

## Supplementary Material

**TABLE SI**  
**Characteristics of the studies that assess the role of BTMs**  
**in predicting the prognosis of bone fracture healing.**

Study	Type of study	Population	Intervention
Oni et al. (1989)	Observational prospective study	50 adult patients with closed tibial shaft fractures were included and after divided in two groups (delayed union and normal bone union) Treatment: conservative	Serum ALP, serum intact OC
Joerring et al. (1992)	Observational prospective study	12 patients with fracture of the tibial diaphysis were allocated in delayed union and normal bone union groups Gender: males and females Age: 23-67 years (males) and 24-72 years (females) Treatment: conservative	Serum PICP (RIA), PIIINP (RIA) and ICTP (RIA) Sample collection: baseline, 1, 2, 6, 12, 18, 26 weeks after fracture Sample storage: -20°C until measurement Delay between sample collection and assay: not mentioned
Emami et al. (1999)	Observational prospective study	23 patients developed normal bone union and 7 with delayed union. Gender: males and females Age: 39.9 years (mean) Treatment: osteosynthesis with intramedullary nails	BALP (wheat germ lectin), OC (RIA), PYD (RIA) Sample collection: baseline, 1, every 3 <sup>rd</sup> week until healing, 26 and 52 weeks after treatment Sample storage: -20°C until measurement Delay between sample collection and assay: not mentioned
Kurdy (2000)	Observational prospective study	17 patients with normal bone union and 3 with delayed union Gender: 19 males and 1 female Age: 33.7 years (mean) Treatment: osteosynthesis with external fixator and conservative methods with casts	BALP (wheat germ lectin), ICTP (RIA), PICP (RIA), PIIINP (RIA) Sample collection: baseline, 4, 8, 14 days and 5, 10, 14, 20 weeks after fracture Sample storage: -20°C until measurement Delay between sample collection and assay: not mentioned
Herrmann et al. (2002)	Observational prospective study	10 patients with normal fracture healing and 4 patients with delayed healing Gender: 9 males and 5 females Age: 43.7 years (mean) Treatment: osteosynthesis	Serum BALP (wheat-germ lectin methods), intact and N-MID fragment OC (ELISA) and $\beta$ -CTX (ELISA) Sample collection: 24 h, 7, 14, 28, 42, 60, 90, 180, 365 days after fracture Sample storage: -20°C until measurement Delay between sample collection and assay: until 90 days
Zimmermann et al. (2005)	Observational prospective study	10 patients with delayed union matched to 10 patients with normal bone healing Gender: 16 males and 4 females Age: 46.6 years (mean) Treatment: osteosynthesis with intramedullary nail or compression plate Matching criteria: gender, age, type of fracture, localization and technique of osteosynthesis	Serum TGF- $\beta$ 1 (ELISA), BMP-2 (ELISA), BMP-4 (ELISA) Sample collection: baseline, 1, 2, 4, 6, 8, 12, 24 after treatment Sample storage: -80°C until measurement Delay between sample collection and assay: not mentioned
Marchelli et al. (2009)	Observational cross-sectional study	16 patients with atrophic non-union, 18 controls that have already healed a same fracture type within 6 months and 14 controls that were recovering from the same fractures type at that moment Gender: male Age: 20-39 years Matching criteria: gender, age, localisation and type of fracture Treatment: not mentioned	Serum intact OC (ELISA), BALP (ELISA), OPG (ELISA), free RANKL (ELISA) Sample collection: single measure at 6-11 months after injury Sample storage: not mentioned Delay between sample collection and assay: not mentioned

TABLE SI (continuation)

Study	Type of study	Population	Intervention
Moghaddam et al. (2011)	Observational prospective study	248 patients initially selected. Included in the study 15 patients with atrophic non-union matched to 15 patients with normal bone healing Gender: 24 males, 6 females Age: mean age was 46.7 years in nonunion and 46.4 years in union group Matching criteria: gender, age, type of fracture, localisation, type of fixation, body mass index, smoker status Treatment: osteosynthesis with compression plates, intramedullary nails, external fixators	Serum BALP (CLIA), TRAP 5b (EAA), $\beta$ -CTX (ECLIA), PINP (ECLIA) Sample collection: 1, 2, 4, 8, 12, 52 weeks after surgical intervention Sample storage: -80°C Delay between sample collection and assay: until 5,6 years
Mukhopadhyay et al. (2011)	Observational prospective study	36 adult patients with long bone fracture were divided in two groups: impaired union and normal bone union. Gender: 25 males and 11 females Age: not mentioned Treatment: osteosynthesis or conservative methods	Serum ALP (enzymatic method), urinary total HYP, urinary free HYP (spectrophotometry) Sample collection: baseline, 3, 8, 12 weeks after fracture Sample storage: not mentioned Delay between sample collection and assay: not mentioned
Sarahrudi et al. (2011)	Observational prospective study	9 patients with normal union matched to 9 patients with impaired bone healing Gender: 13 males and 5 females Age: 50.4 (mean age) Treatment: osteosynthesis compression plates, intramedullary nail, external fixators Matching criteria: gender, age, type of fracture, localization, technique of osteosynthesis and soft-tissue damage	Serum TGF- $\beta$ 1 (ELISA) Sample collection: 1, 2, 4, 6, 8, 12, 24 weeks after treatment Sample storage: -80°C Delay between sample collection and assay: not mentioned
Singh Ajai et al. (2013)	Observational prospective study	69 patients with normal union, 26 patients with impaired bone healing Gender: not mentioned Age: 18-45 years Treatment: conservative	Serum ALP (spectrophotometry) Sample collection: baseline, 2, 3, 4, 6, 8, 12 weeks after treatment and at 6 months Sample storage: not mentioned Delay between sample collection and assay: not mentioned
Baardewijk et al. (2013)	Observational retrospective study	34 patients with normal fracture union and 31 patients with a delayed healing process Gender: 25 males and 40 females Age: 40.7 (mean age, normal union), 43.3 (mean age, delayed union) Treatment: osteosynthesis compression plates, intramedullary nail, external fixators and conservative methods Matching criteria: gender, age, type of fracture, localization, technique of osteosynthesis and diabetes mellitus and smoking status.	Serum BMP-2, 4, 6, 7, 9 (ELISA) Sample collection: 4.9 years after bone healing Sample storage: -80°C Delay between sample collection and assay: not mentioned
Kommenou et al. (2005)	Observational prospective study	35 dogs with normal bone union, 36 with delayed union and 12 with non-union Gender: 49 males and 34 females Age: 2-8 years Treatment: osteosynthesis with nondynamic compression plates	Serum ALP (spectrophotometry) Sample collection: baseline, 10, 20, 30 days and afterward on monthly basis until bone union or signs of non-union were evident Sample storage: not mentioned Delay between sample collection and assay: not mentioned
Sousa et al. (2011)	Observational prospective study	7 dogs with normal bone union, and 2 with non-union process Gender: 4 males and 3 females Age: 18 months to 8 years Treatment: osteosynthesis with compression plates, external fixators and intramedullary nails.	Serum ALP, BALP (ELISA), TRAP Sample collection: baseline, 10, 20, 30, 60, 90 days after surgical intervention Sample storage: -80°C Delay between sample collection and assay: until 6 months

TABLE SI (continuation)

Study	Type of study	Population	Intervention
Southwood et al. (2003)	Experimental study	32 rabbits distributed in non-infected fracture group or infected fracture group Gender: females Age: 9-10 months Treatment: femoral fracture defect was inoculated with colony-forming units of <i>Staphylococcus aureus</i>	Serum BALP (ELISA), intact OC (ELISA), DPD (ELISA) Sample collection: baseline, 4, 8, 12, 16 days after surgical intervention Sample storage: -80°C Delay between sample collection and assay: until 16 weeks
Klein et al. (2004)	Experimental study	7 ewes with appropriated normal bone union process and 7 with a delay in the bone union process Gender: females Age: 2 years Treatment: midshaft tibial osteotomy stabilized with either a monolateral external fixator or an unreamed tibial nail	Serum BALP (RIA), PICP (RIA), PIIINP (RIA) Sample collection: baseline, 1, 2, 3, 4, 5, 6, 7, 8, 9 weeks after surgical intervention Sample storage: -80°C Delay between sample collection and assay: : not mentioned

ALP: Alkaline phosphatase; BALP: Bone specific alkaline phosphatase; OC: Osteocalcin; PINP: Amino-terminal procollagen propeptides of collagen type I; PICP: Carboxy-terminal procollagen propeptides of collagen type I; PIIINP: Amino-terminal procollagen propeptides of collagen type III; CTX: Cross-linked C-terminal telopeptides of type I collagen; ICTP: Carboxy-terminal telopeptide of type I collagen; HYP: Hydroxyproline; DPD: Deoxypyridinoline; PYD: Pyridinoline; ICTP: Carboxy-terminal telopeptide of type I collagen; TRAP5b: Tartrate-resistant acid phosphatase isoenzyme 5b; OPG: Osteoprotegerin; RANK: Receptor activator of nuclear factor NF- $\kappa$ B; TGF- $\beta$ 1: Transforming growth factor beta 1; BMP: Bone morphogenetic protein; ELISA: Enzyme-Linked Immunosorbent Assay; RIA: Radioimmunoassay; ECLIA: Electrochemiluminescent Immunoassays; CLIA: Chemiluminescent Immunoassay; EAA: Enzyme Activity Assay.