



Comparison of different treatment approaches for coronoid process fracture in terrible triad injury: a multicenter, randomized controlled study

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Abstract

Purpose The purpose of the study was to compare the functional results of different treatment approaches for the fracture of the coronoid process in terrible triad injury (TTI).

Methods This prospective randomized controlled trial included participants from seven level-1 trauma centres in China. All patients were randomly assigned to three groups, wherein different approaches were applied to treat coronoid fracture: group A) internal fixation of the coronoid process without external fixation or splint (ORIF group), B) external fixation using a hinged fixator without internal fixation (Exfix group), and C) long-arm plaster for two to three weeks postoperatively without internal fixation of coronoid process (Plaster group). Early active motion exercises within the limits of pain were started immediately after surgery under the supervision of a physical therapist. Outcomes were evaluated at regular intervals over the subsequent 12 months.

Results A total of 65 patients (22 patients in Group A, 21 in Group B, and 22 in Group C) were included in this trial from January 2016 to January 2019. The average arc of elbow motion was $114.1^\circ \pm 8.92^\circ$. The average flexion and flexion contracture were $126.4^\circ \pm 11.2^\circ$ and $12.3^\circ \pm 7.7^\circ$, respectively. The arcs of forearm rotation of the elbow for each group were $145.41^\circ \pm 9.36^\circ$, $143.38^\circ \pm 9.79^\circ$, and $143.86^\circ \pm 10.95^\circ$, respectively. The MEPS for each group were 86.82 ± 9.7 , 86.67 ± 9.92 , and 85.23 ± 8.66 , respectively. The DASH score for each group were 18.26 ± 19.31 , 18.85 ± 15.02 , and 20.19 ± 13.59 , respectively.

Conclusion All three approaches in our trial showed similar functional results in the long-term survey. Patients treated with external fixation without internal fixation of the coronoid process showed less pain during early mobilization and acquired maximum flexion within a short duration after surgery.

Keywords Terrible triad injury · RCT study · Functional outcome · Coronoid process fracture

Abbreviations

TTI Terrible triad injury

ORIF Open reduction and internal fixation

Exfix External fixation

MEPS Mayo Elbow Performance Score

DASH Disabilities of the arm, shoulder, and hand

RCT Randomized control trial

LUCL Lateral ulnar collateral ligament

MCLC Medial collateral ligament complex

HO Heterotopic ossification

HEF Hinged external fixation

RH Radial head

VAS Visual analog scale

ROM Range of motion

Introduction

Terrible triad injury (TTI) of the elbow is defined as a posterior or posterolateral dislocation of the ulna humeral joint with fractures of the radial head (RH) and the coronoid process, which are often associated with injuries of the lateral ulnar collateral ligament (LUCL) and the medial collateral ligament complex (MCLC). Due to the considerable

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influence of elbow stability during the injury, conservative treatment commonly leads to a poor prognosis and a high incidence of treatment failures. According to modern concepts, early surgical intervention to restore damaged structures is the treatment of choice for rebuilding stability and facilitating early exercise. Open reduction and internal fixation of the TTI fracture significantly improved functional outcomes compared with nonoperative therapy. However, surgical treatment of TTIs carries a high risk of complications, including joint stiffness, residual instability, ulnar nerve symptoms, heterotopic ossification (HO), and post-traumatic arthritis [1, 2]. The overall complication rate was approximately 41%, and the average reoperation rate was 22% [3, 4].

Some preliminary studies have suggested a strategy utilizing an additional HEF instead of direct repair of the damaged coronoid process and the MCLC, which achieved a good outcome. However, there have been no comparative studies to further corroborate the superiority and insufficient indications of this alternative method. Therefore, this study aimed to investigate the effectiveness of different approaches after the repair of RH and LUCL.

Materials and methods

Subjects

This prospective randomized controlled trial included 65 participants from seven level-1 trauma centres in China, treated between January 2016 and January 2019.

Inclusion criteria were as follows:

- a) Patients with TTI diagnosed using radiographs and computed tomography (CT) and agreed to participate in the trial.
- b) Age ≥ 16 years and ≤ 65 years.

Exclusion criteria were as follows:

- a) Patients with an associated coronoid process fracture classified as Type III according to the Regan and Morrey classification, such as a fragment that involves $> 50\%$ of the coronoid process.
- b) Open fracture with neurovascular dysfunction
- c) Fractures in children and skeletally immature adolescents
- d) History of elbow fracture or upper limb deformity of the affected limb
- e) Pathological fractures, with additional substantial traumatic injuries of the affected upper limb or shoulder and impaired elbow function (stiff elbow or neurological disorder of the upper limb) before the injury

- f) Charcot disease or severe arthritis of the affected elbow
- g) Steroid use within the previous four weeks
- h) Inability to communicate normally or poor medical compliance

Recruited patients were randomly allocated into three groups using a digital randomization platform to receive two different surgical strategies.

The study was registered in the Chinese Clinical Trial Registry (number: ChiCTR-INC-17014197). All procedures were performed in accordance with the Declaration of Helsinki, and the Institutional Review Board approved all procedures performed (IRB number: YS-2017-89). All participants signed a statement of informed consent after receiving clarification regarding the study objectives and procedures.

Clinical study protocol

Each hospital had an investigator in charge of the study and the data collection. During the initial hospital admission, information was obtained regarding patient demographics, injuries, and patient-reported outcome measures of the Mayo Elbow Performance Score (MEPS) before injury. The follow-up time points were three, 12, and 24 months after surgery. The follow-up measures consisted of radiographs, the visual analog scale (VAS), range of motion (ROM), and MEPS, which was completed by another investigator who was blinded to the previous procedure. In cases where patients did not attend follow-up appointments, information was obtained through an online video or e-mail. Adverse events, including complications, failure, and reoperation, were also documented. Failure was defined as early displacement, nonunion, malfunction, or deep infection. Reoperation was defined as revision surgery for all causes except the planned removal of the implant. The diagnosis of failure and the decision to perform further surgery were made locally at the discretion of the treating surgical team.

Randomization and blinding

All patients were randomly assigned to one of three groups at a 1 : 1 : 1 ratio after admission. The randomization sequence was generated by the trial staff and concealed from the physicians during surgery. Demographic data were collected by an investigator who was blinded to surgery. Postoperative data were collected by another investigator blinded to the previous procedure.

Operative technique

All procedures were performed by or under the direct supervision of a fellowship-trained orthopedic surgeon.

The procedure begins with a lateral approach (Kocher), from which the fractured radial head is exposed. The primary goal was to fix the fracture when only one or two head fragments were present. If a stable anatomic reduction is not feasible due to fracture comminution (> 3 fragments), impaction, cartilage damage, or a combined radial neck fracture, arthroplasty of the radial head with a metal prosthesis is preferred. The LUCL was then repaired with nonabsorbable sutures or suture anchors to provide provisional stability of the elbow joint and restore articulation of the humeroulnar joint.

- Group A: after restoration of lateral structures, an anteromedial “over-the-top” approach was used to expose coronoid process fragments. After reduction, a 3.0-mm cannulated screws or a 2.5-mm buttress plate was used for fixation of the coronoid process fracture.
- Group B: after closure of the lateral incision, Exfix (Orthofix Srl, Bussolengo VR, Italy) was performed as described previously [5–7]. The rotational center of the elbow was initially identified with fluoroscopic imaging, which is a key point in the entire procedure. Next, a 2-mm K-wire was inserted into the centre point of the capitulum humeri, acting as the preset rotational center for Exfix. Consequently, the proximal humeral and distal ulnar Schanz screws of Exfix were implanted before Exfix was fixed to the screws. Subsequently, the K-wire was removed. Finally, reduction of the elbow joint was confirmed by multiple fluoroscopic imaging of the anteroposterior and lateral directions during flexion and extension.
- Group C: after closure of the lateral incision, the elbow was fixed at 90° with a long-arm cast for two weeks.

Postoperative management

Daily pin-track care of the Schanz screws was performed by the patients in Group B. A hinged plastic brace was applied to patients in Group A surgery that lasted for four weeks postoperatively. Under the supervision of an orthopedist or physical therapist, early mobilization of extension, flexion, and pronation or supination was initiated in Groups A and B, typically on the first or second postoperative day, and initiated in Group C two to three weeks postoperatively after removal of the cast. Unrestricted shoulder and wrist motion was encouraged. Indomethacin (25 mg, administered orally three times per day) was prescribed for three weeks to prevent heterotopic ossification. All patients received parecoxib (40 mg twice daily) to relieve pain and allow for early active elbow exercises in the first two weeks. The HEF for patients in Group B was removed six weeks postoperatively.

Statistics

SPSS Statistics software (version 25.0; IBM SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Descriptive statistics were employed to determine the ranges, means, and standard deviations, which were compared among three groups using a one-way ANOVA test. Student’s paired *t*-tests were used to compare postoperative radiographic measurements between the groups with the statistical significance set at $P < 0.01$. Proportions analyzed with the Chi-square test or Fisher’s exact test were used to compare failure and reoperation frequencies with the statistical significance set at $P < 0.05$.

Results

A total of 65 consecutive patients were included in this trial, consisting of 22 patients in Group A, 21 in Group B, and 22 in Group C. Patient demographics and injury classifications are summarized in Table 1. There were no differences between the mean age, percentage of males, mean follow-up time, and other data on patient demographics and injury classifications.

The mean follow-up period was approximately 32 months for each group. The major injury mechanism was fall from height. The majority of RH fractures in the groups were Mason type II and III fractures, and the majority of coronoid process fractures were Reagan–Morrey type II fractures. There were two patients with ulnar palsy in Group A and one each in Groups B and C.

There were two, three, and three patients who underwent arthroplasty in each group, respectively, and the rest of the patients in each group underwent open reduction and internal fixation (Table 2).

One case in Group B was reported in a 51-year-old woman, who sustained fracture dislocation of the left elbow identified by radiograph in AP and lateral views, accompanied by CT reconstruction displaying comminuted fractures of both the coronoid and radial heads (Fig. 1). The patient underwent K-wire insertion and intraoperative fluoroscopy, which revealed that the elbow was reduced after radial head replacement and LUCL repair (Fig. 2). Meanwhile, an external fixator was further applied, and postoperative radiography and CT scans showed that the elbow remained in good reduction with an external fixator (Fig. 3). After 7 months, the radiograph showed no signs of instability, and good elbow function was achieved (Fig. S1).

In Group A, 9 of 22 patients used screws combined with suture-with-anchor for internal fixation of the coronoid fracture, and five of 22 patients used screws combined with suture-with-anchor and a 2.0-mm buttress plate for internal fixation of the coronoid fracture. Also reported was a

Table 1 Patient demographics and injury classifications among three groups

	Group A (n = 22)	Group B (n = 21)	Group C (n = 22)	Total	P value
Mean age	38.00 ± 14.20	39.86 ± 14.42	38.50 ± 11.88	38.77 ± 13.34	0.898*
Gender (male, %)	10 (45.45%)	11 (52.38%)	13 (59.09%)	34 (52.31%)	0.664#
Mean follow-up (months)	31.86 ± 8.48	31.95 ± 11.43	32.95 ± 9.92	32.26 ± 9.85	0.923*
Dominant side affected	16 (72.73%)	18 (85.71%)	18 (81.82%)	52 (80.00%)	0.549#
Mean time elapsed until surgery	5.14 ± 1.58	4.19 ± 1.50	4.59 ± 1.65	4.65 ± 1.60	0.149*
Trauma mechanism				47	0.129#
Fall from height	13	15	19		
Traffic accident	9	6	3	18	
Fracture of radial head				0	0.721#
Mason I	0	0	0		
Mason II	12	9	10	31	
Mason III	10	12	12	34	
Fracture of coronoid process				46	0.605#
Reagan and Morrey I	15	17	14		
Reagan and Morrey II	7	5	8	20	
Reagan and Morrey III	0	0	0	0	
Ulnar/medial nerve palsy	2	1	1	4	0.779#

*Measured by Chi-square test

#Measured by one-way-ANOVA test

Table 2 Injury classification among three groups

	Group A	Group B	Group C	Total	P value
Treatment of radial head				0	0.851
Excision of the fragment	0	0	0		
Open reduction with internal fixation	20	18	19	57	
Prosthesis	2	3	3	8	
Treatment of the coronoid				43	< 0.0001
Conservative	0	21	22		
Suture with anchor = 1	3	0	0	3	
Suture with anchor + screw = 2	9	0	0	9	
Suture with anchor + buttress plate = 3	3	0	0	3	
Suture with anchor + screw + buttress plate = 4	5	0	0	5	
Screw only = 5	2	0	0	2	
Screw + buttress plate = 6	2	0	0	2	
buttress plate only = 7	1	0	0	1	
LUCL repair	22	21	22	65	

39-year-old man with TTI of the left elbow. AP and lateral radiographs and CT scan reconstruction revealed fractures of both the coronoid and the radial head (Fig. 4). The patients underwent radial head internal fixation and LUCL repair using the lateral approach, as well as internal fixation of the coronoid process using the medial approach. Postoperative radiographs showed good reduction of the elbow (Fig. 5). It was recorded that satisfied functional results were noted at the nine month follow-up (Fig. S2).

Detailed clinical recovery indicators are presented in Table 3. The mean flexion–extension arcs were 114.55°,

113.86°, and 113.77° in groups A, B, and C, respectively ($P = 0.953$ by one-way ANOVA test). The mean maximum flexion angles were 127.27°, 126.38°, and 125.55° for each group ($P = 0.882$, one-way ANOVA test). The mean forearm rotations were 145.41°, 143.38°, and 143.86°, respectively ($P = 0.786$ by one-way ANOVA test). Three patients (13.6%) underwent secondary surgical intervention due to complications in Group A, four (19%) in Group B, and four (18.2%) in Group C.

The main VAS pain scores in early mobilization in groups A, B, and C were 5.09, 4.1, and 7.23, respectively, and the duration

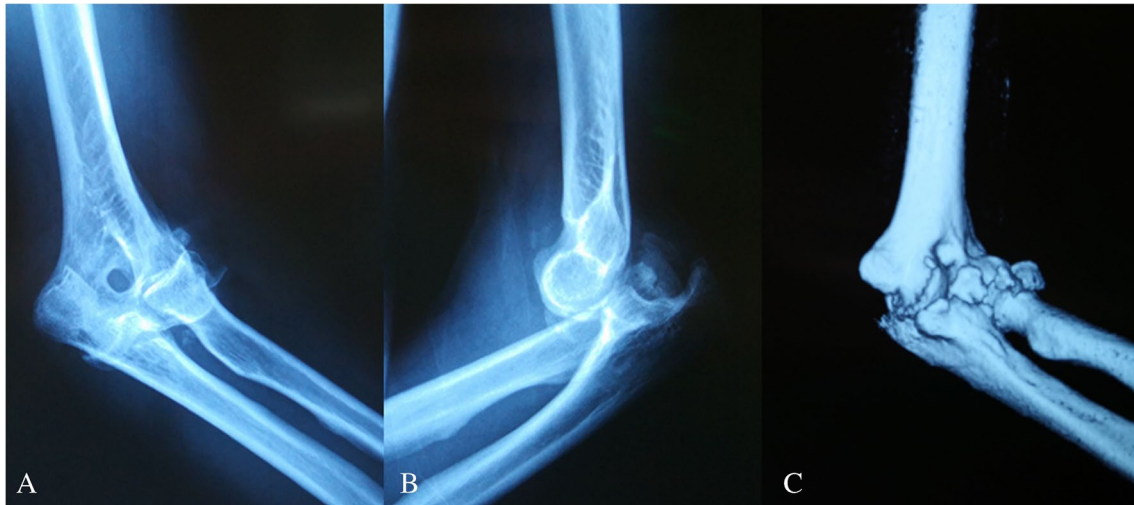
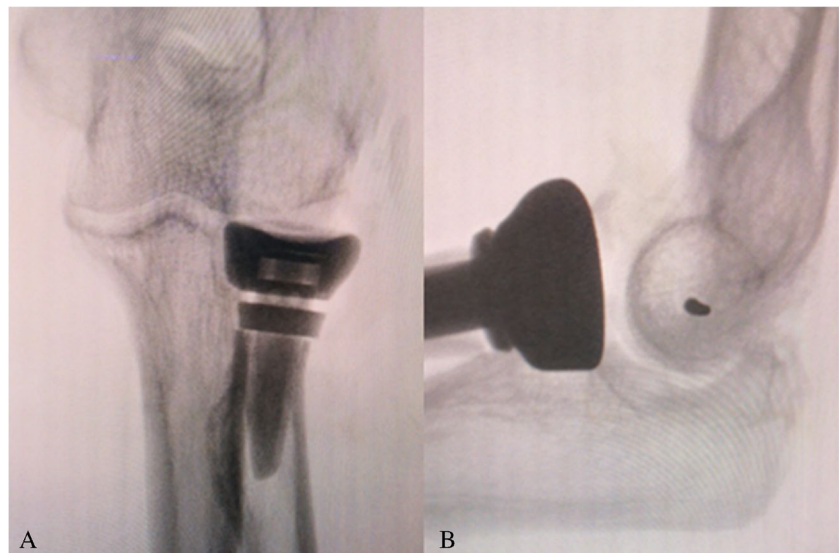


Fig. 1 A 51-year-old female suffered fracture dislocation of left elbow identified by radiograph in AP and lateral view (A, B). CT reconstruction displayed comminuted fractures of both coronoid and radial head (C)

Fig. 2 Intraoperative fluoroscopy presents that elbow be kept in reduction after radial head replacement and LUCL repair



from surgery to acquiring maximum flexion was 34.55, 20.05, and 50.82 weeks, respectively. In groups A, B, and C, at two years, the MEPS scores were 85.91, 85.24, and 84.32, and the DASH scores were 18.26, 18.85, and 20.19, respectively.

The incidence of major complications showed no significant difference between the three groups ($P = 0.853$, chi-square test). The mean range of motion also showed no significant differences among the three groups. Patients in Group B (Exfix group) showed less VAS pain and time from surgery to acquiring maximum flexion compared to other two groups ($P < 0.0001$). Similar results were also found in the MEPS and DASH scores ($P = 0.828$ and 0.921 , respectively, by one-way ANOVA).

Discussion

TTI commonly occurs in adult males, accounting for approximately 30% of all types of elbow dislocation. Compared with nonoperative therapy, open reduction and internal fixation of the TTI significantly improved treatment outcomes. However, surgical treatment of TTIs carries a high risk of complications as well, which majorly include joint stiffness, residual instability, ulnar nerve symptoms, heterotopic ossification (HO), and posttraumatic arthritis [4, 8]. The overall complication rate is approximately 41%, and the average reoperation rate is approximately 22% [9, 10]. In our study, we aimed to determine the relationship

Fig. 3 An external fixator was applied; postoperative radiograph and CT scans show that elbow keep in good reduction with external fixator

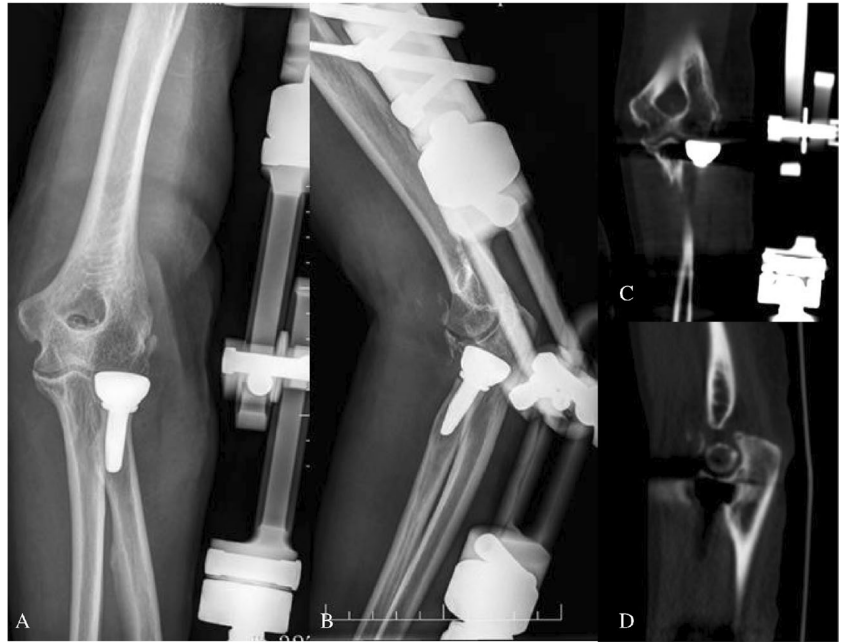


Fig. 4 A 39-year-old male patient who sustained left elbow TTI was admitted. AP and lateral view of radiograph (A, B) and CT scan (C) reconstruction displayed fractures of both coronoid and radial head

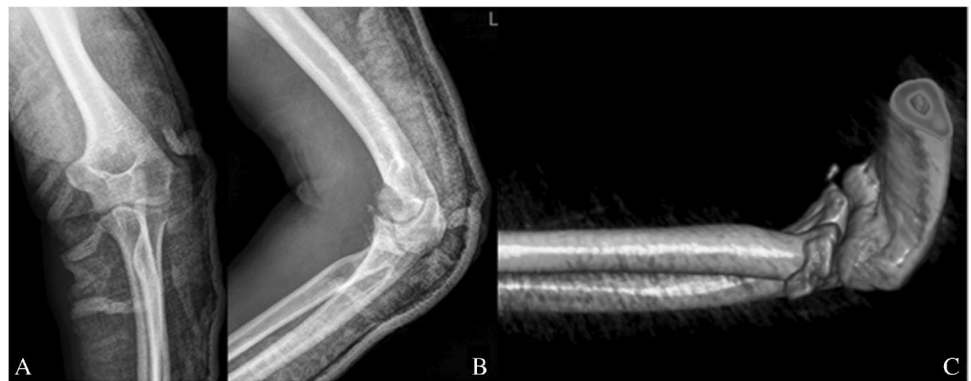


Fig. 5 The patients underwent radial head internal fixation and LUCL repair through lateral approach, as well as internal fixation of coronoid process through medial approach. Postoperative radiograph showed that elbow keep in good reduction

between different approaches for coronoid fractures and their complications.

The TTI, as the name suggests, involves posterior elbow dislocation, coronoid process fracture, and radial head fracture. Stable fixation or replacement of the radial head and repair of the LCL complex are technically feasible; however, the surgical management of coronoid fractures remains challenging. The complexity is due to the fact that coronoid fractures are typically shear rather than avulsion fractures and are often too small for stable fixation [11]. Therefore, Zeiders et al. [12] and Garrigues et al. [13] recently recommended the use of a Lasso suture technique that encircles the small coronoid fragments and coronoid–brachialis capsular–ligamentous complex (CBCC). This approach is regarded as superior to open reduction and internal fixation, which uses lag screw fixation, site-specific plating, and/or suture anchor fixation.

To the best of our knowledge, our trial is the first multicenter RCT study of TTIs, which included seven level-1

Table 3 The detailed clinical recovery indicators among three groups

	Group A	Group B	Group C	Total	P value
Mean arc flexion–extension	114.55 ± 9.23	113.86 ± 10.04	113.77 ± 7.80	114.06 ± 8.92	0.953 [#]
Mean maximum extension	12.73 ± 7.78	12.52 ± 7.49	11.77 ± 8.08	12.34 ± 7.68	0.912 [#]
Mean maximum flexion	127.27 ± 10.71	126.38 ± 11.88	125.55 ± 11.47	126.40 ± 11.20	0.882 [#]
Mean forearm rotation	145.41 ± 9.36	143.38 ± 9.79	143.86 ± 10.95	144.23 ± 9.94	0.786 [#]
Major complication					0.853 [*]
Recurrent instability (secondary subluxation) = 1	2	1	2		
Implant failure = 2	1	1	0		
Pin site infection = 3	0	1	0		
Deep infection = 4	0	1	0		
Heterotopic ossification = 5	2	1	1		
Joint stiffness = 6	1	1	2		
Severe arthritis	2	1	4		
Secondary surgical intervention = 6	4	4	3		
Main pain in early mobilization by visual analog scale (VAS)	5.09 ± 1.57	4.10 ± 1.34	7.23 ± 1.38	5.49 ± 1.93	< 0.0001 [§]
Time from surgery to acquiring maximum flexion	34.55 ± 10.32	20.05 ± 4.85	50.82 ± 5.84	35.37 ± 14.59	< 0.0001 [§]
MEPS	86.82 ± 9.70	86.67 ± 9.92	85.23 ± 8.66	86.23 ± 9.31	0.827 [#]
DASH score	18.26 ± 19.31	18.85 ± 15.02	20.19 ± 13.59	19.10 ± 15.94	0.921 [#]

*Measured by Chi-square test

[#]Measured by one-way-ANOVA test

[§]Measured by Tukey's multiple comparisons test

trauma centers in China. Three alternatives for coronoid fractures have been evaluated: open reduction and internal fixation or arthroplasty, hinged external fixation, and post-operative two to three weeks of long-arm plaster application. According to the demographic results, most TTIs in our study affected the dominant arm, which mostly resulted in a Mason II or III injury of the radial head and Reagan and Morrey I and II fractures of the coronoid process.

According to the results of the long-term survey, there was no significant difference in the active range of motion among the groups. The incidence of secondary surgical interventions was similar. In group A, the reasons for secondary surgical intervention were recurrent instability (one), one consequent implant failure of the coronoid process fracture and recurrent instability (one), heterotopic ossification, and joint stiffness. In group B, the reasons for secondary surgical intervention were as follows: recurrent instability (one), implant failure of the radial head fracture (one), deep infection (one), and heterotopic ossification and joint stiffness (one). In Group C, the reasons for secondary surgical intervention were recurrent instability (one), heterotopic ossification (one), and heterotopic ossification and joint stiffness (one). The rates of secondary intervention were 18.2%, 19%, and 13.6%, which were less than the reported secondary intervention rates in recent literature [12–28]. Therefore, increased knowledge of primary and secondary elbow stabilizers, as well as advances in surgical techniques, have improved the diagnostic and therapeutic protocols in

TTI, which take it for granted that improving clinical outcomes after surgery [12–28]. However, this study has some limitations. The major complications in our study included recurrent instability, heterotopic ossification, and joint stiffness, as noted in most previous reports that adopted current standard surgical protocols. The reported complications include stiffness (0–22%), arthritis (0–19.5%), ulnar nerve entrapment (0–18%), and recurrent instability (4–38%), and the re-intervention rate oscillates between 0 and 55% [3, 12–31]. The incidence of each complication was not significantly different between the groups; thus, we may have judged that these complications were not related to a specific treatment method.

The results of the long-term survey showed similar functional outcomes in each group, including active ROM, MEPS, and DASH scores, indicating that all three alternatives can yield reliable long-term functional results for TTI. Our results are similar to those of recent studies [12–28]. In addition, our results showed that LUCL repair, along with stable fixation of the radial head, provided fundamental stability for TTI. Some authors have advocated primary repair of medial collateral ligaments [32, 33]. However, based on the results of his clinical study, Forthman et al. demonstrated that stability and good functional outcomes of the elbow after fracture and dislocation can be achieved without repairing the medial collateral ligament [34]. We believe that during an injury-causing complex fracture–dislocation of the elbow, the dislocation sometimes hinges around an intact

medial collateral ligament, which may not damage the MCL. Despite the injury, a well-reduced and stable elbow may promote spontaneous healing of the MCL.

According to the short-term survey, patients in the Exfix group experienced less pain during early mobilization than those in the other two groups, indicating that external fixation provides better stability than internal fixation and non-surgical treatment in the early stage of mobilization. This theory also explains the shorter time from surgery to acquiring maximum ROM for patients in the Exfix group than in the other groups. The mechanical support provided by external fixation, as shown by the results of the current studies, can guarantee uneventful bony and soft tissue healing in patients sustained with communicated articular fractures, open fractures with massive soft tissue involvement, and fracture–dislocation [35, 36]. Maniscalco et al. believed that being supported by hinged external fixation can facilitate the postoperative rehabilitation phase (less pain and protected motion) and limit the risk of early or late complications (re-dislocation, stiffness, or chronic instability).

Doornberg et al. [37] reported that anatomical restoration of the trochlear notch and reconstruction of the coronoid process are critical components for successful treatment of these fracture–dislocations of the olecranon. Current optimal management of TTI should restore joint stability and full ROM. However, this is often difficult to achieve surgically because multiple procedures are needed to repair and restore all bony and ligamentous tissues. Moderate to severe swelling around the elbow joint may occur because of the larger area of dissection in surgery. Therefore, minimally invasive surgery that attains mobility and stability is preferred for the treatment of complex elbow fracture–dislocations, which is also technically straightforward.

To date, several studies have demonstrated the application of a hinged external fixator in the treatment of acute and chronic elbow instability [5, 6, 38–40]. Mckee et al. applied hinged external fixator for 16 patients with recurrent complex elbow instability. After treatment, they found the mean range of flexion–extension to be 105 degree and mean Morrey score was 84. The final excellent and good rate was 85% [38]. Jupiter et al. applied hinged external fixator for 5 patients with unreduced elbow dislocation with a result of 123 degree for average arc of flexion and full forearm rotation, and they also noted 89 points for average score on MEPS [39]. Previous studies have included the treatment of residual instability after fixation of fractures and repair of ligament injuries or as an adjunct device to treat chronic instability after injury. Duckworth et al. [7] reviewed 17 cases of unstable elbow dislocations and concluded that the anterior capsuloligamentous structures are allowed to heal and elbow stability can be restored as long as the elbow is held concentric with a hinged external fixator during early mobilization. Yu et al. [6] also reported the outcomes of 20 cases of acute complex instability of the elbow treated with hinged external fixation instead of LCL repair.

The major limitation of our study is the small sample size and short period of follow-up time. A larger RCT is required to obtain more reliable results. Another limitation was the complexity of coronoid fixation and intraoperative localization of the rotation center. A slight deviation may have been caused by intraoperative fluoroscopy and was neglected. Therefore, a better alternative for rotational center localization needs to be explored. The third limitation is that only one investigator was involved in this study, which may cause investigator bias to the results.

Conclusion

External fixation, internal fixation of coronoid process fractures, and postoperative long-arm plaster resulted in similar functional outcomes in the long-term survey. Despite the requirement of good tolerance for the external fixation and the daily care of the pin tract, external fixation can facilitate the postoperative rehabilitation phase with less pain and earlier mobilization compared to other alternatives.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00264-023-05864-0>.

Author contribution JD contributed to the study conception and design. Material preparation, data collection, and analysis were performed by BR. The first draft of the manuscript was written by SL and YW. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval The study was a level-I multicenter RCT study and was registered in the Chinese Clinical Trial Registry (number: ChiCTR-INC-17014197). All procedures were performed according to the Declaration of Helsinki, and the Institutional Review Board approved all procedures performed (IRB number: YS-2017-89).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent to publish The authors affirm that human research participants provided informed consent for publication of the images in Figures.

Competing interests The authors declare no competing interests.

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