Changes in Biochemical Markers after Lower Limb Fractures, Karl Stoffel,1 Hanna Engler,2 Markus Kuster,3 and Walter Riesen2 1Department of Orthopaedic Surgery, University of Western Australia, Fremantle, Western Australia, Australia; 2 Institute of Clinical Chemistry and Hematology and 3 Department of Orthopaedic Surgery, Kantonsspital, St. Gallen, Switzerland; address correspondence to this author at: Institute of Clinical Chemistry and Hematology, Kantonsspital, 9007 St. Gallen, Switzerland; fax 41-71-494-3900, e-mail hanna.engler@ikch.ch)

Background: The bone remodeling sequence after bone fracture changes the concentrations of biochemical bone markers, but the relationships of fracture size and of healing time to changes in biomarkers are unclear. The present pilot study was undertaken to determine the changes found in serum bone markers after plate osteosynthesis of closed distal tibial and malleolar fractures during a study period of 24 weeks.

Methods: We measured tetrathionate acid phosphatase (TRACP 5b), collagen type I C-terminal telopeptide (ICTP), bone-specific alkaline phosphatase (bone ALP), osteocalcin (OC), procollagen type I C-terminal propeptide (PICP), procollagen type III N-terminal propeptide (PIIINP), and human cartilage glycoprotein 39 (YKL-40) in 20 patients with lower limb fractures (10 malleolar, 10 tibia). A physical examination and radiographs were completed to assess evidence of union.

Results: All malleolar fractures healed within 6 weeks, whereas 2 tibial fractures did not show complete bone healing after 24 weeks. Changes were comparable but more pronounced in the tibia group, and marker concentrations remained increased at the end of study (bone ALP, 86 vs 74 U/L; OC, 14.9 vs 7.7 µg/L; ICTP: 5.6 vs 3.3 µg/L at day 84 after osteosynthesis, P <0.05 in tibia; 80 vs 70 U/L, 8 vs 5.2 µg/L, and 3.5 vs 3.2 µg/L, respectively, in the malleolar fracture group).

Conclusions: In normal bone healing, changes in bone turnover markers were primarily dependent on the fracture size. Delayed tibia fracture healing may involve a disturbance in bone remodeling.

Fracture healing can be divided into 3 distinct phases, inflammation, regeneration, and remodeling. The initial hematoma is replaced very rapidly by moderately dense fibrous tissue containing predominately type III collagen that is produced by fibroblastic cells and preosteoblasts. (1). This tissue is later replaced by type I collagen (2), which makes up more than 90% of the mature bone matrix. Human cartilage glycoprotein 39 (YKL-40), a recently described glycoprotein that belongs to the 18 glycosyl hydrolase family (3), was measured for the first time during fracture healing. The function of YKL-40 is not well characterized in bone healing, but it may play a role in tumor invasion and cancer angiogenesis (4). YKL-40 is a growth factor for connective tissue cells (5) and a potent migration factor for endothelial cells (4). It participates in inflammatory states and vascular processes (4, 6) and may also reflect the course of fracture healing, which is influenced by mechanical environment and vascular supply. During fracture healing, the woven bone containing type III collagen is replaced by regular type I collagen containing lamellar bone. Resorption and bone formation are coupled, but resorption is much faster than formation. Immobilization stimulates the resorption process and inhibits bone formation, whereas mechanical load stimulates the bone formation process (7). Markers for osteoblast activity, osteoclast activity, and the liberated breakdown products of type I collagen can be measured in blood or urine (8).

We recruited 20 adult patients [14 males and 6 premenopausal females; mean (SD) age, 43 (15.3) years; interval, 18–65 years] within 24 h of injury. All patients gave written informed consent, and the study procedures were in accordance with the Declaration of Helsinki. Ethics approval was given by the local ethics committee on human research. Only patients requiring plate osteosynthesis were included in the study (see Table 1 in the Data Supplement that accompanies the online version of this technical brief at http://www.clinchem.org/content/vol53/issue1). After fixation of the tibial fracture, patients were limited to only partial weight bearing for the first 6 weeks, whereas patients with malleolar fracture were permitted full, protected weight bearing with crutches after 2 weeks, depending on local wound status and degree of pain. A fracture was considered to be healed once it was clinically stable, the patient was able to bear weight fully without support, or bridging callus was observed on the x-ray. A detailed description of the study design and methods used is available in the online Data Supplement.

We obtained nonfasting blood samples in the emergency room within 24 h of fracture and fasting samples the morning of the surgery in the case of postprimary intervention and on the 1st day after surgery. Subsequent fasting blood samples were collected at 3 and 7 days and at 2, 6, 12, and 24 weeks after surgery. Aliquots of serum samples were stored at −20 °C. Alkaline phosphatase and bone-specific alkaline phosphatase (bone ALP) activities (the latter after wheat germ lectin precipitation) were measured on a Hitachi 917 analyzer. Intact osteocalcin (OC) was measured on an Immulite 2000 analyzer. Procollagen type I C-terminal propeptide (PICP) (Prolagen-C; Metra Biosystems), YKL-40 (Chondrex; Metra Biosystems) and tartrate-resistant acid phosphatase (TRACP 5b) (BoneTRAP; SBA Sciences) were measured by ELISA, and collagen type I C-terminal telopeptide (ICTP) (Incstar Corporation) and procollagen type III N-terminal propeptide (PIIINP) (RIA-gnost P III P; CIS bio international) were measured by RIA. Nonparametric statistical tests were used for all statistical analysis, with the Bonferroni...
correction for multiple comparisons (see online Data Supplement for details).

No infections, wound healing disturbances, or deep venous thromboses were observed. All malleolar fractures healed within 6 weeks. None of the tibial fractures were considered united at 6 weeks; 5 fractures were united at 12 weeks, and 3 were united at 24 weeks. These results are comparable to those of previous studies reporting tibial fracture healing in 80%-90% of cases within 20–24 weeks (9–11). Two tibial fractures failed to unite within the study period of 24 weeks. Both resulted from high-energy trauma (motor vehicle accident), and patients underwent surgery within 4 and 7 days, respectively. In both cases, the tibial fracture was associated with a fibular fracture, in 1 case (high fibular fracture) nonstabilized. Initial serum values at the time of fracture are presented in Table 1, and Table 2 in the online Data Supplement. FT₄, PICP, and OC were significantly higher in the fibular fracture group rather than in the malleolar fracture group but still within the reference intervals.

In the malleolar fracture group, no changes were observed in bone ALP, PICP, TRACP 5b, or PIIINP concentrations between presentation at the emergency room and time of osteosynthesis, whereas OC and ICTP increased and YKL-40 decreased significantly (P < 0.05). The fibrous tissue marker PIIINP increased after osteosynthesis, reaching maximal concentrations after 2 weeks in the malleolar fracture group and after 12 weeks in the tibial group, decreasing thereafter. Normalization of PIIINP preceded radiologic evidence of bone union, which occurred after 6 weeks in the malleolar fractures and between week 12 and week 24 in the tibial fractures (see Fig. 1 in the online Data Supplement). The sustained high concentrations in the tibial group can be explained by the cancellous bone area (9, 12) and the urgent need for adequate callus to stabilize the fracture. Proinflammatory hormones stimulate chondrocyte proliferation (13), resulting in an increase of the cartilage marker YKL-40. A narrow peak, particularly noticeable in the tibial group, was observed immediately after osteosynthesis and might be related to an inflammatory process, vascularization, or articular cartilage turnover (3, 5, 6, 13).

Activation of osteoclasts was indicated by an increase in TRACP 5b activity and ICTP concentrations (Fig. 1, right panels). After osteosynthesis, a marked increase in TRACP 5b concentrations (Fig. 1, top right panel) was observed in both groups (P < 0.05), reaching a maximum after 7 days in the malleolar group and after 2 weeks in the tibial fracture group. Because the assay is specific for the bone-specific isoform TRACP 5b, it is unlikely that this increase is caused by hematoma formation. ICTP showed a biphasic pattern after fracture. A first peak was reached 3 days after osteosynthesis in malleolar fractures and after 3–7 days in the tibial group. A second peak was observed after 6 weeks in both groups. The higher increase in TRACP 5b activity and ICTP concentrations in tibial fractures probably reflects the larger fracture area and the need for more extensive bone remodeling in the tibial group rather than a different period of immobilization, because the values were still increased in both groups after radiologic bone union and full weight bearing.

Osteoblast-like cells respond to bone fracture with a temporary cessation of the cell activity (10, 12). Our data show an early decrease in bone ALP activity and PICP concentrations (Fig. 1, left panels). The lowest concentrations were observed 3 days after surgery; thereafter, the mean values increased in both groups (P < 0.05), with higher concentrations in the tibial compared with the malleolar fracture group (P < 0.05 at the end of the study). OC, a marker of the mineralization process, increased immediately after fracture, reaching a first peak shortly after osteosynthesis, and increased gradually thereafter (Fig. 1, bottom panel). Because most of this protein is incorporated into the bone matrix (14), the initial increase might reflect a sudden liberation in the course of fracture or during the initial stage of bone resorption, as suggested by other studies (10, 11). OC leveled off 14 days after osteosynthesis in malleolar fractures but increased further in tibial fractures. PICP and OC were significantly higher in tibial than malleolar fractures (P < 0.05) from 3 days (OC) and 14 days (PICP) onward. These differences might be explained by the different fracture sizes. Increased OC in normally healed tibial fractures was also reported by

<table>
<thead>
<tr>
<th>Marker</th>
<th>Malleolar fractures (n = 10), median (interval)</th>
<th>Tibial fractures (n = 10), median (interval)</th>
<th>P value</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH, µU/L</td>
<td>1.400 (0.94–3.40)</td>
<td>0.995 (0.800–1.575)</td>
<td>NSa</td>
<td>0.4–4.0</td>
</tr>
<tr>
<td>ft4, pmol/L</td>
<td>13.0 (10.4–14.4)</td>
<td>14.15 (13.1–15.0)</td>
<td>&lt;0.05</td>
<td>8–23</td>
</tr>
<tr>
<td>PTH, ng/L</td>
<td>40.0 (25–46)</td>
<td>43.5 (20.3–60.8)</td>
<td>NS</td>
<td>12–80</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.3 (2.3–2.4)</td>
<td>2.2 (2.2–2.3)</td>
<td>NS</td>
<td>2–2.5</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>1.0 (0.9–1.0)</td>
<td>0.9 (0.8–1.0)</td>
<td>NS</td>
<td>0.8–1.5</td>
</tr>
<tr>
<td>PIIINP, U/mL</td>
<td>0.428 (0.374–0.487)</td>
<td>0.442 (0.437–0.497)</td>
<td>NS</td>
<td>0.3–0.8</td>
</tr>
<tr>
<td>YKL-40, µg/L</td>
<td>114 (68–169)</td>
<td>101 (73–123)</td>
<td>NS</td>
<td>25–93 F; 24–125 mol/L</td>
</tr>
</tbody>
</table>

a NS, not significant.
Oni et al. (15), whereas other studies found no changes (11).

Delayed bone fracture healing was observed in 2 cases with high-energy trauma in which the tibial fracture was associated with a fibular fracture. Early signs of a probable delayed union may be an uncoupling and malfunction of the bone remodeling unit. In one patient, this was indicated by a high increase in osteoblast activity (3.5-fold increase after 12 weeks vs admission level compared with maximal 2.5-fold increase in normally healing tibial bone) and impaired mineralization (OC concentrations unchanged) in conjunction with an insufficient increase in osteoclast function (no changes in TRACP 5b activity, no changes in ICTP concentrations after the initial increase until plate osteosynthesis), results similar to those reported by Hermann et al. (16) (see File and Fig. 2 in the online Data Supplement). The other case showed minor changes in bone ALP and PICP, with high initial OC compared with normally healing tibial fractures (13.5 ng/L vs 7.7 ng/L), a 1.7-fold increase until osteosynthesis, and no further changes, data similar to other reports (11, 12).

In conclusion, bone turnover markers increased after osteosynthesis, with resorption markers reaching peak concentrations before increases in bone formation markers. These results are explained by the coupling between bone resorption and formation, but bone resorption is much faster than bone formation (7). Time for fracture healing and extent of changes in markers of bone metabolism are mainly dependent on fracture size (Fig. 1). The initial decrease in bone formation markers and the marked increase in ICTP immediately after fracture are mediated by immobilization and osteosynthesis after injury. Markers of bone turnover remain increased even after bone union is completed (11, 17–19).

Fig. 1. Kinetics in bone turnover markers after malleolar or tibial fracture.
Bone formation markers are depicted in the left panels, bone resorption markers in the right panels. The top panels show changes in osteoblast (bone ALP) and osteoclast (TRACP 5b) activity. The middle panels show changes in collagen matrix synthesis (PICP) and degradation (ICTP). In the bottom panel, OC is depicted as a marker for mineralized matrix formation. Data are given as medians (25th and 75th percentiles).
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References

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