# Bone Turnover Markers and Glucocorticoid Treatments

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Jean-Pierre Devogelaer, Anne Durnez, Damien Gruson, and Daniel H. Manicourt

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J.-P. Devogelaer (✉) • D.H. Manicourt (✉)
Pôle de Pathologie Rhumatismale, Universite Catholique de Louvain, Brussels, Belgium
e-mail: jean-pierre.devogelaer@uclouvain.be; devogelaer@ruma.ucl.ac.be; daniel.manicourt@uclouvain.be

A. Durnez (✉)
Pôle de Pathologie Rhumatismale, Universite Catholique de Louvain, Brussels, Belgium

AZ Jan Portaels, Vilvoorde, Belgium
e-mail: anne.durnez@uclouvain.be

D. Gruson (✉)
Pôle de Recherche en Endocrinologie, Diabète et Nutrition et Département des Laboratoires Cliniques, Institut de Recherche Expérimentale et Clinique, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Bruxelles, Belgium
e-mail: damien.gruson@uclouvain.be



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**Abstract**

Glucocorticoids (GCs) remain as the cornerstone of therapy in most inflammatory diseases, even if newly developed biological molecules became available. GCs are potent, possess a fast action, and are cheap and relatively easy to prescribe. However, their beneficial therapeutic activity has a nasty counterpart: quite a lot of complications, notably secondary osteoporosis, aseptic bone osteonecrosis, and fractures. The skeleton is continuously remodeling, old bone being resorbed and replaced by new young bone. GCs interfere with the bone turnover and provoke a disequilibrium in favor of bone loss and fragility. The mechanisms of bone fragility consist of a decreased activity and in apoptosis of osteoblasts, as well as an increase in bone resorption. These changes have already been observed histomorphometrically a long time ago in transiliac bone biopsies. Biological parameters of bone turnover, chiefly degradation products of type I collagen, can help to assess atraumatically the bone metabolism. If, in idiopathic osteoporosis, they can have a predictive value of bone loss, they cannot be considered as surrogates for bone mineral density measurements. In GC-OP, the concentrations of the bone turnover markers (BTMs) of bone formation dramatically and rapidly decrease, whereas the BTMs of bone resorption slightly increase. During GC therapy, they cannot be used as predictive tools of bone fragility on an individual basis. Other markers such as RANKL/RANK/osteoprotegerin seem to be promising in this aim, but this still awaits confirmation.

**Keywords**

Glucocorticoid • Bone turnover • Biomarkers • Osteoporosis • Bone mineral density • Bone remodeling • Collagen • Telopeptide

**List of Abbreviations**

| | |
|---|---|
| ALN | Alendronate |
| BDP | Beclomethasone dipropionate |
| BMD | Bone mineral density |
| BSAP | Bone-specific alkaline phosphatase |
| BTMs | Bone turnover markers |
| BUD | Budenoside |
| CD | Crohn's disease |
| COMP | Cartilage oligomeric matrix protein |
| COPD | Chronic obstructive pulmonary disease |
| CTX | Carboxy-terminal cross-linking telopeptide of type I collagen |
| DAS | Disease activity score |
| Dkk-1 | Dickkopf-1 |
| FN | Femoral neck |
| GC | Glucocorticoid |
| ICTP | Carboxy-terminal telopeptide of type I collagen |
| Il-6 | Interleukin-6 |

| | |
|---|---|
| JIA | Juvenile idiopathic arthritis |
| MMP | Metalloproteinase |
| MP | Methylprednisolone |
| NTX | Amino-terminal cross-linking telopeptide of type I collagen |
| OBS | Osteoblasts |
| OC | Osteocalcin |
| OCS | Osteoclasts |
| OP | Osteoporosis |
| OPG | Osteoprotegerin |
| PICP | Procollagen type I C-terminal propeptide |
| PINP | Procollagen type I N-terminal propeptide |
| PTH | Parathyroid hormone |
| RA | Rheumatoid arthritis |
| RANK | Receptor activator of nuclear factor NF-kB |
| RANK-L | Receptor activator of nuclear factor NF-kB-ligand |
| rh | Recombinant human |
| RIS | Risedronate |
| Scl | Sclerostin |
| SLE | Systemic lupus erythematosus |
| TPTD | Teriparatide |
| TRAP | Tartrate-resistant acid phosphatase |
| UC | Ulcerative colitis |
| uDPD | Urinary deoxypyridinoline |
| uPYD | Urinary pyridinoline |
| VF | Vertebral fracture |

## Introduction

Owing to their immunomodulatory, immunosuppressive, and anti-inflammatory properties, glucocorticoids (GCs) are frequently prescribed in many conditions as various as intestinal, locomotor, skin, vascular inflammatory, or allergic diseases, as well in organ transplantation. Besides their favorable actions, GCs can beget a lot of complications such as bruising and skin atrophy, truncal obesity, cataracts, hypertension, salt and fluid retention, and disorders in the glucose and lipids metabolism. One of the most devastating complications consists of the development of osteoporosis and bone fragility leading to an increased incidence of fractures, which can occur soon after the onset of GC therapy (Van Staa et al. 2002; Kanis et al. 2004).

In a retrospective study, the risk of fracture was shown to be commensurate to the daily dose. Compared to controls, the adjusted relative rate for a daily dose of $\leq$ 2.5 mg equivalent predniso(lo)ne increased from 1.17, 1.10, 0.99, and 1.55 for nonvertebral, forearm, hip, and vertebral fractures, respectively, to 1.64, 1.19, 2.27, and 5.18 for a dose of $\geq$ 7.5 mg/day (Van Staa et al. 2000a, b).

Even since the availability of potent biologic agents such as human tumor necrosis factor alpha antibodies, anti-cytotoxic T-lymphocyte antigen 4-immunoglobulin antibody, and recombinant humanized antihuman interleukin 6 receptor monoclonal antibody, GCs are still frequently prescribed, because they are cheap and demonstrate a rapid and potent therapeutic response, all characteristics of particular importance in chronic conditions with severe flares. It is therefore mandatory to systematically consider preventive measures as soon as GC therapy is started. The chapter addresses the role of bone turnover markers (BTMs) in the development of bone loss and bone fragility and in deciding a preventative therapy.

## Available Glucocorticoids in Daily Clinical Practice

In Cushing's syndrome, cortisol (or hydrocortisone) is secreted in excess by the adrenal glands. It has been recognized for years that this condition is complicated by osteoporosis. Hydrocortisone is chiefly utilized for hormone replacement in states of adrenal insufficiency. Derivatives were synthesized in the aim of augmenting the therapeutic potency of GCs without significantly increasing the side effects. Various preparations of GCs are available for a daily clinical use. They are shown in Table 1, with their respective potencies.

## The Routes of Administration of Glucocorticoids

According to the severity of the condition, various routes of administration and dose regimens have been proposed for therapy, such as for systemic administration (daily oral, constant, intermittent, or alternate-day doses; step-up, step-down, intravenous

**Table 1** Equivalence of the more frequently used preparations of glucocorticoids

| | Approximative equivalence in mg | Relative anti-inflammatory potency |
|---|---|---|
| **With a short biologic half-life (8–12 h)** | | |
| Hydrocortisone | 20 | 1 |
| Cortisone | 25 | 0.8 |
| **With a long biologic half-life (36–72 h)** | | |
| Betamethasone | 0.75 | 25 |
| Dexamethasone | 0.75 | 30 |
| **With an intermediate biologic half-life (12–36 h)** | | |
| Prednisone | 5 | 4 |
| Prednisolone | 5 | 4 |
| Oxazolone derivative of prednisone (Deflazacort) | 6 | 4 |
| Methylprednisolone | 4 | 5 |
| Triamcinolone | 4 | 5 |

Modified after Haynes (1990), p. 1447

pulse therapy) or for local administration (inhaled, skin topical, or intra-articular). It became rapidly evident that in most conditions, every route of GC administration could provoke detrimental effects (Nagant de Deuxchaisnes et al. 1984; Emkey et al. 1996; Richy et al. 2003; Dovio et al. 2004; Dhar et al. 2014) on bone mineral density (BMD) and on biochemical markers of bone metabolism.

## The Mechanisms of Action of Glucocorticoids

The potent immunosuppressive and anti-inflammatory actions of GCs are expressed through GC receptors, which are considered to have positive genomic effects on anti-inflammatory proteins (an action so-called transactivation) or negative effects on the production of pro-inflammatory proteins (so-called transrepression). Transactivation is considered accountable for side effects of GCs (notably on the skeleton). Transrepression, on the contrary, is seen as favorable (Stahn and Buttgereit 2008). However, GCs can also exert positive effects through an acute genomic-independent activity (Jiang et al. 2015). This particularity could help to the development of new GCs with nongenomic mechanisms and provoking less adverse effects (Jiang et al. 2015). These promising drugs are not yet clinically available. In the bone tissue, osteoblasts (OBS) seem to be the main target of GCs. This has been histomorphometrically demonstrated already a long time ago (Bressot et al. 1979; Aaron et al. 1989). The number and the longevity of active OBS and the wall thickness of the trabecular plates are dramatically decreased. Furthermore, GCs promote apoptosis of OBS and osteocytes (Weinstein et al. 1998). Moreover, GCs promote osteoclast (OCS) survival (Weinstein et al. 2002). The apoptosis of osteocytes could also favor the malfunction of the mechanostat, potentially leading to GC-induced osteonecrosis (Weinstein et al. 2000).

The discovery of the receptor activator of nuclear factor NF-kB ligand (RANK-L), its receptor RANK, and the decoy receptor of RANK-L, osteoprotegerin (OPG), has further improved our understanding of the bone remodeling in physiologic and pathologic conditions (Manolagas 2000). Various theoretical mechanisms of GC-induced OP and their actions on biomarkers of bone remodeling are summarized in Fig. 1.

## The Bone Turnover Markers

Bone is a tissue which is physiologically and relentlessly remodeled, in order to eliminate the weakened old bone and replace it with new more solid bone. This mechanism in equilibrium in normal conditions allows the maintenance of bone mass and of its mechanical resistance in healthy young males and premenopausal women. During growth, the bone modeling is accompanied by spontaneous changes in the BTMs. This has been studied for some BTMs (Pereira et al. 1999). In healthy adolescent girls (aged 13–18 years) and boys (aged 15–18), a progressive decrease in the BTMs concentration toward adult levels was observed comparatively with

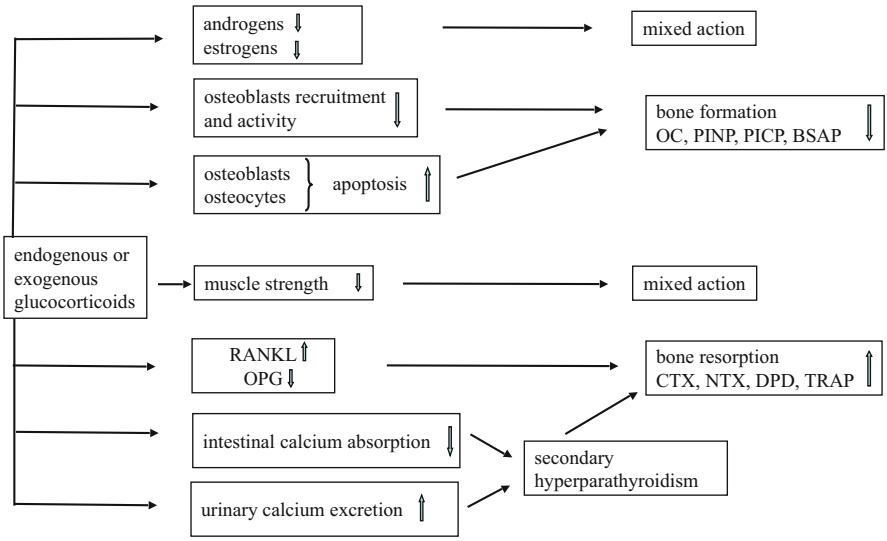


**Fig. 1** Various mechanisms potentially involved in the development of glucocorticoid-induced osteoporosis and action on bone turnover markers. *DPD* deoxypyridinoline, *OPG* osteoprotegerin, *TRAP* tartrate-resistant acid phosphatase

values of children aged 5–12 years (girls) or 5–14 years (boys). A further decrease in BTMs was observed in girls suffering from juvenile idiopathic arthritis (JIA) treated by GCs (Pereira et al. 1999). Beyond menopause in women as well as in elderly in men, there is an acceleration of the bone remodeling, with some preponderance of the markers of resorption over the markers of bone formation (Devogelaer et al. 2011). Pathologic conditions, notably inflammatory diseases and mainly endogenous or exogenous GC excess, modify the bone remodeling. The biomarkers which can be measured in GC-OP are summarized in Tables 2, 3, and 4. These tables include the methods of dosage, the precautions to be respected for sampling, and the possible interferences. First of all, it should be remembered that most BTMs have a circadian rhythm, being high during the night and in the morning and low in the afternoon. It is therefore important to take the blood or urine samples in the early morning and preferably for most of them in a fasting condition or, at the worst, always at the same time of the day in a very same patient (Devogelaer et al. 2011). Moreover, some BTMs demonstrate a seasonal variation, with a zenith in February and a nadir in August (Rapuri et al. 2002). These changes parallel the changes in the level of 25OH vitamin D3 (Rapuri et al. 2002). Furthermore, BTM levels increase after a fracture – a frequent event during GC therapy – and can remain elevated for about 1 year (Ivaska et al. 2007). The practitioner who cares for the patients should have in mind these characteristics when interpreting the clinical significance of BTMs.

**Table 2** The OPG/RANKL/RANK system

| Biomarker | Available assay format | Sampling | Pre-analytical variability | Analytical variability | Biological variability |
|---|---|---|---|---|---|
| **Receptor activator of nuclear factor kappa B ligand (RANKL)** | EIA | Serum | Poorly documented | Intra-laboratory within run CV: below 15%<br>Intra-laboratory between run CV: below 20%<br>Interlaboratory/method CV: ND | Limited circadian rhythmic variation Affected by thyroid function<br>Intra-individual variability: ND |
| **Osteoprotegerin (OPG)** | EIA | Serum<br>Plasma<br>(OPG concentrations are lower in serum than in plasma) | Poorly documented | Intra-laboratory within run CV: below 10%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/method CV: ND<br>OPG concentrations are falsely diminished with hemoglobin interference | Limited circadian rhythmic variation Affected by thyroid function<br>Intra-individual variability: ND |

**Table 3** Biomarkers related to bone resorption: available assays, sampling precautions, pre-analytical factors, analytical and biological variability

| Biomarker | Available assay format | Sampling | Pre-analytical variability | Analytical variability | Biological variability |
|---|---|---|---|---|---|
| Amino-terminal cross-linking telopeptide of type I collagen **(NTX)** | EIA<br>ECi | Serum<br>Plasma<br>Urine | Morning collection<br>Urine: second morning void | Intra-laboratory within run CV: about 10%<br>Intra-laboratory between run CV: about 10%<br>Interlaboratory/ method CV: 40% (urine) | Circadian rhythm<br>Food intake<br>Age<br>Gender<br>Kidney and liver functions<br>Fractures<br>Pregnancy<br>If urinary measurement, must be corrected with urinary creatinine levels<br>Intraindividual variability: about 10% |
| **Carboxy-terminal cross-linking telopeptide of type I collagen (CTX)** | AI<br>ECLIA<br>EIA | Serum<br>Plasma<br>Urine | Morning collection after overnight fast<br>Urine: second morning void | Intra-laboratory within run CV: below 5%<br>Intra-laboratory between run CV: below 8%<br>Interlaboratory/ method: 10–30% | Important circadian rhythm<br>Food intake<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Kidney function<br>If urinary measurement, must be corrected with urinary creatinine levels<br>Intraindividual variability: about 10% |
| **Carboxy-terminal telopeptide of type I collagen (ICTP)** | EIA | Serum | Morning collection after overnight fast | Intra-laboratory within run CV: about 10%<br>Intra-laboratory between run CV: about 10%<br>Interlaboratory/ method CV: 25% | Important circadian rhythm<br>Food intake<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Kidney function<br>Intraindividual variability: ND |

| | | | | | |
|---|---|---|---|---|---|
| **Deoxypyridinoline (DPD)** | EIA<br>AI<br>RIA | Urine | Morning collection<br>Avoid exposure to ultraviolet light<br>Second morning void | Intra-laboratory within run CV: below 8%<br>Intra-laboratory between run CV: below 10%<br>Interlaboratory/ method CV: about 25% | Circadian rhythm<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Measurement must be corrected with urinary creatinine levels<br>Intra-individual variability: about 20% |
| **Dickkopf-related protein-1 (DKK1)** | EIA | Serum | Poorly documented | Intra-laboratory within run CV: about 15%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/ method CV: ND | Poorly documented |
| **Hydroxyproline** | HPLC method | Urine | Second morning void<br>Keep samples refrigerated or frozen until analysis | Intra-laboratory within run CV: about 15%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/ method CV: ND | Nonspecific for the bone<br>Affected by food intake<br>Measurement must be corrected with urinary creatinine levels<br>Intra-individual variability: ND |
| **Osteopontin (OPN)** | EIA | Serum<br>Plasma<br>(Plasma showed higher concentrations than serum) | Poorly documented | Intra-laboratory within run CV: below 10%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/ method CV: ND | Potential circadian rhythmic variation<br>Intra-individual variability: ND |

(*continued*)

**Table 3** (continued)

| Biomarker | Available assay format | Sampling | Pre-analytical variability | Analytical variability | Biological variability |
|---|---|---|---|---|---|
| **Pyridinoline (PYD)** | EIA<br>AI<br>RIA | Urine | Morning collection<br>Avoid exposure to ultraviolet light<br>Second morning void | Intra-laboratory within run CV: about 15%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/ method CV: about 25% | Circadian rhythm<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Liver function<br>Active arthritis<br>Measurement must be corrected with urinary creatinine levels<br>Intraindividual variability: about 20% |
| **Sclerostin (SCL)** | EIA | Serum<br>Plasma<br>Levels reported to be about 30% higher in plasma than in serum | Poorly documented | Intra-laboratory within run CV: below 10%<br>Intra-laboratory between run CV: about 15%<br>Interlaboratory/ method CV: 10–20% | Circadian rhythm<br>Associations reported with age, height, dialysis vintage, troponin, homocysteine, phosphate, PTH<br>Increased in patients with type 1 and type 2 diabetes<br>Higher concentrations observed in hemodialyzed patients |

| | | | | | |
|---|---|---|---|---|---|
| **Secreted frizzled-related protein 1 (sFRP-1)** | EIA | Serum | Poorly documented | Intra-laboratory within run CV: below 15%<br>Intra-laboratory between run CV: about 20%<br>Interlaboratory/ method CV: ND | Poorly documented |
| **Tartrate-resistant acid phosphatase (TRAP)** | EIA<br>AI | Serum | Avoid hemolysis<br>Keep samples frozen until analysis to avoid degradation | Intra-laboratory within run CV: about 10%<br>Intra-laboratory between run CV: about 10%<br>Interlaboratory/ method CV: ND | Circadian rhythm<br>Age<br>Gender<br>Fractures<br>Intraindividual variability: about 25% |

*AI* automated immunoassay, *ECLIA* electrochemiluminescent immunoassay, *EIA* enzyme immunoassay, *ECi* enhanced chemiluminescent assay, *RIA* radioimmunoassay, *HPLC* high-performance liquid chromatography, *CV* coefficient of variation, *ND* not documented

**Table 4** Biomarkers related to bone formation: available assays, sampling precautions, pre-analytical, analytical, and biological variability

| Biomarker | Available assays | Sampling | Pre-analytical variability | Analytical variability | Biological variability |
|---|---|---|---|---|---|
| **Bone-specific alkaline phosphatase (BSAP)** | CLIA<br>EIA<br>AI | Serum | Keep samples refrigerated or frozen until analysis<br>Avoid hemolysis | Intra-laboratory within run CV: below 8%<br>Intra-laboratory between run CV: below 10%<br>Interlaboratory/method CV: 30% | Circadian rhythm (low)<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Intraindividual variability: about 8% |
| **Osteocalcin (OC)** | CLIA<br>EIA<br>ECLIA<br>AI | Serum<br>Urine | Keep samples frozen until analysis to avoid degradation<br>Avoid hemolysis<br>Urine: second morning void | Intra-laboratory within run CV: below 8%<br>Intra-laboratory between run CV: below 10%<br>Interlaboratory/method CV: 10–30% | Circadian rhythm<br>Kidney function<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>If urinary measurement, must be corrected with urinary creatinine levels<br>Intraindividual variability: about 20% |
| **Procollagen type I N-terminal propeptide (PINP)** | ECLIA<br>EIA<br>AI<br>RIA | Serum | Relatively stable biomarker<br>No specific need of fasting | Intra-laboratory within run CV: below 8%<br>Intra-laboratory between run CV: below 10%<br>Interlaboratory/method CV: ND | Circadian rhythm (low)<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Intraindividual variability: about 12% |
| **Procollagen type I C-terminal propeptide (PICP)** | EIA<br>RIA | Serum | Poorly documented | Intra-laboratory within run CV: about 10%<br>Intra-laboratory between run CV: about 10%<br>Interlaboratory CV: ND | Circadian rhythm (low)<br>Age<br>Gender<br>Fractures<br>Pregnancy<br>Intraindividual variability: ND |

*AI* automated immunoassay, *ECLIA* electrochemiluminescent immunoassay, *CLIA* chemiluminescent immunoassay, *EIA* enzyme immunoassay, *RIA* radioimmunoassay, *CV* coefficient of variation, *ND* not documented

## Bone Markers and Inflammatory Diseases Likely to be Treated by Glucocorticoids

In active rheumatoid arthritis (RA), not having started GCs yet, most biomarkers of bone remodeling have been frequently found elevated. Procollagen type I N-terminal propeptide (PINP) and procollagen type I C-terminal propeptide (PICP), both markers of bone formation and CTX a marker of bone resorption, were significantly higher than in controls (Cortet et al. 1998). In this study OC, PINP, and PICP levels were correlated with femoral neck (FN)-BMD. In another study, serum OC was significantly lower in patients with active RA than in normal controls and inactive RA patients (Al-Awadhi et al. 1999). Serum OC was confirmed to be lower than in normal controls in both severe erosive RA and less destructive RA, whereas CTX was more elevated in active RA. Based on the low levels of OC and high CTX levels, the authors concluded to an uncoupling in bone turnover in RA patients, even not taking GCs (Garnero et al. 1999). The predictive value of increased serum levels of CTX, even if associated with radiological progression of the disease, was lower than classical markers of RA such as erythrocyte sedimentation rate, C-reactive protein, and DAS28 score of disease activity (Jansen et al. 2004). The predictive value for an annual radiological progression over 11 years was improved when the baseline values of the RANKL/OPG ratio were added to the classical markers (van Tuyl et al. 2010). However, these markers cannot be used as surrogate markers for radiological endpoints in the follow-up of RA (Syversen et al. 2009).

In 30 female patients with systemic lupus erythematosus (SLE), sOC was found significantly lower than in controls, contrary to the values of BSAP, PICP, ICTP, and uDPD which were not significantly different from controls, whether they had a normal, osteopenic, or osteoporosis BMD (Redlich et al. 2000). OC levels were also found low in another study with 20 SLE females at the time of diagnosis. In this study, however, urinary cross-link excretion was increased (Teichmann et al. 1999). In another study, uPYD and uDPD were significantly higher in postmenopausal women than in premenopausal women with SLE, which was not unexpected (Kipen et al. 1998). The interference of the urinary concentration of creatinine as denominator in the elevation of the urinary marker should be taken into account in such systemic conditions.

In inflammatory bowel diseases (IBD), whether Crohn's disease (CD) or ulcerative colitis (UC), sOC has been found to be low and ICTP to be high (Bischoff et al. 1997), while sOC and PICP, both markers of bone formation, and ICTP, a marker of bone resorption, were not different from controls in another study. However, urinary PYR and uDPD were elevated (Bjarnason et al. 1997). Serum RANKL and OPG levels were found to be increased in CD, with RANK expressed in the mucosa of the colon at a higher level than in normal colon (Franchimont et al. 2004).

It is important to assess the levels of BTMs in the disease states before initiation of GC therapy, in order to be able to appreciate the changes due to the treatment versus the disease activity itself.

## Hyperparathyroidism Caused by Glucocorticoids, Yes or Not?

A theoretical mechanism for the development of GC-OP was attributed a long time ago to a secondary hyperparathyroidism which could occur as GCs provoke a decrease in intestinal calcium absorption and an increase in urinary calcium excretion (Morris et al. 1990; Suzuki et al. 1983). This assumption did not resist to the current availability of modern assays of parathyroid hormone (Paz-Pacheco et al. 1995).

## Effects of Glucocorticoid Therapy on Bone Turnover Markers in Volunteers

The effect on OC of 60 mg of prednisone administered orally at 8 a.m. over 5 consecutive days was studied by Godschalk and Downs (1988) in young volunteer males. They observed a rapid fall in sOC of 32% from baseline already evident 24 h after the first dose. Forty-eight to 96 h after the first dose, serum OC had fallen 63% from baseline values. Twenty-four hours after the last day of dosing, OC remained significantly low (−49%) but reached basal values back 48 h after weaning from GCs. In two other studies, Lems et al. (1995, 1998) treated male volunteers with 10 mg oral prednisone/day for 7 days. Serum OC decreased of – 20% to – 33% from the second to the fourth days of GC therapy, and of – 25% to – 27% the seventh and eighth days, but returned to badeline values 4 days after weaning from GC. A study of dose-ranging effect of GCs on sOC is illustrated in Table 5. In this Table, the study by Godschalk and Down (1988) is compared with studies by Nielsen et al. (1988a) and Kotowicz et al. (1990). Similar changes in OC levels were evidenced after equivalent GC doses in these studies. Kotowicz et al. observed a negative correlation between the prednisone dosage and sOC ($r = -0.7, p < 0.001$) in 50 patients with various rheumatoid disorders receiving long-term prednisone therapy. By using a multiple regression analysis, they demonstrated a significant relationship between sOC and prednisone dosage ($R^2 = 0.72$; $p < 0.001$). In a preliminary study, an inverse correlation between serum undercarboxylated osteocalcin level and oral glucocorticoid dose was also observed in patients suffering from rheumatoid arthritis (Mokuda et al. 2012). As

**Table 5** Effects of various doses of prednisone on serum osteocalcin estimated from three studies

| Daily dose of prednisone | Serum osteocalcin decrease in % | | |
|---|---|---|---|
| | By Godschalk and Downs (1988) | By Nielsen et al. (1988a) | By Kotowicz et al. (1990) |
| 5 mg | NS | (–) | −2% |
| 10 mg | −17% | (–) | −19% |
| 15 mg | −22% | (–) | −32% |
| 20 mg | −26% | (–) | −43% |
| 40 mg | (–) | −74% | (–) |
| 60 mg | −63% | (–) | (–) |

undercarboxylated osteocalcin could influence muscle function in humans, this drop might favor falls and increase the fracture risk by affecting the lower limb muscle strength (Levinger et al. 2014).

The comparison of the slopes of the decreases in sOC levels in volunteers naive to GCs and in patients on long-term GC therapy demonstrates that the reduction in sOC concentration does not continuously decrease and levels off. The OBS remain at the ready to secrete OC again as soon as GC therapy is discontinued. By giving 40 mg prednisone daily to 18 volunteers, Nielsen et al. observed a 74% decrease in sOC the day after the last dose of GC (Nielsen et al. 1988a). The comparative series of numbers mentioned in Table 5 are rather similar.

In another study, Nielsen et al. (1988b) administered 2.5 mg or 10 mg prednisone orally at 08:00 H p.m. or placebo. In the placebo group, the circadian rhythm of serum OC was maintained (rise from 11.30 p.m. and peak at 02:30 a.m., decrease after that time until a nadir around 03:30 p.m). The two doses of prednisone similarly inhibited the circadian rhythm of sOC (lack of increase, even a decrease in the expected nocturnal rise in sOC), with a similar maximal decrease. What was different was the duration of the inhibiting effect of the 10 mg versus the 2.5 mg prednisone dose, which was twice longer for the high dose (12 h versus 6 h). Therefore, even a very low dose of prednisone can inhibit and revert the circadian rhythm of sOC. Administering GCs at night could at long term become more deleterious for the bone than if they had been administered early in the morning. However, it should be recalled that the administration of small doses of predniso(lo)ne (5–7.5 mg) daily at bedtime had been many years ago recommended in rheumatoid arthritis with the aim of reducing the morning stiffness, with apparently clinical benefits and not more side effects (de Andrade et al. 1964).

Intra-articular injection of 40 mg triamcinolone acetonide provoked a decrease of 50% in the OC concentration within 24 h after injection, with a reincrease after 7 days and normalization after 14 days (Emkey et al. 1996). Intra-articular injections of triamcinolone hexacetonide in the knee provoked a similar decrease in sOC within 24 h in 20 patients suffering from RA. If the rheumatoid patients were submitted to a bed rest of 24 h, a slightly larger decrease of serum cartilage oligomeric matrix protein (COMP) was observed, suggesting a better cartilage protective effect (Weitoft et al. 2005). The bone resorption marker uDPD was not affected. Such a short bed rest time of 1 day did not apparently influence the bone remodeling.

Intravenous pulse therapies of large GC doses provoke also a rapid drop in sOC values, as well as also in PICP (Lems et al. 1993), but unexpectedly in ICTP too (Lems et al. 1993, 1996), with a normalization in a few weeks. In our experience and that of others, this kind of GC administration is generally devoid of deleterious effect on BMD, provided of course if the pulse injections are not too numerous (Devogelaer 2006; Frediani et al. 2004). Compared with oral methylprednisolone (MP) 16 mg/day for 1 month, followed by a slow tapering down to 4 mg/day, three intravenous pulses of 1000 mg on alternate days did not provoke any significant bone loss after 1 year versus a significant loss amounting to – 9.3%, – 7.8%, and – 10.0%, respectively, at the lumbar spine, whole body, and femoral neck BMD for the oral doses. Bone-specific alkaline phosphatase (BSAP) showed a significant

decrease in the oral group only (−56%, $p < 0.01$) after 1 year (Frediani et al. 2004).

Inhaled glucocorticoids (InGCs) are more and more frequently used in the treatment of asthma and chronic obstructive pulmonary diseases (COPD). A preliminary remark is that the crude effects of InGCs on bone metabolism were difficult to determine, because, as in daily clinical practice, a lot of patients were either simultaneously on oral GCs, had received GCs for a while just before initiation of InGCs, or received oral GCs for treating flares during InGC therapy. In the majority of studies with InGCs, a significant decrease in sOC was observed (Hanania et al. 1995; Wisniewski et al. 1997; Jones et al. 2002; Richy et al. 2003), within some studies, a larger decrease in sOC and a larger increase in uDPD and uPYR provoked by beclomethasone dipropionate (BDP) compared with budesonide (BUD) administered at equivalent doses (Struis and Mulder 1997; Tattersfield et al. 2001). There was no correlation between the changes in BTMs and the changes in BMD, but a negative correlation between the cumulative doses of InGCs and BMD was observed in some studies (Hanania et al. 1995; Wisniewski et al. 1997).

## Other Biomarkers of Bone

Other biomarkers of bone formation such as BSAP, PINP, and PICP have been much less frequently studied. In general, they have demonstrated directionally the same behavior as sOC, but with a much lower magnitude of changes. Such a comparative example is shown in healthy postmenopausal women on 5 mg prednisone per day for 6 weeks (Table 6) (Ton et al. 2005).

As a rapid bone loss occurs soon after initiation of GCs therapy, particularly in the first year of therapy, an elevation of the biomarkers of bone resorption is expected. Urinary and serum amino telopeptide of type I collagen (uNTX), urinary and serum carboxy-terminal telopeptide of type I collagen (CTX), serum cross-linked telopeptide domain of type I collagen (ICTP), serum tartrate-resistant acid

**Table 6** Effects of low dose of prednisone (5 mg/day for 6 weeks) on bone markers in healthy postmenopausal volunteers (percentage from baseline before therapy)

| Markers | Week 2 | Week 4 | Week 6 | Week 8 | p from baseline |
|---|---|---|---|---|---|
| **OC** | −19%* | −23%* | −26%* | + 1%* | <0.01 |
| **BSAP** | −40% | −12.6% | −16% | −4% | =0.06 |
| **PICP** | −15%* | −19%* | −8%** | +7%* | <0.01 |
| **PINP** | −7%* | −16%* | −11% | +4% | <0.01 |
| **sNTX** | +5% | +1% | −2% | −11% | =NS |
| **uNTX/cr** | +5% | −2% | −6% | −2% | =NS |
| **uDPD/cr** | −10% | −12.6% | −14% | −4% | =NS |

*OC* osteocalcin, *BSAP* bone-specific alkaline phosphatase, *PICP* type I carboxyl-terminal propeptide, *PINP* type I amino-terminal propeptide, *sNTX* serum type I collagen N-telopeptide, *uNTX* urinary type I collagen N-telopeptide, *Cr* creatinine, *uDPD* urinary free deoxypyridinoline. Values estimated from graphs of Ton 2005

*$p < 0.01$ from placebo

phosphatase (TRAP), urinary pyridinoline (uPYD), and deoxypyridinoline (DPD) were the most frequently measured. Discordant results were observed according to the time period of the studies.

ICTP levels decreased on GC in the studies of Lems et al. 1993, 1996, 1998, as well as uPYD (Lems et al. 1996) and uNTX (Lems et al. 2006). Serum CTX increased significantly of 149–248% in the studies of Paglia et al. 2001 and Dovio et al. 2004. Such a discrepancy in the response to GCs in sCTX and sICTP has been observed also in another study in patients suffering from rheumatoid arthritis and treated with oral prednisolone (7.5 mg per day) (Engvall et al. 2013). The increase in CTX and the decrease in ICTP releases are due to the fact that the liberation of these collagen fragments is generated by different proteinases (Garnero et al. 2003). CTX is released by cathepsin K and ICTP by matrix metalloproteinases (MMPs) MMP-2, MMP-9, MMP-13, or MMP-14. This partly explains that small doses of prednisolone can retard the progression of inflammatory erosions in rheumatoid hands and feet (van Everdingen et al. 2002) but are still deleterious for bone mass (Devogelaer 2006).

Serum NTX did not change (Fujii et al. 2007). Urinary NTX, however, increased significantly in another study (+60%) simultaneously with a decrease in sOC (−40%) (Kaji et al. 2010a). No correlation between the change in the levels of biomarkers and BMD changes was observed. These authors found in another study that uDPD levels were significantly higher in women with vertebral fractures (VF) than in women without VF. Furthermore, uDPD was a factor linked to prevalent VF in postmenopausal women only (Kaji et al. 2010b). Such a cross-sectional study does not allow to conclude to the utility of the measurement of uDPD in the aim at measuring the risk of fracture in patients treated by GCs. It should be recalled that biological parameters of bone turnover may remain elevated at long term after a fracture.

Urinary PYD did not change in other studies (Hanania et al. 1995; Siomou et al. 2003). Urinary DPD did not change significantly either (Hanania et al. 1995; Bornefalk et al. 1998). The last authors also observed a significant decrease in iCTP (−19%) in patients older than 65 years and suffering from asthma treated with oral GCs, but no change was seen in younger patients. The unexpected results of uNTX, uPYD, and uDPD could simply be explained by the correction for urinary creatinine. However, it was suggested that this was potentially attributable to a decrease of 62% in the level of Il-6 (Bornefalk et al. 1998). This hypothesis needs confirmation. Further prospective studies with fracture and biomarkers as endpoints should therefore be implemented in order to be able to confer a prognostic value on changes in parameters of bone remodeling induced by GCs.

## RANK, RANKL, and OPG System

In several studies with patients suffering from pathologic conditions necessitating GC therapy (e.g., renal diseases (Sasaki et al. 2001, 2002), Crohn's disease (von Tirpitz et al. 2003), cardiac transplantation (Fahrleitner et al. 2003), and various

rheumatic conditions (Brabnikova Maresova et al. 2013)), OPG significantly decreased after the initiation of the GC treatment. The decrease in OPG confirms in vivo what was observed in preclinical studies (Hofbauer et al. 1999). Rather unexpectedly, patients suffering from Cushing's syndrome, if they have low sOC values, were shown to have significantly higher values of OPG (Ueland et al. 2001), even persisting for 6–18 months after the cure of the condition. Contrary to OPG, sOC levels were already rapidly normalized (Camozzi et al. 2010). In another study, OPG levels were shown to be the only independent predictor of BMD changes at femoral neck and lumbar spine, using multiple regressions ($r = 0.98$, $p < 0.001$) compared with changes in serum OPG, CTX and creatinine, body mass index, months since heart transplantation, cumulative dose of prednisolone, renal function, and parathyroid hormone (Fahrleitner et al. 2003). Patients with vertebral fractures had levels of OPG 2.9-fold lower than patients without fracture. OPG was shown to have a predictive value for prevalent vertebral fractures, contrary to age, body mass index, serum creatinine, femoral neck BMD, and months since heart transplantation. A decrease by 20 or 30% in OPG levels increased the risk of prevalent fractures by 7.9 or 22 times, respectively (Fahrleitner et al. 2003). These interesting predictive values for fracture await confirmative studies before to recommend OPG dosage in patients receiving GC treatment.

The explanations of the apparently inconsistent data stem from the small number of studied patients, the various doses of GC used, and our ignorance about the correlation between the dosages and the biological activity of the biomarkers in peripheral blood and their local concentration and action at the cellular level. Therefore, the results observed in Dickkopf-1 (Dkk-1) and sclerostin (Scl) levels should be interpreted with caution (Brabnikova Maresova et al. 2013). The significant decrease in Scl and the nonsignificant increase in Dkk-1 observed within 4 days after initiation of GC therapy await confirmation. Indeed, another small clinical study showed on the contrary an increase in Scl and a decrease in Dkk-1 after 12 months of GC therapy (Gifre et al. 2013). Sclerostin deficiency in humans is complicated by sclerosteosis, a condition which consists of a skeleton with a high bone mass (Balemans et al. 2001). Expression of DKK-1 in cultured human osteoblasts has been shown to be enhanced by GCs (Ohnaka et al. 2004) and to attenuate the induction of apoptosis of osteoblasts (Wang et al. 2008), an action of this cytokine of possible interest in the prevention of bone complications in GC therapy.

## Treatment of Glucocorticoid-Induced Osteoporosis

Vitamin D and calcium supplementation are weak antiresorptive agents. In a study of patients suffering from various rheumatic conditions and necessitating GC treatment, we compared the effect of calcium supplementation (800 mg) alone, with intravenous disodium pamidronate (90 mg at once at the start of therapy) and quarterly disodium pamidronate 30 mg. The results are shown in Table 7. The depressive effect of pamidronate was far more marked than that of calcium on sOC and BSAP, on top of the known depressive effect of GCs on bone formation.

**Table 7** Preventive therapy in patients starting glucocorticoids. Percentage change from the start

| | | Calcium alone 800 mg/day | Cumulative GC dose mg equivalent prednisone | Pamidronate 90 mg at the start only | Cumulative GC dose mg equivalent prednisone | Pamidronate 90 mg at the start + 30 mg/ 3 months | Cumulative GC dose mg equivalent prednisone |
|---|---|---|---|---|---|---|---|
| **Initial GC dose** | | 19 (16) | | 28 (25) | | 25 (23) | |
| **Parameters** | | | | | | | |
| **BSAP µg/L** | Baseline | 9.4 (8.1) | | 6.5 (1.6) | | 8.6 (6.2) | |
| | +3 months | −22% | 1185 (311) | −45% | 1976 (1736) | −45% | 1548 (652) |
| | +6 months | −47% | 2060 (657) | −49% | 2954 (2064) | −57% | 3004 (1794) |
| | +9 months | −40% | 2734 (1081) | −46% | 3603 (2430) | −47% | 3959 (2488) |
| | +12 months | −42% | 3233 (1401) | −1.5% | 3993 (2787) | −51% | 4962 (3300) |
| **OC ng/mL** | Baseline | 9.3 (3.3) | | 10.5 (7.4) | | 12.1 (8.3) | |
| | +3 months | −23% | 1185 (311) | −70% | 1976 (1736) | −70% | 1548 (652) |
| | +6 months | −33% | 2060 (657) | −54% | 2954 (2064) | −67% | 3004 (1794) |
| | +9 months | −33% | 2734 (1081) | −39% | 3603 (2430) | −63% | 3959 (2488) |
| | +12 months | −2% | 3233 (1401 | −20% | 3993 (2787) | −73% | 4962 (3300) |
| **CTX pM/L** | Baseline | 2648 (1115) | | 2702 (1279) | | 2828 (2488) | |
| | +3 months | −25% | 1185 (311) | −56% | 1976 (1736) | −56% | 1548 (652) |
| | +6 months | −45% | 2060 (657) | −53% | 2954 (2064) | −65% | 3004 (1794) |
| | +9 months | −62% | 2734 (1081) | −21% | 3603 (2430) | −57% | 3959 (2488) |
| | +12 months | −41% | 3233 (1401 | −10% | 3993 (2787) | −71% | 4962 (3300) |

Modified after Boutsen et al. (2001)

*GC* glucocorticoids, *BSAP* bone-specific alkaline phosphatase, *OC* osteocalcin, *CTX* C-telopeptide (mean +/− SD)

The antiresorptive effect of pamidronate was much more marked than that of calcium (cf CTX). Lumbar BMD increased 1.7 (2.2)% and 2.3 (3.4)% after 12 months on single pamidronate infusion and quarterly pamidronate, respectively, whereas it decreased 4.6 (2.9)% on calcium alone. Similar changes were observed at the hip regions. There was no correlation between the changes in BMD and BTMs (Boutsen et al. 2001).

Most commercially available bisphosphonates (BPs) proved to maintain and even increase BMD in patients treated by GCs, as it is the case in the treatment of postmenopausal osteoporosis. Alendronate (ALN) and risedronate (RIS) have been the most frequently studied and became the standard care of GC-OP. Moreover, they have served as comparative agents for new molecules in trials of prevention and treatment of GC-OP. For example, zoledronic acid has been compared with risedronate in preventive and curative treatment of glucocorticoid-induced OP (Devogelaer et al. 2013). Figure 2 shows the changes in the median concentrations of bone resorption and bone formation markers in treatment and prevention in males and females already treated with GCs. A more marked decrease in the levels of serum CTX, PINP, BSAP, and uNTX was observed in patients on zoledronic acid compared with risedronate, both in males and in pre- or postmenopausal females, independently of the GC dose (Devogelaer et al. 2013).

As the main effect of GCs consists of a decrease in bone formation, and secondarily an increase in bone loss, it is consistent to prescribe teriparatide (TPTD), a recombinant human parathyroid hormone (rhPTH 1–34), a bone anabolic drug (Saag et al. 2009). The study of 3 years compared subcutaneous injections of TPTD (20 μg/day) with oral ALN (10 mg/day) in patients on daily minimum 5 mg prednisone or its equivalent for at least 3 months and put on daily calcium (1000 mg) and vitamin D (800 IU). BMD increased more in the TPTD group than in the ALN group after 36 months (11.0% versus 5.3% at the lumbar spine, 5.2% versus 2.7% at the total hip, and 6.3% versus 3.4% at the femoral neck, respectively). Incident VF was observed in 1.7% of patients on TPTD versus 7.7% on ALN, most of VF occurring in the first 18 months (Saag et al. 2009). Figure 3 shows the median percent changes from baseline of biomarkers (PINP, BSAP, OC, PICP, and CTX). The time course of changes in their concentrations is interesting to scrutinize. A significant increase in all markers was observed from the first month in the TPTD group, with highest values for OC and PICP already after 1 month and for BSAP, PINP, and CTX after 6 months. PICP became not significantly different from baseline values after 6 months, 18 months, and 36 months. CTX became not different from baseline at 18 and 36 months. In the ALN group, PICP, PINP, and CTX levels decreased significantly from 1 month, with a nadir after 6 months for PINP; after 18 months for BSAP, PICP, and CTX; and after 36 months for OC. For all biomarkers, there was a significant difference between the TPTD and ALN groups at all illustrated times (Saag et al. 2009). Gains in BMD on TPTD were pursued even when a fading in the concentrations of biomarkers was observed. It should be noted, however, that the balance between markers of bone formation and of bone resorption was all along the

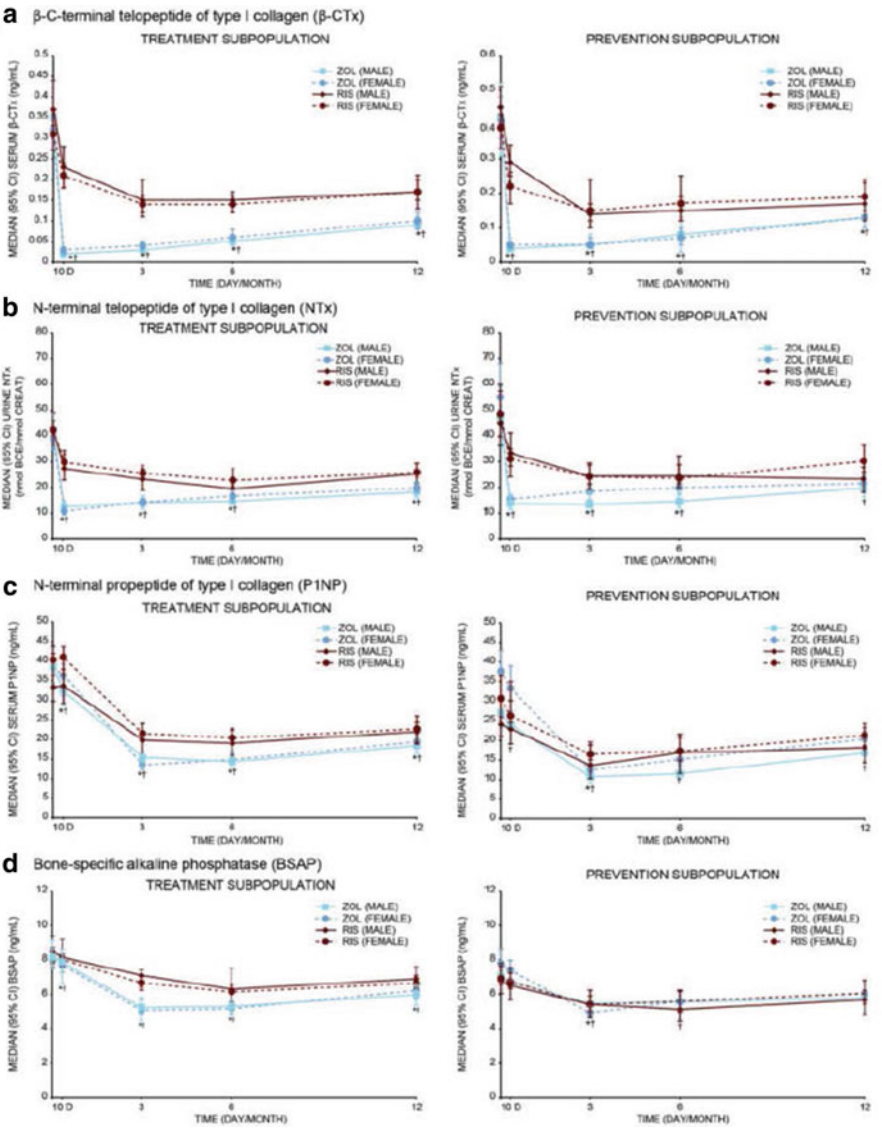


**Fig. 2** Changes in the concentrations (median) of bone resorption and bone formation markers. Bone resorption markers [serum β-CTx (**A**)] and urine NTx (**B**) and bone formation markers [serum PINP (**C**) and serum BSAP (**D**)], overtime in the male and female subgroups of the treatment and prevention subpopulations. $P < 0.05$ shows statistical significance; $^{*}P < 0.05$ (male subjects), $^{\dagger}P < 0.05$ (female subjects). Error bars represent 95 % Cls. *ZOL* zoledronic acid, *RIS* risedronate (Reprint from Devogelaer JP et al (2013). Rheumatology (Oxford) 52(6), 1058-1069 with permission)

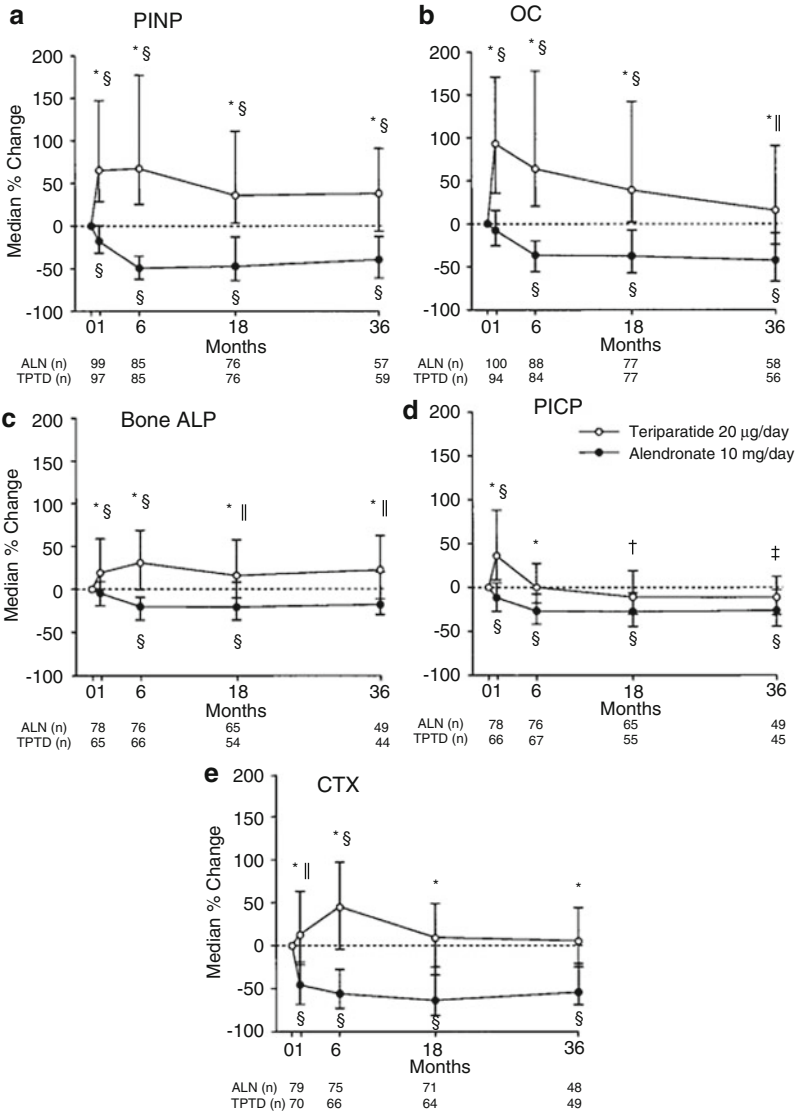


**Fig. 3** Teriparatide versus alendronate in glucocorticoid-induced OP. Effect on BTMs concentrations: median changes from baseline. *PINP* N-terminal type I procollagen propeptide, *OC* osteocalcin, *bone ALP* bone alkaline phosphatase, *PICP* C-terminal type I procollagen propeptide, *CTX* C-terminal telopeptide of type I collagen (Reprint from Saag KG et al. (2009) Arthritis Rheum 60(11), 3346–3355, with permission)

study in favor of TPTD treatment, probably explaining the continuous BMD gain. On the contrary, there was some leveling off in lumbar BMD during the months 24–36 in the ALN group. It should be noted, however, that if an increase in lumbar BMD and hip BMD was observed across various baseline GC doses both in TPTD

and ALN groups, lumbar BMD increased significantly more in patients with a low-dose GC (<5 mg/day) treated with TPTD, compared with patients on medium (>5 and <15 mg/day) and high GC-dose (≥ 15 mg/day) group (Devogelaer et al. 2010). ALN therapy increased lumbar BMD the same way at any GC dose. Such a blunting of the anabolic effect of PTH (1–34) by simultaneous GC treatment had already been shown in a rat model (Oxlund et al. 2006). No weakness of bone mechanical resistance attributable to this blunting was observed, however. As the biomarkers of bone formation decrease during therapy in the ALN group, it seems unlikely that the increase in BMD should be the consequence of new bone formation (Eastell et al. 2010). The increase in BMD is probably due to an increased mineralization of bone, similarly as it is observed in the treatment of postmenopausal osteoporosis (Boivin et al. 2000). In the ALN group, contrary to the TPTD group, the increase in the femoral neck BMD after 18 months was correlated with the baseline biomarkers concentrations ($p < 0.05$). There was also a negative correlation between changes in CTX at 1 month and the increase in lumbar BMD in the ALN group (Burshell et al. 2010). In the TPTD group, the early changes in PINP were significantly correlated with later increases in BMD. PINP could be, therefore, a useful marker for monitoring the BMD response to TPTD (Burshell et al. 2010). Further prospective studies should, however, be necessary before recommending the use of biomarkers of the bone for the follow-up of patients on GC therapy.

## Conclusion

Generally speaking, the biomarkers of bone remodeling are valuable scientific instruments. They have been frequently used in clinical trials. They can help to assess the compliance of the patients and could be considered in the evaluation of the response or failure to therapy. The currently available data remain, however, not sufficient to predict the increase or the reduction in fracture risk. The frequency of bone complications in GC therapy is that high that it precludes the use of such biomarkers to settle the initiation of a preventative therapy. Most of them cannot predict bone loss. When anti-osteoporosis therapy has been initiated, the changes in biomarkers are directionally similar to the changes observed in idiopathic osteoporosis, in which the utility of the use of markers has so far not yet encountered unanimity.

## Potential Applications to Prognosis, Other Diseases, or Conditions

Bone complications begotten by glucocorticoid therapy are extremely frequent and may occur soon after the initiation of therapy. Bone loss is rapid particularly in the first 6–12 months of treatment. It is therefore recommended to prescribe preventative therapies (calcium, vitamin D, bisphosphonates, teriparatide) early after the GC-start, particularly if GCs are foreseen to be maintained for a long time. This is

mandatory in the daily clinical practice, without waiting for the results of bone turnover markers. They chiefly serve eventually to follow the compliance of the patients to the antiresorptive or bone anabolic agents. As the concentrations of BTMs are different in the various conditions justifying glucocorticoid treatments, it is commonsense to measure some of them before initiating a preventative therapy, if one desires to assess the compliance of the patient, in order to be able to demonstrate significant changes in their values. They cannot so far constitute surrogates of BMD measurements to evaluate the fracture risk. In pivotal studies of medicines for prevention and/or therapy of glucocorticoid-induced osteoporosis, they have been used to add rapid biological changes to slow BMD changes observed by dual energy X-ray absorptiometry. If the predictive value of OPG dosage for fractures is confirmed, this dosage could be used in the future for a rapid decision of preventive therapy, even in patients necessitating only low doses of GC.

Moreover, in the perspective of the clinical development of selective glucocorticoid receptor agonists (SEGRAs) with potentially less bone side effects, the BTMs could be used to rapidly demonstrate a lesser toxicity to the bone from these dissociated GCs, compared with predniso(lo)ne.

## Summary Points

- Glucocorticoids remain nowadays the cornerstone of therapy for most inflammatory diseases.
- They are cheap and potent and possess a fast therapeutic action.
- Their use can provoke multiple complications notably bone fragility, fractures, and aseptic osteonecrosis.
- Glucocorticoids rapidly induce a decrease in bone formation evidenced by a drop in the concentration of osteocalcin and other biomarkers of bone formation such as PINP, PICP, and BSAP.
- Glucocorticoids also provoke an increase in bone resorption evidenced by an elevation in the concentration of serum and urine CTX, NTX, DPD, and a decrease in the levels of osteoprotegerin.
- The combination of the abovementioned actions on bone turnover markers begets a rapid bone loss leading to bone fragility.

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