prognosis. There was a significant association between caspase-1 and IL-1β levels and Mazur scores. Furthermore, serum levels of Caspase-1 could potentially serve as a potential diagnostic biomarker for poor prognosis in patients with high-energy pilon fractures. The current study may provide novel targets and a comprehensive approach to protecting patients with high-energy pilon fractures.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XG collected and analyzed the data and wrote the manuscript. FL, GW and YG interpreted the results and collected the data. XS designed and supervised the research, interpreted and discussed the data and wrote and submitted the manuscript. All authors read and approved the final manuscript. XG and XS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the ethic committee of Ordos Central Hospital (approval no. IRB2015-OCHOS-116). Written informed consent was obtained by all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zelle BA, Dang KH and Ornell SS: High-energy tibial pilon fractures: An instructional review. Int J Orthop 43: 1939-1950, 2019.
2. Bone LB: Fractures of the tibial plafond. The pilon fracture. Orthop Clin North Am 18: 95-104, 1987.
3. Sirkin M and Sanders R: The treatment of pilon fractures. Orthop Clin North Am 32: 91-102, 2001.
4. Kottmeier SA, Madison RD and Divaris N: Pilon fracture: Preventing complications. J Am Acad Orthop Surg 26: 640-651, 2018.
5. Qiu XS, Li XG, Qi XY, Wang Z and Chen YX: What is the most reliable classification system to assess tibial pilon fractures? J Foot Ankle Surg 59: 48-52, 2020.
6. Wiebking U: Pilon fractures: Review of diagnostics and classification. Unfallchirurg 120: 632-639, 2017 (In German).
7. Leonetti D and Tigani D: Pilon fractures: A new classification system based on CT-scan. Injury 48: 2311-2317, 2017.

8. Chowdhry M and Porter K: The pilon fracture. Trauma 12: 89-103, 2010.
9. He X, Hu Y, Ye P, Huang L, Zhang F and Ruan Y: The operative treatment of complex pilon fractures: A strategy of soft tissue control. Indian J Orthop 47: 487-492, 2013.
10. Dombrowsky A, Abyar E, McGwin G and Johnson M: Is definitive plate fixation overlap with external fixator pin sites a risk factor for infection in pilon fractures? J Orthop Trauma 35: e7-e12, 2021.
11. Hebert-Davies J, Kleweno CP and Nork SE: Contemporary strategies in pilon fixation. J Orthop Trauma 34 (Suppl 1): S14-S20, 2020.
12. Osman W, Alaya Z, Kaziz H, Hassini L, Braiki M, Naouar N and Ben Ayeche ML: Treatment of high-energy pilon fractures using the ILIZAROV treatment. Pan Afr Med J 27: 199, 2017.
13. Yao ND and Wang FL: Delayed operation on treatment of high-energy distal tibia Pilon fracture. Zhongguo Gu Shang 24: 256-258, 2011 (In Chinese).
14. Martin KD: Posterior arthroscopic reduction and internal fixation for treatment of posterior malleolus fractures. Foot Ankle Int 41: 115-120, 2020.
15. Barbera J, Selverian S, Courington R, Mikhail C and Colvin A: The top 50 most influential articles in hip arthroscopy. Arthroscopy 36: 716-722, 2020.
16. Luo H, Chen L, Liu K, Peng S, Zhang J and Yi Y: Minimally invasive treatment of tibial pilon fractures through arthroscopy and external fixator-assisted reduction. Springerplus 5: 1923, 2016.
17. El-Mowafi H, El-Hawary A and Kandil Y: The management of tibial pilon fractures with the Ilizarov fixator: The role of ankle arthroscopy. Foot (Edinb) 25: 238-243, 2015.
18. Zhu X, Zhang K, Lu K, Shi T, Shen S, Chen X, Dong J, Gong W, Bao Z, Shi Y, *et al*: Inhibition of pyroptosis attenuates *Staphylococcus aureus*-induced bone injury in traumatic osteomyelitis. Ann Transl Med 7: 170, 2019.
19. Liu J, Liu W, Lv P, Wang Y and Ouyang X: Activation of nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 6 by Porphyromonas gingivalis regulates programmed cell death in epithelium. J Dent Sci 18: 1867-1875, 2023.
20. Tsuchiya K: Inflammasome-associated cell death: Pyroptosis, apoptosis, and physiological implications. Microbiol Immunol 64: 252-269, 2020.
21. Cao H, Liang J, Liu J, He Y, Ke Y, Sun Y, Jiang S and Lin J: Novel effects of combination therapy through inhibition of caspase-1/gasdermin D induced-pyroptosis in lupus nephritis. Front Immunol 12: 720877, 2021.
22. Zhou Y, Chen Z, Yang X, Cao X, Liang Z, Ma H and Zhao J: Morin attenuates pyroptosis of nucleus pulposus cells and ameliorates intervertebral disc degeneration via inhibition of the TXNIP/NLRP3/Caspase-1/IL-1β signaling pathway. Biochem Biophys Res Commun 559: 106-112, 2021.
23. Zhang J and Wei K: Necrosulfonamide reverses pyroptosis-induced inhibition of proliferation and differentiation of osteoblasts through the NLRP3/caspase-1/GSDMD pathway. Exp Cell Res 405: 112648, 2021.
24. Luo TD, Eady JM, Aneja A and Miller AN: Classifications in brief: Rüedi-Allgöwer classification of tibial plafond fractures. Clin Orthop Relat Res 475: 1923-1928, 2017.
25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
26. Wu D, Peng C, Ren G, Yuan B and Liu H: Novel anterior curved incision combined with MIPO for Pilon fracture treatment. BMC Musculoskelet Disord 21: 176, 2020.
27. Zhao L, Li H, Zhang S, Dong Z and Cui Q: Serum HMGB1 levels and its clinical significance in elderly patients with intertrochanteric fractures after intramedullary fixation surgery. Medicine (Baltimore) 102: e32873, 2023.
28. Meyer U, de Jong JJ, Bours SGP, Keszei AP, Arts JJ, Brink PRG, Menheere P, van Geel TA, van Rietbergen B, van den Bergh JP, *et al*: Early changes in bone density, microarchitecture, bone resorption, and inflammation predict the clinical outcome 12 weeks after conservatively treated distal radius fractures: An exploratory study. J Bone Miner Res 29: 2065-2073, 2014.
29. Saribal D, Hocaoglu-Emre FS, Erdogan S, Bahtiyar N, Caglar Okur S and Mert M: Inflammatory cytokines IL-6 and TNF-α in patients with hip fracture. Osteoporos Int 30: 1025-1031, 2019.
30. Rüedi TP and Allgöwer M: Fractures of the lower end of the tibia into the ankle-joint. Injury 1: 92-99, 1969.
31. Tarkin IS, Clare MP, Marcantonio A and Pape HC: An update on the management of high-energy pilon fractures. Injury 39: 142-154, 2008.
32. Sirkin MS: Plating of tibial pilon fractures. Am J Orthop (Belle Mead NJ) 36 (12 Suppl 2): S13-S17, 2007.
33. Crist BD, Khazzam M, Murtha YM and Della Rocca G: Pilon fractures: Advances in surgical management. J Am Acad Orthop Surg 19: 612-622, 2011.
34. Dial DM and Ryan M: Locking plate technology and its use in foot and ankle surgery. Clin Podiatr Med Surg 28: 619-631, 2011.
35. Wang C, Xu C, Li M, Li H, Wang L, Zhong D and Liu H: Arthroscopic ankle fusion only has a limited advantage over the open operation if osseous operation type is the same: A retrospective comparative study. J Orthop Surg Res 15: 80, 2020.
36. Atesok K, Doral MN, Whipple T, Mann G, Mei-Dan O, Atay OA, Beer Y, Lowe J, Soudry M and Schemitsch EH: Arthroscopy-assisted fracture fixation. Knee Surg Sports Traumatol Arthrosc 19: 320-329, 2011.
37. Liangjun J, Qiang Z, Hang L and Zhijun P: Injury mechanism, fracture characteristics and clinical treatment of pilon fracture with intact fibula-A retrospective study of 23 pilon fractures. J Clin Orthop Trauma 8 (Suppl 2): S9-S15, 2017.
38. Galante V, Vicenti G, Corina G, Mori C, Abate A, Picca G, Conserva V, Speciale D, Scialpi L, Tartaglia N, *et al*: Hybrid external fixation in the treatment of tibial pilon fractures: A retrospective analysis of 162 fractures. Injury 16 (Suppl 4): S131-S137, 2016.
39. Son S, Yoon SH, Chae BJ, Hwang I, Shim DW, Choe YH, Hyun YM and Yu JW: Neutrophils facilitate prolonged inflammasome response in the DAMP-rich inflammatory milieu. Front Immunol 12: 746032, 2021.
40. Wright SS, Vasudevan SO and Rathinam VA: Mechanisms and consequences of noncanonical inflammasome-mediated pyroptosis. J Mol Biol 434: 167245, 2022.
41. Yang L, Liu J, Shan Q, Geng G and Shao P: High glucose inhibits proliferation and differentiation of osteoblast in alveolar bone by inducing pyroptosis. Biochem Biophys Res Commun 522: 471-478, 2020.