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Original Article

Day-14 inflammatory–coagulation risk stratification and local carboxytherapy in patients with pelvic and lower-limb fractures: A prospective study

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Abstract

Objectives: To identify early immunological and inflammatory–coagulation predictors of complicated healing after fractures of the pelvis, femur and tibia, and to evaluate local carboxytherapy in patients at high risk.

Methods: We prospectively enrolled 124 consecutive patients aged 18–70 years with operatively treated pelvic (n = 19), femoral (n = 44) and tibial (n = 61) fractures (2022–2024). Interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), D-dimer, 25-hydroxyvitamin D and osteocalcin were measured on admission and on days 14, 30 and 45. On day 14, patients were classified as high inflammatory–coagulation risk (HIR) or standard risk. HIR patients without contraindications received local carboxytherapy from day 15.

Results: Open, unstable, comminuted and segmental fractures were associated with higher IL-2, TNF-α, CRP and D-dimer and lower 25-hydroxyvitamin D and osteocalcin ($p \leq 0.011$). On day 14, 50 patients (40.3%) were HIR; 36 received carboxytherapy and 14 did not. By day 45, CRP <10 mg/L was reached in 92% vs 50% ($p = 0.009$) and D-dimer <1.3 mg/L in 94% vs 64% ($p = 0.028$), and osteocalcin was higher with carboxytherapy (22 ± 7 vs 16 ± 6 ng/mL; $p = 0.041$). Delayed union occurred in 16.7% vs 28.6% ($p = 0.041$), nonunion in 2.8% vs 21.4% ($p = 0.080$) and thromboembolic events in 8.3% vs 21.4% ($p = 0.18$). Day-14 D-dimer ≥1.5 mg/L (OR 3.6; 95% CI 1.3–10.2), CRP >20 mg/L (3.1; 1.1–8.9) and open or comminuted fracture (2.5; 1.0–7.2) independently predicted late complications, whereas carboxytherapy was protective (0.33; 0.11–0.95).

Conclusions: Day 14 after injury is a key checkpoint for risk stratification based on IL-2, TNF-α, CRP and D-dimer combined with fracture morphology. In high-risk patients without active infection, local carboxytherapy was associated with faster resolution of systemic inflammation and hypercoagulation and with fewer late complications.

Keywords: Carboxytherapy; Fracture healing; Interleukin-2; Pelvis; Tumor necrosis factor-alpha; Venous thromboembolism

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Introduction

In 2019, 178 million new fractures occurred worldwide, and 455 million people had acute or long-term consequences of fractures.[1] Fractures of the pelvis, femur and tibia carry the greatest medical and social burden. They usually result from high-energy trauma, require long and complex treatment, and often end in persistent disability.

The risk of early and late complications persists throughout recovery. Fracture-related infection, venous thromboembolism (VTE), delayed union, nonunion and functional failure of the limb remain the main problems.[2–4] The outcome depends largely on the site and morphology of the fracture, but the severity of the systemic response to injury plays an equally important role. Trauma triggers cytokine-mediated inflammation and activation of coagulation, and an excessive or prolonged response impairs microcirculation and reparative osteogenesis.[5–8] Vitamin D deficiency and reduced osteoblast activity further limit repair.[9,10]

Early diagnosis of complicated healing remains insufficiently developed. Reliable immunological and inflammatory–coagulation markers of risk have not been defined, and prevention and rehabilitation are rarely tailored to the individual patient. In our retrospective analysis of 284 patients treated in 2018–2022, complications occurred in 21.8% of cases despite technically correct osteosynthesis, and outcomes differed even when fracture type and fixation were the same. This suggested that, besides the mechanics of repair, the individual immuno-inflammatory reactivity of the patient influences the course of healing.

Local carbon dioxide (CO_2) therapy improves microcirculation and stimulates angiogenesis in the zone of bone injury in experimental models of bone defects, fractures and distraction osteogenesis,[11–13] and has been used in conditions with impaired microcirculation and chronic wounds.[14] However, it has not been applied systematically in trauma, particularly in tibial and femoral fractures.

The aim of this study was to assess the immunological and inflammatory–coagulation response after fractures of the pelvis, femur and tibia, to identify early predictors of complicated healing on day 14 after injury, and to evaluate local carboxytherapy in patients at high inflammatory–coagulation risk.

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Methods

Study design and setting

This prospective study included 124 patients treated at the Republican Scientific Center for Emergency Medical Care and its Bukhara branch, Uzbekistan, in 2022–2024. Patients were enrolled consecutively in order of admission, provided they consented to participate and complete clinical and diagnostic data were available. All patients were followed until discharge and then for 6–12 months. The study was performed within the research plan of Bukhara State Medical Institute (no. 01.2022.DSc.152). Reporting follows the STROBE statement.[15]

Participants

Inclusion criteria were fractures of the pelvis, femur or tibia; diaphyseal or metaphyseal fractures of the femur or tibia, or unstable pelvic ring fractures; open or closed fractures; age 18–70 years at injury; indication for surgical treatment; the possibility of clinical, laboratory and instrumental follow-up at the scheduled time points; and written informed consent.

Exclusion criteria were pathological fractures, including tumor-related and metastatic fractures; fractures associated with severe systemic bone disease; age below 18 or above 70 years; severe decompensated comorbidity precluding standardized surgical treatment and follow-up; death in the early post-traumatic period; refusal to participate or inability to obtain informed consent; and non-adherence to the follow-up protocol or loss to follow-up.

Clinical and radiological assessment

On admission we recorded fracture site, open or closed status, stability, fracture-line type (transverse, oblique, comminuted or segmental), mechanism of injury and time from injury to admission. Radiographs were obtained in two standard projections, with inlet and outlet views for pelvic fractures. Multislice computed tomography was performed for intra-articular and unstable pelvic fractures to classify the injury and plan surgery. Pelvic ring stability was graded by the Tile classification.[16]

Surgical treatment and thromboprophylaxis

Unstable pelvic ring injuries were treated with external fixation, with open reduction and internal fixation with plates and screws, or with both; the approach was chosen according to Tile stability, displacement and the state of the soft tissues. In massive bleeding, damage-control principles with a pelvic compression device and staged stabilization were applied. Femoral shaft fractures were treated with closed reduction and locked intramedullary nailing; femoral neck fractures in patients older than 60 years with hip arthroplasty; and proximal femoral fractures in working-age patients with cannulated screws or a dynamic hip screw. Open and unstable tibial fractures were treated with Ilizarov transosseous fixation, closed diaphyseal fractures with locked intramedullary nailing, and metaphyseal fractures with open reduction and plating.

VTE prophylaxis was selected with an automated program that calculated the individual VTE risk score. Low-risk patients received compression and early mobilization, moderate-risk patients combined prophylaxis, and high-risk patients low-molecular-weight heparin with D-dimer monitoring and dose adjustment.

Laboratory and immunological methods

Blood was drawn on admission before surgery and premedication, and on days 14, 30 and 45. Serum was separated by centrifugation at 2000 g for 10 min. CRP and D-dimer were measured by immunoturbidimetry; D-dimer above 1.5 mg/L was regarded as hypercoagulation. 25-hydroxyvitamin D [25(OH)D] was measured by chemiluminescent immunoassay and osteocalcin by enzyme-linked immunosorbent assay (ELISA). IL-2 and TNF-α were measured by solid-phase ELISA. Samples for cytokine assays were stored at −80 °C without repeated freeze–thaw cycles and analyzed in batches with calibrators and control samples.

Risk stratification on day 14

Thresholds were defined before enrollment on the basis of published data and the physiology of the inflammatory response: IL-2 ≥16 pg/mL (marked T-cell activation), TNF-α ≥20 pg/mL (moderate to high systemic inflammation), CRP >20 mg/L (intense acute-phase response) and D-dimer ≥1.5 mg/L (vascular hypoperfusion and microthrombosis). On day 14, patients were assigned to the high inflammatory–coagulation risk (HIR) group if they met two or more of these criteria, or if they had a severe injury pattern (open fracture, Tile type C pelvic injury or comminuted fracture). The remaining patients formed the standard-risk (SR) group.

Local carboxytherapy

Local carboxytherapy was given to HIR patients with marked cytokine activation (IL-2 ≥16 pg/mL and/or TNF-α ≥20 pg/mL) together with elevated CRP or D-dimer, or with these findings combined with severe fracture morphology. It was used as a module to optimize microcirculation, in addition to standard treatment and not as an alternative to surgery. Injectable carboxytherapy with medical CO_2 was performed in the fracture zone with a GD-CDT Carboxy device twice weekly for 30 days, starting on day 15. It was given outside acute infection and after screening for contraindications: active infection, marked local inflammation and contraindications to injections. CO_2 was preferred to oxygen because oxygenation in the presence of inflammatory edema may increase oxidative stress.

Of the 50 HIR patients, 36 received carboxytherapy (HIR + CO_2). The other 14 patients, with a comparable laboratory profile, did not receive it because of limitations, contraindications or inability to complete the protocol (HIR without CO_2). SR patients received standard treatment only.

Outcomes

Laboratory outcomes were the course of IL-2, TNF-α, CRP, D-dimer, 25(OH)D and osteocalcin from day 14 to day 45, and the proportions of patients with CRP <10 mg/L and D-dimer <1.3 mg/L on day 45. Clinical outcomes were late complications after stratification: delayed union (radiographic signs of delayed callus formation at 6–8 weeks), nonunion (no radiographic consolidation at 8 weeks or later, requiring repeat surgery) and thromboembolic events. Union was recorded when callus bridged at least two of four cortices on anteroposterior and lateral radiographs without pathological mobility. Radiographs were read independently by two specialists, with a consensus decision in case of disagreement. Deep vein thrombosis (DVT) was confirmed by duplex ultrasonography and pulmonary embolism (PE) by computed tomography angiography.

Statistical analysis

The distribution of variables was tested with the Kolmogorov–Smirnov test. Normally distributed data are presented as mean ± standard deviation (SD) and compared with Student's t-test; otherwise the Mann–Whitney U test was used. Paired comparisons used the paired t-test or Wilcoxon test, and comparisons of more than two groups used ANOVA or the Kruskal–Wallis test. Categorical data were compared with Pearson's χ^2 test, or Fisher's exact test when expected frequencies were below 5, and odds ratios (OR) with 95% confidence intervals (CI) were calculated. Correlations were assessed with Pearson's or Spearman's coefficient.

Independent predictors of late complications were identified by backward stepwise logistic regression. The initial model included day-14 IL-2, TNF-α, CRP, D-dimer and 25(OH)D, fracture morphology (type, open fracture, comminution) and carboxytherapy; only predictors with $p < 0.05$ were retained. Model stability was checked by k-fold cross-validation and bootstrap resampling, and calibration with the Hosmer–Lemeshow test. Analyses were performed with Statistica 12.0 (StatSoft, USA) and SPSS 25.0 (IBM, USA). Statistical significance was set at $p < 0.05$.

Results

Patient characteristics

The cohort included 78 men (63%) and 46 women (37%). Working-age patients predominated; the largest age group was 30–49 years (33.9%) (Table 1). There were 61 tibial (49.2%), 44 femoral (35.5%) and 19 pelvic (15.3%) fractures; 33 were open and 91 closed. Road traffic accidents (48.4%) and falls from height (28.2%) accounted for more than three quarters of injuries, and 112 patients (90.3%) were admitted within 24 h of injury. The study design is shown in Figure 1.

Table 1: Characteristics of the study cohort (n = 124).

Characteristic	Category	n (%)
Sex	Male	78 (63.0)
	Female	46 (37.0)
Age, years	18–29	27 (21.8)
	30–49	42 (33.9)
	50–64	34 (27.4)
	≥65	21 (16.9)
Place of residence	Urban	77 (62.1)
	Rural	47 (37.9)
Fracture site	Tibia	61 (49.2)
	Femur	44 (35.5)
	Pelvis	19 (15.3)
Type of fracture	Closed	91 (73.4)
	Open	33 (26.6)
Fracture line	Transverse	31 (25.0)
	Oblique	27 (21.8)
	Comminuted	49 (39.5)
	Segmental	17 (13.7)
Mechanism of injury	Road traffic accident	60 (48.4)
	Fall from height	35 (28.2)
	Occupational injury	15 (12.1)
	Sports injury	8 (6.5)
	Assault	6 (4.8)
Time to admission	First 24 h	112 (90.3)
	Days 2–3	12 (9.7)

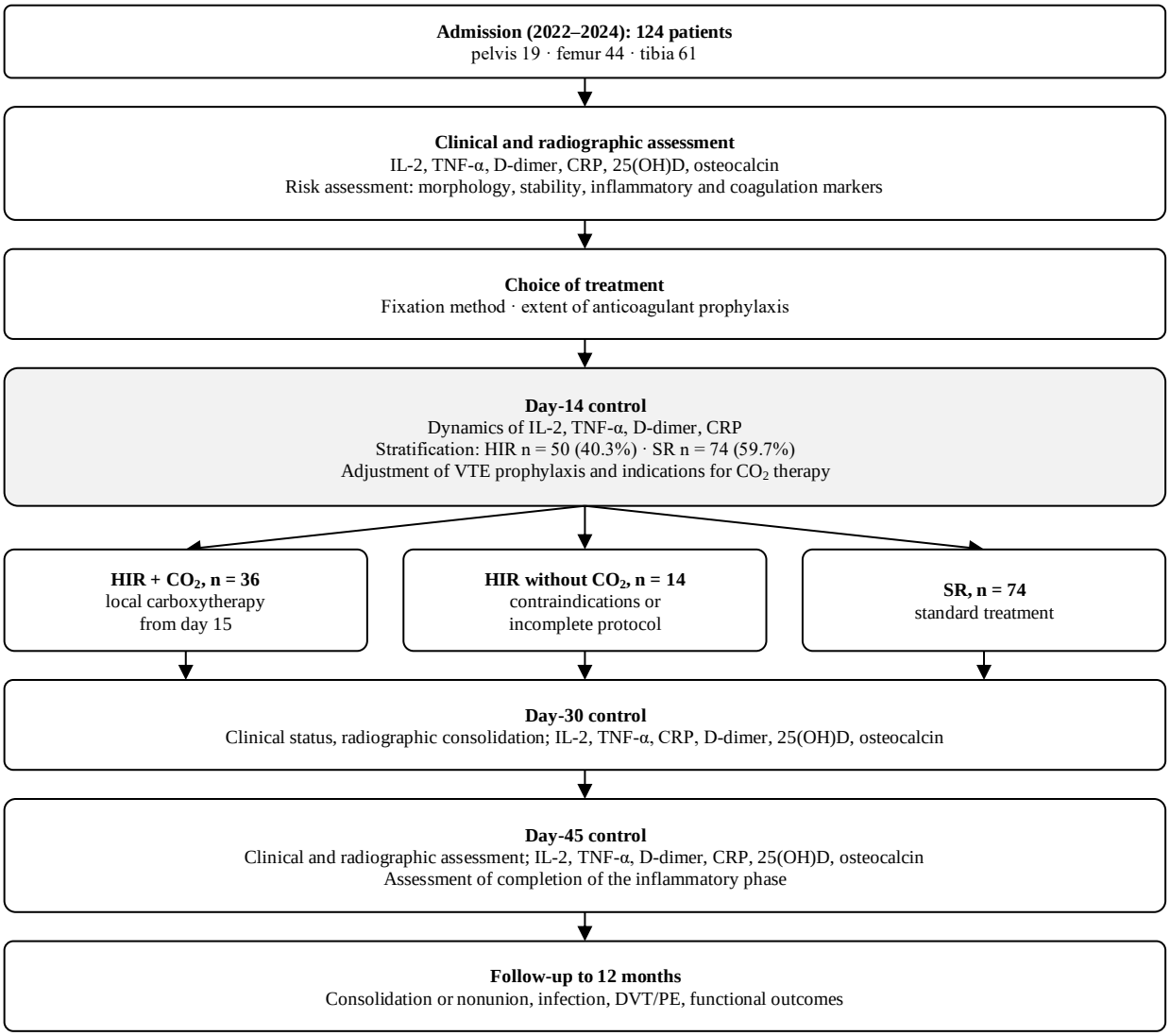


Figure 1: Design of the prospective study and observation points. HIR, high inflammatory–coagulation risk; SR, standard risk; CO_2, local carboxytherapy; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism.

Admission profile

Pro-inflammatory cytokines were highest in pelvic fractures (IL-2 17.9 ± 5.2 pg/mL, TNF-α 21.9 ± 5.6 pg/mL, CRP 25.7 ± 8.1 mg/L, D-dimer 1.89 ± 0.51 mg/L), but differences between fracture sites were not significant (p = 0.06–0.61). Open fractures had 20–25% higher IL-2 and TNF-α, higher CRP and D-dimer, and lower 25(OH)D and osteocalcin than closed fractures (Table 2).

Table 2: Laboratory and immunological parameters on admission in closed and open fractures (M ± SD).

Parameter	Closed (n = 91)	Open (n = 33)	p
IL-2, pg/mL	14.9 ± 3.8	18.6 ± 4.2	<0.001
TNF-α, pg/mL	18.2 ± 4.6	22.7 ± 5.1	<0.001
CRP, mg/L	20.4 ± 6.2	27.9 ± 7.4	<0.001
D-dimer, mg/L	1.47 ± 0.39	1.91 ± 0.48	<0.001
25(OH)D, ng/mL	23.9 ± 5.7	20.8 ± 6.4	0.008
Osteocalcin, ng/mL	12.8 ± 3.5	10.9 ± 3.7	0.011

Mann–Whitney U test. IL-2, interleukin-2; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein; 25(OH)D, 25-hydroxyvitamin D.

Unstable fractures had 20–25% higher IL-2 and TNF-α than stable fractures at all three sites (p < 0.001), with parallel increases in CRP and D-dimer and lower 25(OH)D (p = 0.006) and osteocalcin (p = 0.008). Marker levels increased with the complexity of fracture morphology: from transverse to segmental fractures, IL-2 rose from 14.8 ± 3.6 to 19.3 ± 4.8 pg/mL, CRP from 19.1 ± 6.0 to 27.1 ± 7.4 mg/L and D-dimer from 1.36 ± 0.33 to 1.94 ± 0.50 mg/L (p < 0.001), while osteocalcin fell from 13.4 ± 3.6 to 10.6 ± 3.9 ng/mL (p = 0.006). Road traffic injuries produced higher IL-2 (17.9 ± 4.4 vs 15.6 ± 3.9 pg/mL; p = 0.002), CRP (26.2 ± 7.0 vs 21.4 ± 6.1 mg/L; p < 0.001) and D-dimer (1.84 ± 0.47 vs 1.53 ± 0.41 mg/L; p < 0.001) than falls.

Course from admission to day 14

In pelvic fractures, the systemic response on day 14 increased with Tile type. Type C injuries had IL-2 of 18 ± 5 pg/mL, TNF-α 25 ± 7 pg/mL, CRP 35 ± 10 mg/L and D-dimer 2.5 ± 0.8 mg/L, compared with 12 ± 3 pg/mL, 15 ± 5 pg/mL, 10 ± 4 mg/L and 1.0 ± 0.4 mg/L in type A injuries (p < 0.05). Early thromboembolic events (by day 14) occurred in none of 10 type A, 1 of 6 type B (16.7%) and 1 of 3 type C patients (33.3%; p = 0.045 for type C vs types A and B combined), although all patients received prophylaxis.

In femoral fractures, open injuries had higher CRP (30 ± 10 vs 18 ± 7 mg/L; p = 0.041) and D-dimer (2.0 ± 0.7 vs 1.4 ± 0.5

mg/L; p = 0.038) on day 14 than closed injuries, with a trend for IL-2 and TNF-α. In tibial fractures, open injuries had higher IL-2 (16 ± 4 vs 12 ± 3 pg/mL; p = 0.021), TNF-α (24 ± 7 vs 18 ± 6 pg/mL; p = 0.035), CRP (25 ± 8 vs 12 ± 5 mg/L; p = 0.008) and D-dimer (1.6 ± 0.5 vs 0.8 ± 0.3 mg/L; p = 0.012). At both sites, all cases with CRP above 30 mg/L on day 14 were comminuted fractures.

Day-14 stratification

On day 14, 50 patients (40.3%) were assigned to the HIR group and 74 (59.7%) to the SR group. Open and comminuted fractures and unstable pelvic injuries predominated in the HIR group. The proportion of HIR patients was significantly higher in Tile type C pelvic injuries and in open tibial fractures, with a similar trend in open femoral fractures (Table 3).

Carboxytherapy was given to 6 of 8 HIR patients with pelvic fractures, 11 of 15 with femoral fractures and 19 of 27 with tibial fractures (72.0% overall; p > 0.1 between sites). On day 14, the HIR + CO_2 and HIR without CO_2 subgroups had similar levels of all markers (p > 0.1), and both differed from the SR group (Table 4).

Table 3: Distribution of patients by risk group on day 14 according to fracture site and type.

Site	Type of injury	n	HIR, n (%)	SR, n (%)	p
Pelvis	Tile A/B (stable)	13	4 (31)	9 (69)	0.046
	Tile C (unstable)	6	4 (67)	2 (33)	
Femur	Closed	32	9 (28)	23 (72)	0.087
	Open	12	6 (50)	6 (50)	
Tibia	Closed	34	11 (32)	23 (68)	0.031
	Open	27	16 (59)	11 (41)	

p, comparison of the proportion of HIR patients between injury types within each site (χ^2 test). HIR, high inflammatory–coagulation risk; SR, standard risk.

Table 4: Laboratory parameters on day 14 before the start of carboxytherapy (M ± SD).

Parameter	HIR + CO_2 (n = 36)	HIR without CO_2 (n = 14)	SR (n = 74)	p
IL-2, pg/mL	18.2 ± 4.6	17.9 ± 4.3	12.1 ± 3.0	<0.001
TNF-α, pg/mL	24.2 ± 5.9	24.7 ± 6.2	15.3 ± 5.1	<0.001
CRP, mg/L	28.6 ± 9.5	29.4 ± 10.2	8.3 ± 5.0	<0.001
D-dimer, mg/L	1.79 ± 0.44	1.83 ± 0.46	0.82 ± 0.29	<0.001
25(OH)D, ng/mL	21 ± 7	20 ± 6	26 ± 8	0.049
Osteocalcin, ng/mL	9 ± 3	9 ± 3	15 ± 5	0.006

No differences between the HIR + CO_2 and HIR without CO_2 subgroups (p > 0.1 for all parameters); both differ from SR. CO_2, local carboxytherapy.

Course from day 14 to day 45

Two weeks after the start of carboxytherapy (day 30), IL-2 in the HIR + CO_2 subgroup had fallen from 18.5 to 12.0 pg/mL (p < 0.05), whereas in the HIR without CO_2 subgroup it decreased only slightly, from 17.8 to 15.0 pg/mL. By day 45, all inflammatory and coagulation markers were lower in treated patients: IL-2 8.0 ± 3.0 vs 12.0 ± 4.0 pg/mL (p = 0.028), CRP 6.0 ± 4.0 vs 12.0 ± 6.0 mg/L (p = 0.034) and D-dimer 0.8 ± 0.3 vs 1.2 ± 0.4 mg/L (p = 0.045), with a trend for TNF-α (14.0 ± 4.0 vs 18.0 ± 5.0 pg/mL; p = 0.054) (Table 5, Figure 2).

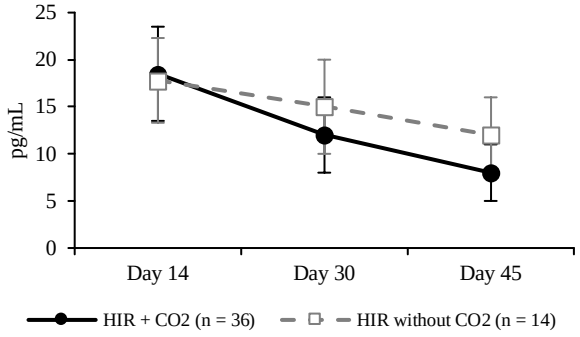
By day 45, CRP <10 mg/L was reached in 33 of 36 treated patients (92%) and 7 of 14 untreated patients (50%; p = 0.009), and D-dimer <1.3 mg/L in 34 of 36 (94%) and 9 of 14 (64%; p = 0.028). 25(OH)D did not change significantly in either subgroup. Osteocalcin increased in both subgroups but more with carboxytherapy, reaching 22 ± 7 vs 16 ± 6 ng/mL on day 45 (p = 0.041).

Table 5: Course of laboratory parameters in HIR patients with and without carboxytherapy (M ± SD).

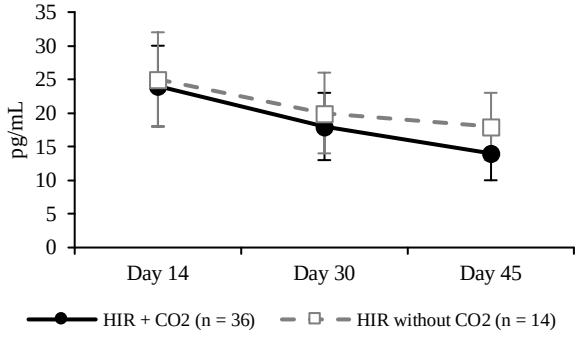
Parameter	HIR + CO_2, day 14	HIR + CO_2, day 30	HIR + CO_2, day 45	HIR without CO_2, day 14	HIR without CO_2, day 30	HIR without CO_2, day 45	p, day 45
IL-2, pg/mL	18.5 ± 5.0	12.0 ± 4.0*	8.0 ± 3.0*	17.8 ± 4.5	15.0 ± 5.0	12.0 ± 4.0*	0.028
TNF-α, pg/mL	24.0 ± 6.0	18.0 ± 5.0*	14.0 ± 4.0*	25.0 ± 7.0	20.0 ± 6.0	18.0 ± 5.0	0.054
CRP, mg/L	28.0 ± 10.0	15.0 ± 8.0*	6.0 ± 4.0*	30.0 ± 12.0	20.0 ± 10.0	12.0 ± 6.0*	0.034
D-dimer, mg/L	1.8 ± 0.5	1.2 ± 0.4*	0.8 ± 0.3*	1.9 ± 0.6	1.5 ± 0.5	1.2 ± 0.4*	0.045
25(OH)D, ng/mL	21 ± 7	20 ± 6	19 ± 6	20 ± 6	18 ± 6	17 ± 5	0.27
Osteocalcin, ng/mL	9 ± 3	15 ± 5*	22 ± 7*	8 ± 4	12 ± 5*	16 ± 6*	0.041
CRP <10 mg/L on day 45, n (%)			33 (92)			7 (50)	0.009
D-dimer <1.3 mg/L on day 45, n (%)			34 (94)			9 (64)	0.028

*p < 0.05 versus day 14 within the subgroup (paired test). p, day 45: comparison between subgroups; proportions compared with the χ^2 test.

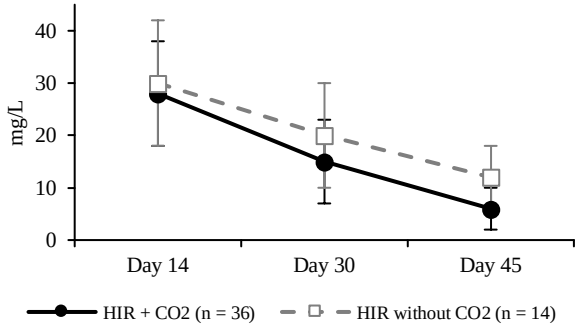
A. IL-2



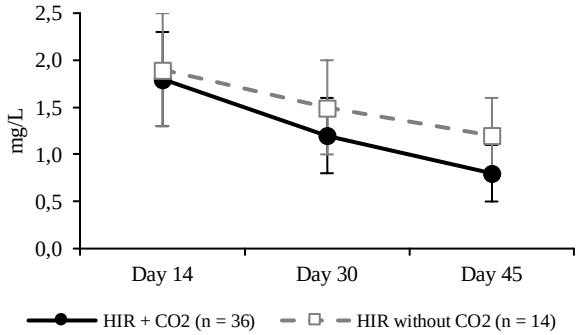
B. TNF-α



C. CRP



D. D-dimer



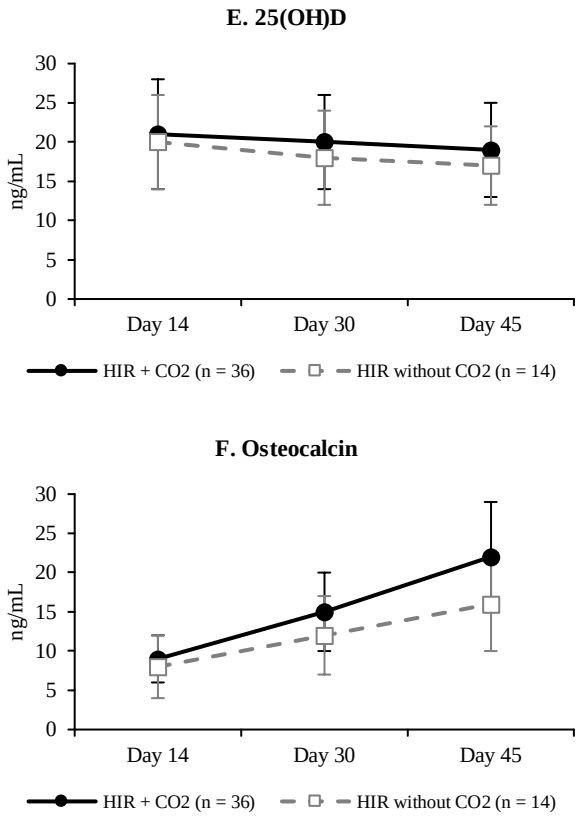


Figure 2: Course of IL-2 (A), TNF-α (B), CRP (C), D-dimer (D), 25(OH)D (E) and osteocalcin (F) in HIR patients with carboxytherapy (solid line, n = 36) and without it (dashed line, n = 14) from day 14 to day 45. Mean ± SD. p values compare the subgroups on day 45: IL-2 0.028, TNF-α 0.054, CRP 0.034, D-dimer 0.045, 25(OH)D 0.27, osteocalcin 0.041.

Late complications

Delayed union occurred in 6 of 36 HIR + CO_2 patients (16.7%), 4 of 14 HIR without CO_2 patients (28.6%) and 2 of 74 SR patients (2.7%) (p = 0.041 for HIR + CO_2 vs HIR without CO_2). Nonunion occurred in 1 (2.8%), 3 (21.4%) and 1 (1.4%) patients, respectively (p = 0.080). Thromboembolic events occurred in 3 (8.3%), 3 (21.4%) and 2 (2.7%) patients (p = 0.18); in the HIR without CO_2 subgroup they comprised two calf DVTs and one femoral vein thrombosis, and in the HIR + CO_2 subgroup mainly asymptomatic muscular vein thromboses (Table 6, Figure 3).

Table 6: Late complications after day-14 stratification, n (%).

Complication	HIR + CO_2 (n = 36)	HIR without CO_2 (n = 14)	SR (n = 74)	p*
Delayed union	6 (16.7)	4 (28.6)	2 (2.7)	0.041
Nonunion	1 (2.8)	3 (21.4)	1 (1.4)	0.080
Thromboembolic events (DVT/PE)	3 (8.3)	3 (21.4)	2 (2.7)	0.18

*HIR + CO_2 versus HIR without CO_2. DVT, deep vein thrombosis; PE, pulmonary embolism.

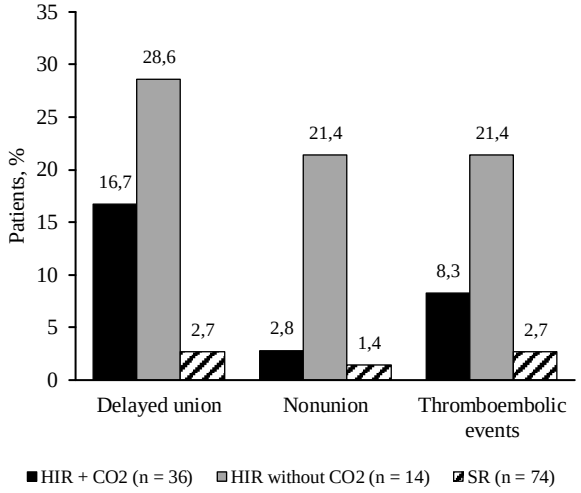


Figure 3: Late complications in HIR patients with carboxytherapy (n = 36), HIR patients without carboxytherapy (n = 14) and SR patients (n = 74), % of patients. HIR + CO_2 vs HIR without CO_2: delayed union p = 0.041, nonunion p = 0.080, thromboembolic events p = 0.18.

In multivariable analysis, D-dimer ≥1.5 mg/L on day 14 increased the odds of late complications 3.6-fold, CRP >20 mg/L 3.1-fold, and an open or comminuted fracture 2.5-fold. Carboxytherapy had an independent protective effect (OR 0.33; 95% CI 0.11–0.95; p = 0.028), corresponding to a 67% reduction in the odds of complications at the same level of inflammation and injury severity. IL-2, TNF-α and 25(OH)D did not remain in the final model (Table 7).

Table 7: Independent predictors of late complications (delayed union or nonunion, thromboembolic events).

Factor on day 14	OR (95% CI)	p
D-dimer ≥1.5 mg/L	3.6 (1.3–10.2)	0.017
CRP >20 mg/L	3.1 (1.1–8.9)	0.032
Open or comminuted fracture	2.5 (1.0–7.2)	0.049
Carboxytherapy (CO_2)	0.33 (0.11–0.95)	0.028
Other (IL-2, TNF-α, 25(OH)D)	–	n.s.

Backward stepwise logistic regression (likelihood ratio). The initial model included laboratory and morphological parameters reflecting the inflammatory–coagulation status and the use of CO_2 therapy; only predictors with p < 0.05 were retained. OR, odds ratio; CI, confidence interval; n.s., not significant.

Discussion

This prospective study showed that the systemic inflammatory–coagulation response after fractures of the pelvis, femur and tibia is linked to injury severity, that re-assessment on day 14 identifies a high-risk group of patients, and that local carboxytherapy in this group was associated with faster resolution of inflammation and hypercoagulation and with fewer late complications.

Open, unstable, comminuted and segmental fractures were accompanied by higher IL-2, TNF-α, CRP and D-dimer and lower 25(OH)D and osteocalcin. This agrees with the concept that tissue damage drives the systemic immune response through damage-associated molecular patterns, which in turn activate coagulation,[5] and that excessive inflammation impairs bone repair.[6–8] The simultaneous rise of inflammatory and coagulation markers and fall of osteotropic factors indicates that inflammation, hypercoagulation and impaired reparative osteogenesis are coupled. Low vitamin D status, which is known to affect fracture healing and post-traumatic bone turnover, adds to this unfavorable background.[9,10]

Day 14 proved to be a key checkpoint for stratification. By this time, SR patients had near-normal values (IL-2 ≈12 pg/mL, TNF-α ≈15 pg/mL, CRP ≈8 mg/L, D-dimer ≈0.8 mg/L), whereas HIR patients retained marked activation. The most severe and persistent response occurred in Tile type C pelvic injuries, which also had the highest rate of early thromboembolic events. These findings support early stabilization of the pelvic ring, intensified thromboprophylaxis and dynamic laboratory monitoring in such patients.[4,16,17]

D-dimer and CRP on day 14 were independent predictors of late complications. IL-2 and TNF-α did not enter the final model, but their pathophysiological role is not diminished. IL-2 reflects T-cell activity, and TNF-α is linked to the innate immune response and the start of the inflammatory–coagulation cascade. Their rise precedes the increase in CRP and the disturbance of coagulation, so they remain useful early markers of immune activation, particularly in combination with other signs.

In HIR patients, carboxytherapy was associated with a faster decline in cytokines, earlier normalization of CRP and D-dimer, and a greater rise in osteocalcin, suggesting stimulation of reparative osteogenesis. These laboratory changes were accompanied by fewer cases of delayed union, a trend toward fewer nonunions, and fewer thromboembolic events (8.3% vs 21.4%), although the last difference was not significant. The effect is consistent with experimental data showing that CO_2 application increases blood flow and angiogenesis and accelerates endochondral ossification in bone defects, fractures and distraction osteogenesis.[11–13] The reduction in thrombotic events may be related to improved regional microcirculation and endothelial function.

In practice, local carboxytherapy can be recommended as part of a comprehensive rehabilitation program for HIR patients without active infection, marked local inflammation or contraindications to injection. It is best started after the acute phase of traumatic inflammation has stabilized and should be monitored by clinical status, CRP, D-dimer and the cytokine profile. In high-risk patients, pharmacological and mechanical VTE prophylaxis, correction of vitamin D deficiency, monitoring of inflammatory–coagulation markers and early functional activation should be combined, taking into account the stability of fixation.

This study has limitations. Carboxytherapy was not allocated randomly: patients in the comparison subgroup had limitations, contraindications or could not complete the protocol, and the subgroup was small (n = 14). The study was conducted in two centers of one region. The laboratory thresholds were taken from published data and require external validation, and outcome assessment was not blinded. Randomized controlled trials are needed to confirm the effect of carboxytherapy on clinical outcomes.

Conclusions

Open, unstable and comminuted fractures of the pelvis, femur and tibia are accompanied by elevated IL-2, TNF-α, CRP and D-dimer and reduced 25(OH)D and osteocalcin. Day 14 after injury is a key checkpoint: combined assessment of clinical and radiological signs with these markers identified a high-risk group (40.3% of patients). In this group, local carboxytherapy was associated with faster regression of systemic inflammation and hypercoagulation, with normalization of CRP in 92% and of D-dimer in 94% of patients by day 45, and independently reduced the odds of late complications (OR 0.33).

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Authors' contributions

Bakhtiyor Z. Khamdamov: Conceptualization, Methodology, Supervision, Writing – review & editing. Azim S. Muminov: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Claude (Anthropic) to translate and condense the dissertation text into English manuscript form. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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