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REVIEW

Fracture-induced changes in biomarkers CTX, PINP, OC, and BAP—a systematic review

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Abstract

To assess the time from fracture until bone turnover markers (BTM), which are biochemical markers reflecting in vivo bone formation and resorptive activity, have returned to a stable level since BTM have been shown to be at least as good as bone mineral density in monitoring the effect of anti-resorptive treatment in osteoporosis. This study searched for articles in PUBMED, CINAHL, Medline, EM-BASE, and Cochrane, and identified 3486 unique articles. These articles were screened based on predefined inclusion and exclusion criteria. Seven articles addressing time to normalization of either CTX, PINP, osteocalcin, or bone-specific alkaline phosphatase after a recent fracture were identified and these were analyzed qualitatively. CTX appeared to return to baseline within 6 months. PINP appeared to return to baseline within 6 months and interestingly dip below baseline after a year. Osteocalcin was elevated throughout the first year after a fracture, with most changes in the first 6 months. Bone-specific alkaline phosphatase (BAP) was increased for up to a year, however with a discrepancy between used assays. Seven studies were identified, showing CTX and PINP to return to baseline within 6 months. OC was elevated for 12 months. BAP was increased for up to a year. However, none of these studies had fasting patients and a long follow-up period with regular measurements. The studies could indicate that the BTM CTX and PINP have returned to baseline within 6 months; however, further studies are needed assessing pre-analytical factors while having a long follow-up. Bone turnover markers appear as good as or better than bone mineral density in monitoring the effect of anti-resorptive medication in osteoporosis. This study tries to identify the time from fracture until BTM are back at baseline. Most studies did not however take pre-analytical variation into consideration. Further research is therefore needed.

Keywords Bone turnover markers · Fracture · Osteoporosis · Systematic review

Introduction

Bone is a dynamic tissue undergoing continuous anabolic and catabolic turnover. Under normal conditions, there is a balance between bone formation and bone resorption. In contrast, in conditions affecting bone metabolism, e.g., osteoporosis or Mb. Paget, the bone turnover is often changed [1, 2].

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The turnover of bone can be assessed by measuring bone turnover markers (BTM). BTM can be subdivided into bone formation markers [3, 4] and bone resorption markers. Bone formation markers primarily reflect osteoblastic bone-forming activity. During bone formation, type 1 collagen replaces type 3 collagen and takes part of the bone matrix [5]. This, in turn, yields fragments from the formation of type 1 collagen from procollagen: type-1 c-terminal propeptide, PICP [6, 7], and type-1 n-terminal propeptide, PINP [8, 9]. Several articles have suggested that PINP is a more sensitive bone formation marker than the others. As such, it is preferable with regard to monitoring the effect of anti-resorptive medication and bone anabolic medication [10].

During enzymatic degradation of bone matrix by the osteoclasts, the bone resorption marker C-terminal collagen cross-links, CTX, which consists of cross-linked telopeptides from collagen, are released [11].

Table 1 Search strategy. The individual queries were all searched individually. The verticals were combined by an OR operator in the search engines and the different queries were combined by an AND operator

Biomarker query	Osteoporosis query	Fracture query
PINP (ti/ab)	Osteoporosis (ti/ab)	Fracture (ti/ab)
P1NP (ti/ab)	Osteopenia (ti/ab)	
Procollagen (adj3) Propeptide (ti/ab)	Osteopor$ (ti/ab)	
Procollagen (adj3) peptide (ti/ab)	Low bone mass (ti/ab)	
Collagen (adj3) propeptide (ti/ab)		
BASP OR BALP OR BAP AND bone OR bones OR biomarker$ OR biological marker$) (ti/ab)		
Crosslaps (ti/ab)		
Telopeptide$ (adj3) collagen (ti/ab)		
n-Telopeptide$ (adj3) collagen (ti/ab)		
c-Telopeptide$ (adj3) collagen (ti/ab)		
Bone turnover marker$ (ti/ab)		
Bone metabolic marker$ (ti/ab)		
Biological markers and exp "Bone and Bones" (ti/ab)		
Biochemical marker$ or biomarker$ or biological marker$ (adj2) bone$ (ti/ab)		
Bone marker$ (ti/ab)		
Osteocalci$ (ti/ab)		
BGLAP (ti/ab)		
Bone gamma (adj4) protein (ti/ab)		

In 2011, a working group from the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry (IFCC) recommended CTX and PINP as the BTM of choice to be used in fracture risk prediction and monitoring anti-resorptive treatment in osteoporosis (e.g., bisphosphonates) [12], and furthermore recommended the use of CTX and PINP in clinical studies regarding osteoporosis. Studies measuring both bone mineral density (BMD) and BTM in association with fracture risk in osteoporotic patients have shown that changes in S-CTX and S-PINP are at least as good as BMD in predicting fracture risk reduction in patients receiving anti-resorptive treatment [13–15].

Moreover, a working group from IOF and European Calcified Tissue Society (ECTS) has also recommended the use of PINP and CTX in the screening of adherence to treatment with bisphosphonates [16].

Therefore, using BTM could be beneficial; however, a number of pre-analytical factors affect the variability on the analytes.

Although CTX is the recommended bone resorption marker, a lot of factors influence the concentration. Among those are the circadian rhythm of CTX with peaks in concentration around 5 a.m. and nadir around 2 p.m. [17–19]. In contrast, PINP has a less pronounced circadian rhythm with a smaller diurnal variation [20]. In addition, other factors like food intake [17, 21], renal insufficiency [22], age [23], and exercise [24] influence the concentrations of CTX. Therefore, measurements of CTX should be performed on fasting patients in the morning (i.e., before 10 a.m.) in order to reduce the pre-analytical variation as much as possible [17]. As a consequence of the small circadian rhythm of PINP, the time of day can be ignored if PINP is the only BTM analyzed [10, 20, 25]. PINP and osteocalcin (OC) are influenced by food intake as well, but to a lesser degree than CTX. In contrast, bone-specific alkaline phosphatase (BAP) is not affected by food intake. Thus, if PINP is the only BTM analyzed, fasting is however not necessary [10]. Vigorous exercise should be avoided the day before a blood sample, in order to reduce variation [26]. In order to ensure the stability until analysis of the two BTM, OC, and CTX, blood should be collected in EDTA-containing tubes, whereas PINP and BAP are stable in serum [27]. The samples should be processed immediately or frozen in order to ensure the quality [27].

Another pre-analytical factor potentially affecting levels of BTM is recent fracture and the healing of bone following a fracture. Bone healing can be subdivided into four phases: an initial inflammation phase in which a hematoma is formed around the fracture [28]. This hematoma and inflammation persists for roughly 2 weeks after which a soft callus forms around the fracture [28]. Later, a hard callus is formed, which persists up until 4 months after fracture. During this period, new bone is formed in the gap of the fracture by endochondral ossification. Once the fracture is solidly united, remodeling is initiated in the fracture area [28]. This remodeling can take up to several years and is characterized by osteoclast and osteoblast activity replacing the woven bone in the fracture area with lamellar bone [28]. Thus, the bone healing process can

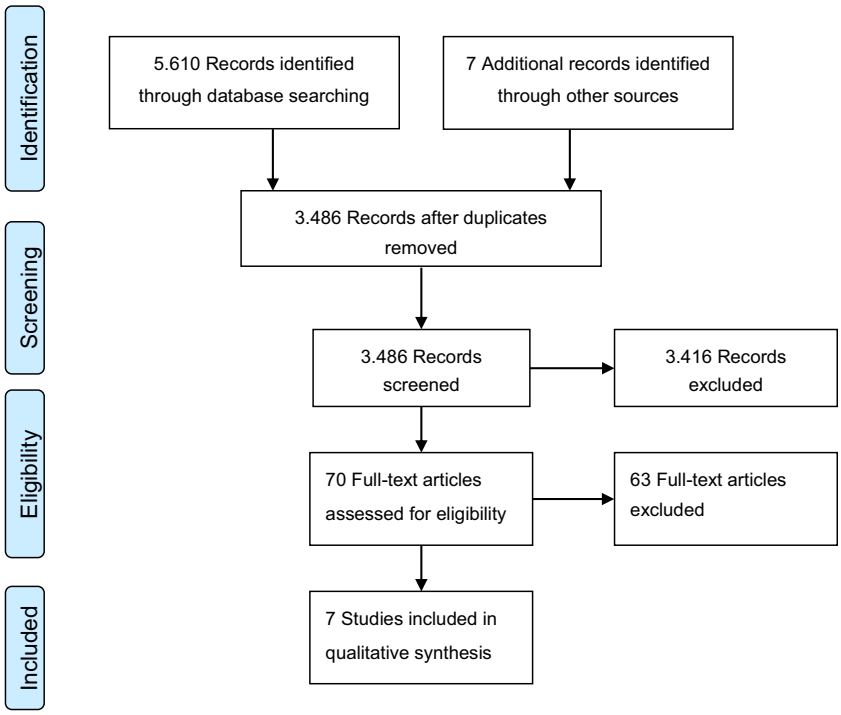

Fig. 1 PRISMA flow chart of search strategy

potentially affect the levels of bone turnover markers long time after the fracture has initially stabilized. Therefore, the aim of this study was to systematically review studies analyzing the concentration of BTM following fractures and find the time from fracture until the concentration of BTM has returned to pre-fracture levels and furthermore has stabilized. This is important in order to be able to know when and how BTM can be used in monitoring anti-osteoporotic again after a fracture.

Method

Search strategy

A systematic literature search was performed on CINAHL, EMBASE, Medline, PUBMED, and Cochrane Libraries on the 22nd of February 2018 to obtain every report regarding BTM, osteoporosis, and fractures in combination. In summary, the strategy shown in Table 1, where the search queries in the columns were combined by an OR-operator and the rows were combined by an AND-operator, was followed. MESH-terms were deliberately avoided in order to include the newest articles. On the 11th of June 2018 and again on March 15, 2019, the search was conducted a second and third time in order to include articles published in between the two searches. Publications from 1947 until week 23 of 2018 were included. Articles were included in English, German, and French.

Selection process

The first author conducted the search and entered the articles into EndNote (EndNote X8;

Thomson Reuters, Philadelphia, PA), where duplicates were removed. The articles were imported to the online software platform Covidence (www.covidence.com), where the first author screened the articles based on the inclusion and exclusion criteria. In the case of articles that did not explicitly fulfill the inclusion criteria, the last author was consulted.

The reference lists of chosen articles were screened for missed titles.

The inclusion criteria of articles were as follows:

- Studies describing any BTM following fracture or trauma
- Studies with a population of patients with osteoporosis
- Studies comparing “baseline” BTM measurements with one or more follow-up measurements
- Studies with a follow-up time of 24 weeks or more

The exclusion criteria were as follows:

- Studies where all or a majority of patients were treated with anti-resorptive pharmaceuticals (e.g., zoledronic acid)
- Studies where BTM were measured in animals
- Conference abstracts

All inclusion criteria and none of the exclusion criteria had to be fulfilled for the article to be included.

Table 2 Included studies of the systematic review. All biomarkers analyzed in the respective studies are listed regardless of whether they are relevant to the review

Author	Biomarker(s)	# of patients	Men/women	Age range (mean)	Length of follow-up	Fracture type
Hitz et al. 2007 [29]	• S-PINP • S-OC • S-ICTP	122	21/101	Unclear range (70.0)	1 year	Hip or upper extremities
Ingle et al. 1999 [30]	• BAP • OC • PINP • TRAcP • DPD • NTX	14	7/7	51–86 (63)	1 year	Ankle
Ingle et al. 1999 [31]	• BAP • OC • PINP • TRAcP • DPD • NTX	20	0/20	47–79 (63)	1 year	Distal forearm
Ivaska et al. 2007 [32]	• S-CTX • S-TRAcP • S-PINP • S-OC • U-DPD • U-OC	113	0/113	Unclear range (77.9)	1 year	Hip, distal forearm, vertebral, and others
Ohishi et al. 2008 [33]	• S-CTX • S-OC • U-Pyr • U-DPD • U-CTX	33	8/25	Unclear range (71.8)	48 weeks	Vertebral
Veitch et al. 2006 [34]	• S-BAP • S-OC • S-PINP • S-CTX • S-PIIINP	18	16/2	18–78 (34)	24 weeks	Tibial
Yan et al. 2017 [35]	• S-CTX • S-PINP • S-POstn	261	0/261	76–84 (80)	1 year	Femoral

S biomarkers measured in serum, *U* biomarkers measured in urine, *BAP* bone-specific alkaline phosphatase, *CTX* carboxy-terminal telopeptide cross-links, *DPD* deoxypyridinoline, *NTX* amino-terminal telopeptide cross-links, *OC* osteocalcin, *Pyr* pyridinoline, *PICP* type-1 c-terminal propeptide, *PINP* type-1 n-terminal propeptide, *PIIINP* type-3 n-terminal propeptide, *POstn* periostin, *TRAcP* tartate-resistant acid phosphatase

Quality assessment of studies

No quality assessment tool was found as the perfect tool for assessing bias and quality in the included studies. Therefore, the assessment of quality was based on whether the study made an effort to identify and eliminate pre-analytical factors, the length of follow-up, and the characteristics of the included patients.

Results

Study selection

The PRISMA flow chart of the search strategy can be seen in Fig. 1. The search strategy yielded 7 articles. Main characteristics of the 7 articles are presented briefly in Table 2. All 7 included studies were prospective cohort studies. A total of 687 patients were included in the studies of which 80.5% (553) had a fracture. In total, 10 biomarkers were investigated in the 7 articles; however, not all of these were bone markers and most were only analyzed in one study. Thus, four biomarkers were chosen for further analysis: BAP, PINP, CTX, and OC. The studies are listed in Table 3 with a summary of how the biomarkers are deviating from the baseline in the studies.

Changes in CTX

The studies from Ivaska et al., Ohishi et al., Veitch et al., and Yan et al. all measured CTX after fracture [32–35]. Ivaska et al. found that CTX increased during 2 weeks following fracture and decreased in the following 2–3 months. After 6 months, they had returned to a stable level close to pre-

Table 3 Summary of concentration of biomarkers measured in the included studies. Columns are denoting the deviation compared to the baseline established in the study. ↑: increased concentration $p < 0.05$, ↓: decreased concentration $p < 0.05$, ↑ NC: increase but not calculated if it is significant, ↓ NC: decrease but not calculated if it is significant, 0: nonsignificant deviation, ND: not done at this point of time

Author	1 week	2 weeks	4 weeks	6 weeks	2 months	3 months	4 months	6 months	12 months
CTX									
Ivaska et al. [32]	0	ND	ND	ND	ND	ND	↑	ND	↑ NC
Ohishi et al. [33]	ND	↑	↑	ND	ND	0	ND	0	0
Veitch et al. [34]	↑	↑	↑	ND	↑	↑	↑	↑	ND
Yan et al. [35]	↑ NC	↑ NC	↑ NC	ND	ND	ND	ND	ND	↑ NC
PINP									
Hitz et al. [29]	ND	ND	↑	ND	ND	↑ NC	ND	ND	↓
Ingle et al. [30]	↑	↑	↑	↑	ND	↑	ND	↑	0
Ingle et al. [31]	↑	↑	↑	↑	ND	↑	ND	↑	↑
Ivaska et al. [32]	–	ND	ND	ND	ND	ND	↑	ND	↑ NC
Veitch et al. [34]	↑	↑	↑	ND	↑	↑	↑	↑	ND
Yan et al. [35]	↑ NC	↑ NC	↑ NC	ND	ND	ND	ND	ND	↑ NC
OC									
Hitz et al. [29]	ND	ND	↑ NC	ND	ND	↑ NC	ND	ND	↑ NC
Ingle et al. [30]	↑	↑	0	↑	ND	↑	ND	↑	0
Ingle et al. [31]	0	0	0	↑	ND	↑	ND	↑	↑
Ivaska et al. [32]	0	ND	ND	ND	ND	ND	↑	ND	↑ NC
Ohishi et al. [33]	ND	0	↑	ND	ND	↑	↑	ND	↑
Veitch et al. [34]	↑	↑	↑	ND	↑	↑	↑	↑	ND
BAP (ELISA)									
Ingle et al. [30]	0	0	↑	↑	ND	0	ND	0	0
Ingle et al. [31]	0	↑	↑	↑	ND	↑	ND	↑	↑

fracture levels, although it was elevated throughout the 1-year follow-up. Ohishi et al. found a significant increase in both u- and s-CTX in the first 4 weeks but found no significant deviation after the 12th week. Furthermore, they found no significant difference in CTX when comparing patients with non-union of the spine and patients with normal union of the spine. Veitch et al. found elevated levels of CTX throughout the 24-week follow-up period following a tibial shaft fracture. Lastly, Yan et al. found an increase in CTX in the first 14 days of the study followed by a steady decline throughout a yearlong follow-up. However, it did not return to baseline within the follow-up period.

Changes in PINP

Hitz et al., Ingle et al., Ivaska et al., Veitch et al., and Yan et al. all analyzed PINP after fracture [29–32, 34, 35]. Hitz et al. found PINP to rise during the early period after fracture, but it had returned to baseline after 4 months. Interestingly, a dip below their baseline was detected after 1 year. They did however not calculate whether the deviation from baseline was statistically significant. Ingle et al. found that for ankle fractures, PINP was significantly higher within the first 180 days. For forearm fractures, they found PINP to be significantly elevated up to and including 360 days after the fracture. Ivaska et al. found that like CTX, PINP increased in 2 weeks following fracture and decreased in the following 2–3 months with a complete return to a stable level close to pre-fracture levels within 6 months. Veitch et al. found a significant increase in PINP of 100% within the first 12 weeks which remained elevated compared to baseline throughout the 52-week follow-up. Lastly, Yan et al. found a gradual increase of PINP in the first 30 days followed by a steady decline throughout the study period of a year.

Changes in OC

Hitz et al., Ingle et al., Ivaska et al., Ohishi et al., and Veitch et al. measured OC after fracture [29–34]. Hitz et al. found OC to rise throughout the 12-month follow-up; however, like for PINP, they did not calculate whether this deviation was statistically significant. In patients with ankle fractures, Ingle et al. found OC to be above baseline level between 7 and 180 days after the fracture, but no significant increase in OC after 360 days could be detected. In patients with distal forearm fractures, they found OC to be significantly increased compared to baseline level between 42 and 360 days post fracture. Ivaska et al. found serum OC to peak later than other

Table 4 Assessments of strengths and weaknesses. The studies were screened for whether they assessed the pre-analytical variations and if they did anything to reduce these. Based on these observations and general strengths and weaknesses in studies (e.g., long follow-up as a strength and few patients as a weakness), a summary of strengths and weaknesses are made in the rightmost columns

Author	Fasting	Time of day of blood sample	Timing of first blood sample	Strengths	Weaknesses
Hitz et al. 2007 [29]	No information in article	Blood samples drawn before noon within a 3-h window to reduce effect of circadian rhythm	Varying time of inclusion. Some inclusion of patients through mail, yielding a slow process	• Follow-up blood samples drawn in a 3-h window	• Varying time of inclusion • Unclear whether patients were fasting
Ingle et al. 1999 [30]	Baseline blood sample was not in fasting state. Follow-up samples was drawn in fasting state	Unclear time of baseline sample. Follow-up blood samples were drawn in the morning	Baseline blood samples drawn within 24 h of fracture	• 52-week follow-up • Relatively fast inclusion of patients after fracture	• Few ($n = 14$) patients in study • Comparison of non-fasting baseline with fasting follow-up blood samples
Ingle et al. 1999 [31]	Baseline blood sample was not in fasting state. Follow-up samples was drawn in fasting state.	Unclear time of baseline sample. Follow-up blood samples were drawn in the morning	Baseline blood samples drawn within 24 h of fracture	• 52-week follow-up • Relatively fast inclusion of patients after fracture	• Few ($n = 20$) patients in study • Comparison of non-fasting baseline with fasting follow-up blood samples
Ivaska et al. 2007 [32]	Patients were not fasting when the blood samples were drawn	Blood samples were collected between 1:15 a.m. and 11:30 p.m. at baseline and between 8:30 and 11:35 a.m. on follow-up	Baseline blood samples drawn within 26.4 h (mean: 6.8 h) of fracture	• Pre-injury baseline blood and urine samples • Sporadic follow-up with varying intervals between samples	• Blood samples collected were non-fasting
Ohishi et al. 2008 [33]	No information in article	Blood samples drawn between 9:00 a.m. and 11:00 a.m.	Blood samples drawn between 9:00 a.m. and 11:00 a.m.	• Investigates whether there is a difference in normal and delayed union	• Unclear whether patients are fasting
Veitch et al. 2006 [34]	Baseline blood samples drawn in non-fasting state. Follow-up blood samples in fasting state	Blood samples drawn between 8:00 a.m. and 10:00 a.m.	Baseline blood sample drawn within first 48 h	• Follow-up blood samples drawn in a short time interval and in fasting patients	• Baseline blood sample drawn in non-fasting state • Few ($n = 18$) patients
Yan et al. 2017 [35]	Blood samples drawn in fasting state	Blood samples drawn in the morning	Baseline blood sample drawn in morning after admission between 7:00 a.m. and 9:00 a.m.	• Large cohort ($n = 261$) • Large control group ($n = 106$) • All blood sampled in one batch • Fasting patients	• No analysis between 1 and 12 months

measured BTM (PINP, CTX, and TRACP5b) and found OC levels to be elevated for a longer period. They concluded, that most changes in BTM seem to take place in the first 6 months, and that the BTM was elevated throughout the 1-year follow-up period. Ohishi et al. found OC to be significantly elevated between weeks 4 and 48 post fracture. They also found OC to peak 24 weeks post fracture. Lastly, Veitch et al. found a significant increase in OC peaking 12 weeks after fracture. They attributed the relatively small increase compared to PINP to cortisol-mediated suppression of OC, based on their findings of 45% elevation of cortisol in fracture patients during the first week.

Changes in BAP

Ingle et al. measured BAP after fracture in two separate studies. Ingle et al. found an increase in BAP between days 28 and 42 when analyzing with ELISA and between days 28 and 90 when analyzing with IRMA in patients with ankle fracture and an increase in BAP from day 14 and throughout the yearlong

Table 5 Included articles sorted by biomarker

Biomarker	Articles
PINP	• Bone mineral density and bone markers in patients with a recent low-energy fracture: effect if 1-year treatment with calcium and vitamin D1–3 [29] • Changes in bone mass and bone turnover following ankle fracture [30] • Changes in bone mass and bone turnover following distal forearm fracture [31] • Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women [32] • Changes in bone mass and bone turnover following tibial shaft fracture [34] • Circulating periostin levels increase in association with bone density loss and healing progression during the early phase of hip fracture in Chinese older women [35]
CTX	• Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women [32] • Sequential changes of bone metabolism in normal and delayed union of the spine [33] • Changes in bone mass and bone turnover following tibial shaft fracture [34] • Circulating periostin levels increase in association with bone density loss and healing progression during the early phase of hip fracture in Chinese older women [35]
OC	• Bone mineral density and bone markers in patients with a recent low-energy fracture: effect if 1-year treatment with calcium and vitamin D1–3 (33) • Changes in bone mass and bone turnover following ankle fracture [30] • Changes in bone mass and bone turnover following distal forearm fracture [31] • Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women [32] • Sequential changes of bone metabolism in normal and delayed union of the spine [33] • Changes in bone mass and bone turnover following tibial shaft fracture [34]
BAP	• Changes in bone mass and bone turnover following ankle fracture (34) • Changes in bone mass and bone turnover following distal forearm fracture (35) • Changes in bone mass and bone turnover following tibial shaft fracture (31) [30, 31, 34]

follow-up period when analyzing with ELISA and an increase between days 7 and 180 when analyzing with IRMA in patients with a forearm fracture. However, they did not offer an explanation on why the different assays provided different results when analyzing the same BTM.

Discussion

This systematic review found that CTX generally returns to a stable concentration close to baseline within 6 months in the included studies. However, a few studies even showed a complete return to baseline in an even shorter period of time. PINP was generally found to return to a stable concentration close to baseline within 6 months as well, but a few studies found it to dip significantly below the baseline concentration between 1- and 2-years post fracture. The studies found most changes in OC to take place within the first 6 months, but also that the changes in OC concentration in general appeared slower than other BTMs. Lastly, BAP was found increased from day 28 to day 42 in patients with an ankle fracture and from day 14 and throughout the yearlong follow-up in forearm fractures when analyzed with ELISA. When analyzed with IRMA, the increase was found from day 28 to 90 in ankle fractures and from day 7 to day 180 in forearm fractures. However, no explanation was offered on why the two assays provided different results. Furthermore, this is based on two studies from the same author group, further establishing the need for additional studies on the subject. Therefore, no firm conclusions can be made regarding BAP.

The strengths and weaknesses of the 7 studies are listed in Table 4 as well as details about the fasting state of patients and timing of blood samples, since these two factors are paramount in reducing pre-analytical variation [17–19, 21].

As seen in Table 4, only 1 of the 7 studies, and Yan et al. [35], analyzed blood from fasting patients and drew the blood samples in the morning, thus reducing the influence of the circadian rhythm on the variation in bone turnover markers.

Since PINP and CTX are the two recommended BTM in monitoring adherence to anti-resorptive medication [16], reducing pre-analytical variation in those is crucial. Therefore, the strength of studies in which patients are not fasting and where blood samples are obtained sporadically throughout the day is lower than studies, which have either eliminated or reduced the impact of these factors.

The main challenge in designing the perfect study is that it is difficult to establish a true baseline of the biomarkers in fracture patients, since including patients pre-fracture and waiting for the patients to have a fracture episode, like the design in Ivaska et al. [32], is costly and would require an enormous cohort of patients in order to have enough power in the fracture group. Secondly, the timing of the blood sample at fracture is important as well. In a study where patients are included when admitted to the hospital, a possibility could be

to obtain the blood samples at admission and the morning after, thus having both samples shortly after the fracture where bone turnover might not have been affected already and fasting morning blood samples the day after the fracture (Table 5).

A study like Yan et al. [35] could be seen as a compromise between reducing factors potentially introducing bias caused by pre-analytical errors and reducing the cost by not establishing a "true" baseline like Ivaska et al. [32], but instead establish a proxy baseline by obtaining blood in the first morning post fracture. Yan et al. did however not obtain blood samples between days 30 and 360. Since most other studies found CTX and PINP to return to a stable level with insignificant deviation from baseline in approximately 6 months, a study with a similar design, but with more frequent blood sampling between day 30 and day 360 would be favorable [29–35].

Studies measuring BTM in low-energy femoral and vertebral fractures could be interesting as well, since these patient groups are osteoporotic by definition. These patients would be expected to start anti-osteoporotic medication post fracture, if they are not already receiving it. Since IOF and ECTS recommend using PINP and CTX in screening adherence to treatment with bisphosphonates, establishing the time from fracture until the BTM have returned to a stable level is of clinical relevance.

Although several studies were identified, a study has yet to be made, in which the blood samples were drawn in the morning on fasting patients, and where the follow-up period was long enough for the biomarkers to return to pre-fracture baseline levels. Such a study could be done on both patients receiving anti-resorptive medication and patients who do not. Furthermore, in the design of such a study, the blood samples should be drawn at least quarterly in order to establish the time from fracture until the bone markers have returned to a stable concentration with insignificant deviation from pre-fracture baseline.

In conclusion, there is a lack of studies taking the pre-analytical variations of the bone turnover markers into account. Since the bone turnover markers are of clinical relevance, further research is needed.

Compliance with ethical standards

Conflict of interest None.

Abbreviations S, biomarkers measured in serum; U, biomarkers measured in urine; BAP, bone-specific alkaline phosphatase; CTX, carboxy-terminal telopeptide cross-links; DPD, deoxypyridinoline; ECTS, European Calcified Tissue Society; ICTP, cross-linked carboxy-terminal telopeptide of type I collagen; IOF, International Osteoporosis Foundation; IFCC, International Federation of Clinical Chemistry; NTX, amino-terminal telopeptide cross-links; OC, osteocalcin; Pyr, pyridinoline; PICP, type-1 c-terminal propeptide; PINP, type-1 n-terminal propeptide; PIIINP, type-3 n-terminal propeptide; POstn, periostin; TRAcP, tartate-resistant acid phosphatase

References

1. Kurland ES, Cosman F, McMahon DJ, Rosen CJ, Lindsay R, Bilezikian JP (2000) Parathyroid hormone as a therapy for idiopathic osteoporosis in men: effects on bone mineral density and bone markers. J Clin Endocrinol Metab 85(9):3069–3076
2. Alvarez L, Guanabens N, Peris P, Monegal A, Bedini JL, Deulofeu R et al (1995) Discriminative value of biochemical markers of bone turnover in assessing the activity of Paget's disease. J Bone Miner Res 10(3):458–465
3. Civitelli R, Armamento-Villareal R, Napoli N (2009) Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporos Int 20(6):843–851
4. van Beek EM, de Vries TJ, Mulder L, Schoenmaker T, Hoeben KA, Matozaki T, Langenbach GEJ, Kraal G, Everts V, van den Berg TK (2009) Inhibitory regulation of osteoclast bone resorption by signal regulatory protein alpha. FASEB J 23(12):4081–4090
5. Carter DH, Sloan P, Aaron JE (1991) Immunolocalization of collagen types I and III, tenascin, and fibronectin in intramembranous bone. J Histochem Cytochem 39(5):599–606
6. Kurdy NM, Bowles S, Marsh DR, Davies A, France M (1998) Serology of collagen types I and III in normal healing of tibial shaft fractures. J Orthop Trauma 12(2):122–126
7. Joerring S, Jensen LT, Andersen GR, Johansen JS (1992) Types I and III procollagen extension peptides in serum respond to fracture in humans. Arch Orthop Trauma Surg 111(5):265–267
8. Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A et al (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 22(2):391–420
9. Terreni A, Pezzati P (2012) Biochemical markers in the follow-up of the osteoporotic patients. Clin Cases Miner Bone Metab 9(2):80–84
10. Szulc P, Naylor K, Hoyle NR, Eastell R, Leary ET (2017) Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. Osteoporos Int 28(9):2541–2556
11. Talwar S. Bone markers in osteoporosis Medscape 2017 [Available from: https://emedicine.medscape.com/article/128567-overview. Accessed 01 Jan 2019
12. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T et al (2011) International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med 49(8):1271–1274
13. Bauer DC, Black DM, Garnero P, Hochberg M, Ott S, Orloff J, Thompson DE, Ewing SK, Delmas PD, for the Fracture Intervention Trial Study Group (2004) Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. J Bone Miner Res 19(8):1250–1258
14. Jacques R, Boonen S, Cosman F, Reid I, Bauer D, Black D et al (2012) Relationship of changes in total hip bone mineral density to vertebral and nonvertebral fracture risk in women with postmenopausal osteoporosis treated with once-yearly zoledronic acid 5 mg: the HORIZON-Pivotal Fracture Trial (PFT). J Bone Min Res [Internet] 27(8) 1627–34 pp. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/691/CN-00969691/frame.html. Accessed 01 Jan 2019
15. Eastell R, Hannon R, Wenderoth D, Rodriguez-Moreno J, Sawicki A (2011) Effect of stopping risedronate after long-term treatment on bone turnover. J Clin Endocrinol Metab [Internet] 96(11) 3367–73 pp. Available from: http://onlinelibrary.wiley.com/o/cochrane/

clcentral/articles/053/CN-01017053/frame.html. Accessed 01 Jan 2019
16. Diez-Perez A, Naylor KE, Abrahamsen B, Agnusdei D, Brandi ML, Cooper C et al (2017) International Osteoporosis Foundation and European Calcified Tissue Society Working Group. Recommendations for the screening of adherence to oral bisphosphonates. Osteoporos Int 28(3):767–774
17. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C (2002) Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. Bone. 31(1):57–61
18. Szulc P, Delmas PD (2008) Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis. Osteoporos Int 19(12):1683–1704
19. Chubb SA (2012) Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. Clin Biochem 45(12):928–935
20. Vasikaran SD, Chubb SP, Ebeling PR, Jenkins N, Jones GR, Kotowicz MA et al (2014) Harmonised Australian reference intervals for serum PINP and CTX in adults. Clin Biochem Rev 35(4): 237–242
21. Schlemmer A, Hassager C (1999) Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. Eur J Endocrinol 140(4):332–337
22. Woitge HW, Pecherstorfer M, Li Y, Keck AV, Horn E, Ziegler R et al (1999) Novel serum markers of bone resorption: clinical assessment and comparison with established urinary indices. J Bone Miner Res 14(5):792–801
23. Szulc P, Seeman E, Delmas PD (2000) Biochemical measurements of bone turnover in children and adolescents. Osteoporos Int 11(4): 281–294
24. Herrmann M, Müller M, Scharhag J, Sand-Hill M, Kindermann W, Herrmann W (2007) The effect of endurance exercise-induced lactacidosis on biochemical markers of bone turnover. Clin Chem Lab Med 45(10):1381–1389
25. Redmond J, Fulford AJ, Jarjou L, Zhou B, Prentice A, Schoenmakers I (2016) Diurnal rhythms of bone turnover markers in three ethnic groups. J Clin Endocrinol Metab 101(8):3222–3230
26. Weiler R, Keen R, Wolman R (2012) Changes in bone turnover markers during the close season in elite football (soccer) players. J Sci Med Sport 15(3):255–258
27. Stokes FJ, Ivanov P, Bailey LM, Fraser WD (2011) The effects of sampling procedures and storage conditions on short-term stability of blood-based biochemical markers of bone metabolism. Clin Chem 57(1):138–140
28. Hodgson S (2009) AO Principles of Fracture Management. Annals of The Royal College of Surgeons of England 91(5):448–449. https://doi.org/10.1308/003588409X432419f
29. Hitz MF, Jensen JE, Eskildsen PC (2007) Bone mineral density and bone markers in patients with a recent low-energy fracture: effect of 1 y of treatment with calcium and vitamin D. Am J Clin Nutr 86(1): 251–259
30. Ingle BM, Hay SM, Bottjer HM, Eastell R (1999) Changes in bone mass and bone turnover following ankle fracture. Osteoporos Int 10(5):408–415
31. Ingle BM, Hay SM, Bottjer HM, Eastell R (1999) Changes in bone mass and bone turnover following distal forearm fracture. Osteoporos Int 10(5):399–407
32. Ivaska KK, Gerdhem P, Akesson K, Garnero P, Obrant KJ (2007) Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women. J Bone Miner Res 22(8):1155–1164
33. Ohishi T, Takahashi M, Yamanashi A, Suzuki D, Nagano A (2008) Sequential changes of bone metabolism in normal and delayed union of the spine. Clin Orthop Relat Res 466(2):402–410
34. Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM (2006) Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int 17(3):364–372
35. Yan J, Liu HJ, Li H, Chen L, Bian YQ, Zhao B, Han HX, Han SZ, Han LR, Wang DW, Yang XF (2017) Circulating periostin levels increase in association with bone density loss and healing progression during the early phase of hip fracture in Chinese older women. Osteoporos Int 28(8):2335–2341