Biomarkers of Bisphosphonate Failure in Osteoporosis

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Abstract

Bisphosphonates are the first-line agents for the management of osteoporosis. Through the suppression of bone turnover, they are able to significantly reduce fracture risk in patients with an adequate calcium and vitamin D supplementation. Bisphosphonate failure can be assumed when two or more fragility fractures occur in the course of treatment, but surrogate markers of the efficacy of bisphosphonate treatment are the variations of bone mineral density (BMD) and

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of bone turnover markers (BTM). Indeed, the demonstration of a significant decrease in BMD and the absence of a significant decrease in BTM while on therapy are considered as indicators of treatment failure. Moreover, other biochemical, clinical, and genetic parameters can be predictive of an inadequate response to bisphosphonate treatment.

Keywords

Osteoporosis • Fracture • Bisphosphonate • Treatment failure • Bone turnover • Bone mineral density

List of Abbreviations

AFF	Atypical femoral fracture
ALP	Alkaline phosphatase
BALP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
BSP	Bone sialoprotein
BTM	Bone turnover marker
CTX	Carboxy-terminal cross-linking telopeptide of type I collagen
DPD	Deoxypyridinoline
FDFT1	Squalene synthase
FPPS	Farnesyl pyrophosphate synthase
GGPS	Geranylgeranyl diphosphate synthase
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IOF	International Osteoporosis Foundation
LRP5	Low-density lipoprotein receptor-related protein
LSC	Least significant change
MVK	Mevalonate kinase
NTX	Amino-terminal cross-linking telopeptide of type I collagen
OC	Osteocalcin
ONJ	Osteonecrosis of the jaw
PINP	Amino-terminal propeptide of type I procollagen
VDR	Vitamin D receptor

Key Facts of Bisphosphonates

- Osteoporosis is a skeletal disorder characterized by reduced bone mineral density and disruption of bone microarchitecture, which leads to impaired bone strength and an increased risk of fractures.
- Fragility fractures are an important cause of morbidity and mortality, and the aim of any osteoporosis treatment is the fracture risk reduction.
- Bisphosphonates are first-line drugs used for the treatment of osteoporosis since randomized clinical trials have demonstrated that they are able to reduce the risk

of fractures in association with an adequate calcium and vitamin D supplementation.

- Their action is mediated by the suppression of bone resorption which is obtained through the inhibition of farnesyl pyrophosphate synthase in the osteoclasts.
- Osteoporosis-approved bisphosphonates are alendronate, risedronate, ibandronate, and zoledronic acid. They differ from each other in terms of route of administration, dosing schedule, and antiresorptive potency.
- Potential side effects of bisphosphonates include esophageal irritation for those administered orally and acute-phase reaction for those administered intravenously.
- Rare but serious adverse effects of long-term bisphosphonate therapy are atypical femoral fractures and osteonecrosis of the jaw.

Definitions of Words and Terms

Osteoporosis	A systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue which leads to increased bone fragility and susceptibility to fracture.
Bone mineral density (BMD)	The amount of bone mass per unit volume (volumetric density) or per unit area (areal density) which can be measured in vivo by densitometric techniques.
Bone turnover	The metabolic process of bone remodeling which occurs throughout life and which consists of the dissolution of bone matrix by osteoclasts (bone resorption) followed by the deposition in the resorption cavities of new bone by osteoblasts (bone formation).
Bone turnover markers	Biochemical products of cellular and noncellular elements of the bone which are indicative of the metabolic activity of the bone. They can be usually measured in blood or urine and they are divided in markers of bone resorption and markers of bone formation, according to the phase of bone turnover they reflect.
Osteoclasts	Bone-specific multinucleated giant cells derived from the monocyte/macrophage hematopoietic lineage which have the task of bone resorption.
Fragility fracture	A fracture that occurs without any identifiable trauma or as a result of a minimal trauma that would be insufficient to fracture a normal bone (e.g., a fall from a standing height or less).

Adherence	A term including both the concepts of persistence and compliance: persistence is the duration of time from initiation to discontinuation of therapy; compliance is the degree to which a patient takes the medication as prescribed.
Least significant change (LSC)	The least variation of a specific parameter that can be considered statistically significant, that is, it represents a meaningful biological change within an individual. It depends on the analytical (CV_a) and intraindividual (CV_i) coefficients of variability. The recommended formula for calculating the LSC with a 95% level of confidence is the following: $1.96 \times \sqrt{2} \times \sqrt{(CV_a^2 + CV_i^2)}$.
Genetic polymorphism	A variation in the DNA sequence of a gene that occurs in a population with a frequency of 1% or more.
Osteonecrosis of the jaw (ONJ)	The appearance of exposed bone in the maxillofacial region that persists for at least 8 weeks in the absence of previous radiotherapy in the craniofacial region.
Atypical femoral fracture (AFF)	A subtrochanteric or femoral shaft fracture in the presence of minimal trauma, lateral cortex origin and transverse appearance, complete extension through both cortices, periosteal or endosteal cortical thickening, and minimal comminution at most.

Introduction

The aim of any osteoporosis treatment is the fracture risk reduction. However, no available treatment is able to eliminate fracture risk. According to this essential concept, the occurrence of a fragility fracture while on therapy for at least 6 months does not necessarily mean that the treatment has failed. Thus, the definition of treatment failure in osteoporosis is more complex and less obvious than expected. In 2012 a working group of the Committee of Scientific Advisors of the International Osteoporosis Foundation (IOF) recommended that a treatment failure may be postulated when two or more incident fractures have occurred during treatment (Díez-Pérez et al. 2012a). Surrogate markers of the response to the treatment are the variations in terms of bone mineral density (BMD) and bone turnover markers (BTM).

Bisphosphonates are the most frequently used agents for the management of postmenopausal, male, and glucocorticoid-induced osteoporosis. Their anti-fracture action is mediated by the suppression of bone resorption through the inhibition in the osteoclasts of farnesyl pyrophosphate synthase (FPPS), an enzyme in the

mevalonate-to-cholesterol pathway (Fig. 1), which induces the detachment of the osteoclasts from the bone surface and their apoptosis (Favus 2010).

Approved bisphosphonates for osteoporosis therapy are alendronate, ibandronate, risedronate, and zoledronic acid. They differ from each other on the basis of route of administration, dosing schedule, bone-binding affinity, and antiresorptive potency

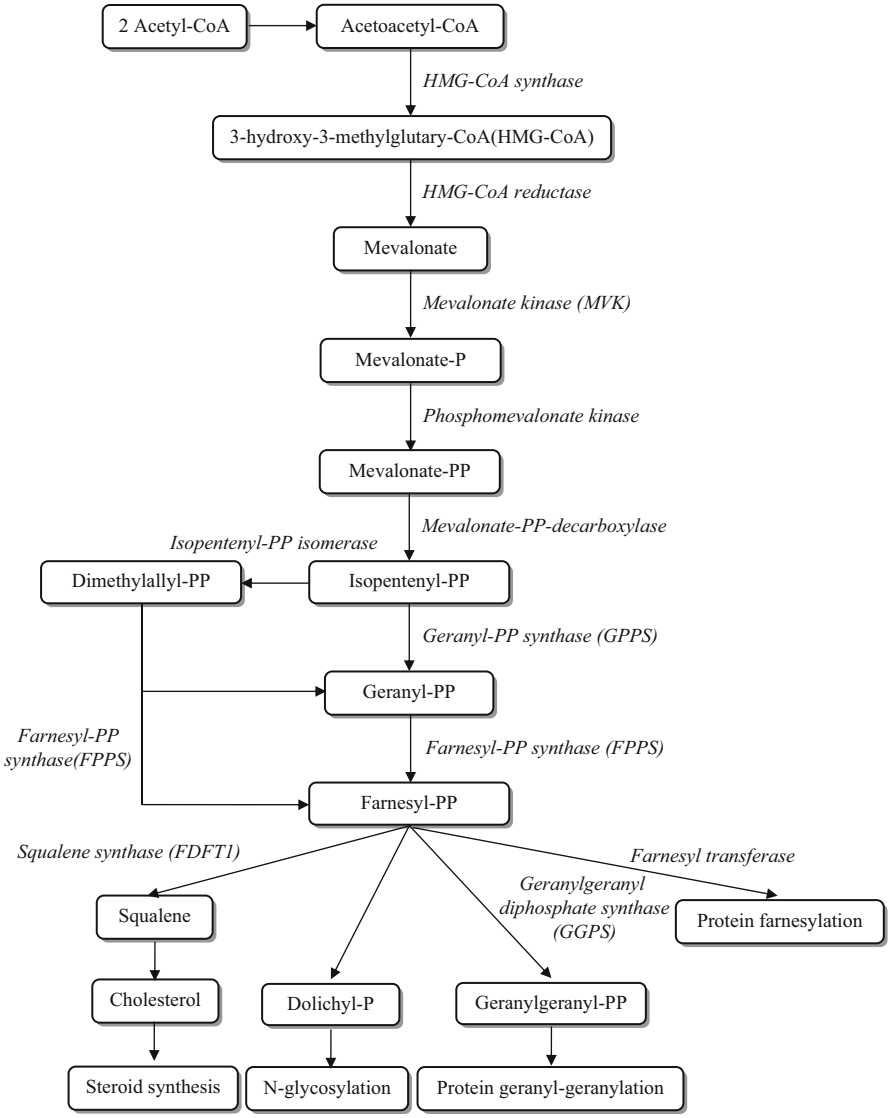

Fig. 1 The mevalonate-to-cholesterol pathway and the metabolic pathway in which bisphosphonates intervene, inhibiting the farnesyl-PP synthase (*FPPS*). *CoA* coenzyme A, *HMG* 3-hydroxy-3-methylglutaryl, *P* phosphate, *PP* pyrophosphate. *N-glycosylation* amino-glycosylation

Table 1 Bisphosphonates approved for the treatment of postmenopausal osteoporosis and their main features. Large, randomized, placebo-controlled, clinical trials demonstrated the fracture risk reduction with bisphosphonates (those administered orally in daily dose) in association with an adequate calcium and vitamin D supplementation. The comparability between daily oral doses and weekly or monthly doses has been subsequently established by assessment of comparative changes in bone mineral density and bone turnover markers

				Fracture risk reduction	
Bisphosphonates	Dosage	Dosing schedule	Route	Hip	Vertebral
Alendronate	70 mg	Weekly	Oral	x	x
Risedronate	35 mg 150 mg	Weekly Monthly	Oral Oral	x	x
Ibandronate	150 mg 3 mg	Monthly Quarterly	Oral Intravenous		x
Zoledronic acid	5 mg	Yearly	Intravenous	x	x

(Favus 2010). An overview of the features of osteoporosis-approved bisphosphonates is presented in Table 1. Randomized, placebo-controlled trials have demonstrated that these drugs are able to determine a significant reduction of the fracture risk, provided that an adequate adherence and calcium and vitamin D supplementation have been guaranteed (Harris et al. 1999; Black et al. 2000, 2007). Indeed, a poor compliance and a scarce intake of calcium and vitamin D are the most likely reasons for a suboptimal response to antiresorptive therapies (Lewiecki 2003). In addition, the presence of a secondary cause of osteoporosis can make ineffective the medical treatment (Fitzpatrick 2002). This is of utmost importance considering that a secondary cause of osteoporosis is present in up to 40% of patients with apparent primary osteoporosis (Eller-Vainicher et al. 2013). An overview of these established causes of suboptimal response to bisphosphonate treatment is presented in Table 2. However, even when these conditions are excluded, some patients do not adequately respond to bisphosphonate therapy, and two or more fragility fractures occur during treatment.

Since a fragility fracture is an important cause of disability and it is the undesirable event that the physician tries to prevent, the availability in the clinical practice of markers of bisphosphonate failure would give a considerable help to the clinician for predicting a priori which patient would have much likelihood to respond to this therapy and for understanding during treatment who should be switched to a different drug before the fracture occurs. Scientific data have demonstrated that the variations of BMD and of BTM can be used as surrogate markers of the efficacy of bisphosphonate treatment, but genetic factors and other biochemical and clinical parameters can be predictive of treatment failure.

Bone Mineral Density

Osteoporosis is, by definition, a condition characterized by bone loss (NIH Consensus Development Panel on osteoporosis prevention, diagnosis, and therapy 2001), and BMD was demonstrated to be able to predict fragility fractures (Marshall

Table 2 Established causes of suboptimal response to bisphosphonate treatment and the main causes of secondary osteoporosis

Poor adherence	
Scarce intake of calcium and vitamin D	
Secondary osteoporosis	*Endocrine diseases* Acromegaly, diabetes mellitus, growth hormone deficit, hypogonadism, hypercortisolism, hyperparathyroidism, hyperthyroidism *Gastrointestinal diseases* Celiac disease, chronic liver disease, inflammatory bowel disease, malabsorption syndromes *Hematologic diseases* Lymphoproliferative and myeloproliferative disorders, multiple myeloma, systemic mastocytosis *Renal diseases* Chronic kidney disease, idiopathic hypercalciuria, renal tubular acidosis *Rheumatologic diseases* Ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus *Organ transplantation* Bone marrow, heart, kidney, liver, lung *Drugs* Anticonvulsants, aromatase inhibitors, chemotherapy, glucocorticoids, gonadotropin-releasing hormone agonists, immunosuppressants, thiazolidinediones *Miscellaneous conditions* Chronic obstructive pulmonary disease, eating disorders, prolonged immobilization, severe disability

et al. 1996). Thus, it could appear presumable that a BMD increase during treatment would be a sign of the efficacy of the therapy and, conversely, a BMD reduction a sign of a useless therapy.

The working group of the Committee of Scientific Advisors of the IOF proposed that a decrease in BMD greater than the least significant change (LSC) with a 95% level of confidence is considered as an indicator of failure to respond to treatment (Díez-Pérez et al. 2012a). The LSC is a parameter which defines the change in BMD that can be confidently detected, depending on the precision error of the technique applied and on the confidence needed to assume a change.

However, a patient could benefit from a reduction in fracture risk even in the presence of a BMD decrease while on treatment, as demonstrated in the Fracture Intervention Trial (FIT), where, for similar decreases in BMD, a decrease in fracture risk in alendronate-treated patients was demonstrated compared with those receiving placebo (Chapurlat et al. 2005).

Indeed, osteoporosis is also a disease characterized by alteration of the bone quality, and bisphosphonates induce not only a BMD increase but also changes in bone microarchitecture (Díez-Pérez and González-Macías 2008). Thus, BMD variations explain only a limited part of the anti-fracture efficacy of these drugs, and treatment-induced changes in microarchitecture and in other parameters of bone quality can significantly influence the fracture risk (Seeman 2007).

Bone Turnover Markers

Markers of bone turnover are biochemical products derived from cellular and noncellular compartments of the bone which can be measured usually in blood or urine and reflect the metabolic activity of the bone. They are usually divided, according to the metabolic phase of bone turnover they reflect, in markers of bone resorption and markers of bone formation. Markers of bone resorption include degradation products of bone collagen, such as the carboxy-terminal cross-linking telopeptide of type I collagen (CTX); noncollagenous proteins, such as the bone sialoprotein (BSP); and osteoclast-derived enzymes, such as cathepsin K and L. Markers of bone formation are products of active osteoblasts expressed during different phases of osteoblast development, such as the bone-specific alkaline phosphatase (BALP), osteocalcin (OC), and amino-terminal propeptide of type I procollagen (PINP) (Seibel 2005). The most important BTM are summarized in Table 3.

The management of osteoporosis with antiresorptive treatments, like bisphosphonates, is associated with an early decrease of markers of bone resorption (already visible after 1 month on treatment with a plateau from 3 months onward) and a later decrease of markers of bone formation after a delay of about 4 weeks due to the coupling of bone resorption and formation (Fig. 2). According to this pharmacologic effect of reduction of the bone turnover, it could be conceivable

Table 3 Nomenclature of markers of bone turnover, their abbreviation, and the biological sample in which they can be measured. They are divided into markers of bone resorption, which include degradation products of bone collagen, noncollagenous proteins, and osteoclast-derived enzymes, and markers of bone formation, which are products of osteoblast activity. *MMP* matrix metalloproteinases

Markers of bone resorption	**Abbreviation**	**Sample**
Amino-terminal cross-linking telopeptide of type I collagen	NTX	Serum Urine
Carboxy-terminal cross-linking telopeptide of type I collagen	CTX	Serum Urine
Carboxy-terminal cross-linking telopeptide of type 1 collagen (generated by MMP)	ICTP or CTX-MMP	Serum
Deoxypyridinoline	DPD	Urine
Pyridinoline	PYD	Urine
Bone sialoprotein	BSP	Serum
Tartrate-resistant acid phosphatase	TRACP	Serum
Cathepsins (K, L)		Serum
Markers of bone formation	**Abbreviation**	**Sample**
Osteocalcin	OC	Serum Urine
Bone-specific alkaline phosphatase	BALP	Serum
Carboxy-terminal propeptide of type I procollagen	PICP	Serum
Amino-terminal propeptide of type I procollagen	PINP	Serum

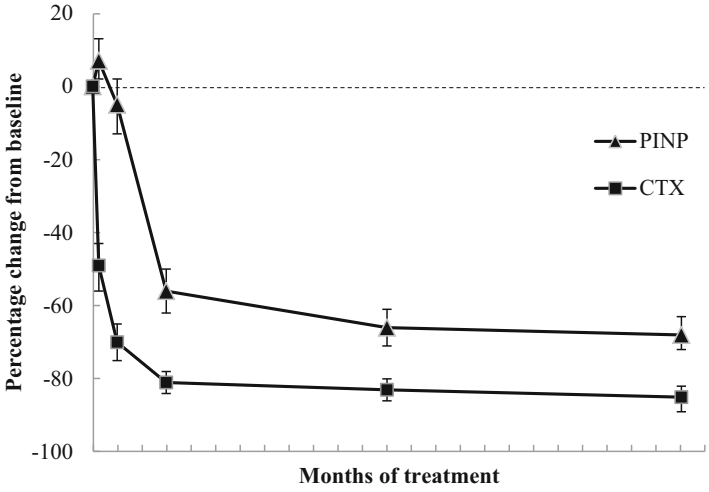

Fig. 2 The direction and magnitude of changes (expressed as mean percentage change from baseline with 95% confidence interval) for a bone formation marker (*PINP*) and a bone resorption marker (*CTX*) in response to bisphosphonate treatment (oral alendronate 70 mg once a week) over 2 years (Figure based on data from Naylor et al. 2016). *PINP* carboxy-terminal propeptide of type I procollagen, *CTX* carboxy-terminal cross-linking telopeptide of type I collagen

that the higher the baseline bone turnover, the greater the expected therapeutic response. However, the accelerated bone turnover itself may be an independent risk factor for fracture (Kanis 2002). Thus, since the available studies used different BTM and different methodologies for their assessment, it is not surprising that conflicting results about the influence of baseline bone turnover on treatment efficacy were found. One study found that risedronate treatment reduced the incident vertebral fractures in women with postmenopausal osteoporosis independent of pretreatment bone resorption as evaluated by the urinary excretion of deoxypyridinoline (DPD) (Seibel et al. 2004). In a post hoc analysis of the FIT, higher baseline levels of PINP were associated with a greater reduction in non-vertebral fracture risk in response to alendronate as compared to lower baseline levels of PINP, but the same association was not found for vertebral fracture risk (Bauer et al. 2006). Finally, another study found that, in postmenopausal women presenting baseline alkaline phosphatase (ALP) values within the upper half of the normal range for premenopausal women, the risk of an inadequate response to bisphosphonate treatment was fourfold increased as compared to women with baseline ALP values in the lower half of the normal range (Cairoli et al. 2014). Therefore, according to these not conclusive results, currently the assessment of baseline bone turnover does not seem to be crucial for treatment decision. However, the availability of baseline values of BTM could be useful in the subsequent treatment follow-up. Indeed, since many studies explored the changes of BTM in the course of therapy for monitoring the effects of treatment, a baseline assessment and another measurement at some defined point during treatment are required. The change in a BTM is then usually expressed

as a percentage of variation of the baseline values, and it is considered significant when exceeding the LSC, a parameter which takes into account both the analytical and intraindividual variability. Alternatively, if baseline levels are unknown, the variation in a BTM can be assumed significant if the value returns in the lower half of the reference interval for premenopausal women, although it is clear that this method can be unreliable since it subtends a significant reduction of BTM only if the pretreatment values were at least in the higher part of the range or abnormally high, a condition that occurs only in a limited percentage of osteoporotic subjects (Vasikaran et al. 2011).

The short-term decrease in BTM under antiresorptive therapy is strongly related to the long-term BMD increase. This association was clearly demonstrated for the hormonal replacement therapy, but a significant relationship has also been reported during bisphosphonate treatment in various studies.

In elderly osteoporotic women treated with alendronate, the changes in urinary CTX at 6 months correlated with long-term BMD changes at the hip, spine, and total body, and the patients with the greatest drop in urinary CTX ($\geq$65%) demonstrated the greatest BMD gains (Greenspan et al. 1998).

In postmenopausal women treated with alendronate, the bone-specific ALP (BALP) levels and the percent BALP change at 6 months were found to be independent predictors of long-term positive BMD response, defined as >3% increase in lumbar BMD at 2 years. Moreover, the combined use of both parameters in a logistic model allowed an accurate identification of nonresponder patients to alendronate treatment in terms of BMD gain (Garnero et al. 1999). A further study observed that in alendronate-treated women, the change from baseline at 6 months in urinary amino-terminal cross-linking telopeptide of type I collagen (NTX) and OC correlated with lumbar and femoral BMD change from baseline at 2 years, although the lack of decrease below a specific threshold in NTX or OC failed to identify women experiencing a bone loss during alendronate treatment (Ravn et al. 1999).

More recently, the TRIO study compared the effects of oral alendronate, ibandronate, and risedronate over 2 years on BMD results and BTM responses. Postmenopausal women who reached the target for response for PINP (considered as a reduction greater than the LSC) by 12 weeks of bisphosphonate treatment experienced a greater increase in lumbar spine BMD at 2 years than those that failed to reach the target for treatment (Naylor et al. 2016). A similar conclusion was obtained in another study for urinary NTX where a poor response in urinary NTX (considered as a change in urinary NTX/creatinine lower than the LSC) at 4 months was proposed to be a useful early indicator in clinical practice of a low response in lumbar BMD after 18 months of risedronate or alendronate treatment (Baxter et al. 2013). Similarly, a French study on a small sample of postmenopausal osteoporotic women demonstrated that a significant change in serum CTX after 4 months of alendronate therapy was predictive of a significant increase in lumbar BMD after 12 months of treatment (Fink et al. 2000).

Overall these studies indicate that during bisphosphonate treatment, a significant decrease of BTM is associated with a subsequent significant BMD gain and,

conversely, that small or no changes in BTM are highly suggestive of a subsequent poor BMD response to treatment.

However, an intrinsic limitation of all these studies is that their primary endpoint is to assess the ability of BTM variations under therapy to predict the BMD change, which in turn is only a surrogate marker of anti-fracture efficacy of antiresorptive treatments, as previously explained.

Post hoc analysis of randomized clinical trials on bisphosphonates assessed the correlation between the BTM changes under treatment and the fracture risk, and in general, they showed that the larger the decrease in BTM, the larger the reduction in fracture risk. In more detail, data from the FIT showed that greater reductions in one or more BTM after 1-year alendronate treatment were associated with a lower risk of vertebral, hip, and non-vertebral fractures (Bauer et al. 2004), suggesting that the measurements of bone turnover in the course of treatment may help to identify alendronate-treated women with suboptimal response. Similarly, in the Vertebral Efficacy with Risedronate Therapy (VERT) study, the reductions in urinary CTX and NTX at 3–6 months of risedronate treatment were significantly associated with the reduction in vertebral fracture risk, and the changes in these bone resorption markers accounted for a percentage of fracture risk reduction with risedronate between 49% and 77%, depending on the marker and the fracture type (vertebral or non-vertebral) (Eastell et al. 2003). Moreover, in this trial the lowest fracture risk was reached when the urinary CTX was below a level equivalent to the mean value for premenopausal women (Eastell et al. 2007). Finally, the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON) study found that lower levels of PINP 1 year after an infusion of 5 mg zoledronic acid were associated with a lower risk of clinical fractures (Delmas et al. 2009), stressing the importance of bone turnover reduction as a key mechanism to reduce fracture risk in patients treated with bisphosphonates, not only orally but also intravenously.

Further studies assessed the relationship between BTM and fracture risk reduction and similar data were reported. One of these is the Improving Measurements of Persistence on ACtonel Treatment (IMPACT) study, a multinational prospective, open-label, cluster-randomized study of postmenopausal women on oral risedronate for 52 weeks, which showed that in patients with a reduction in urinary NTX or serum CTX levels greater than 30%, the incidence of both non-vertebral and all fractures (vertebral and non-vertebral) was significantly lower compared with patients with a 30% or less reduction of BTM. This association was confirmed also in a subgroup analysis of women with good adherence (>80%) (Eastell et al. 2011).

However, beyond the results of these trials, to date it is not completely clear how the BTM should be used in the clinical practice. Indeed, although the available trials seem to suggest a clear relationship between BTM changes under antiresorptive treatment and fracture risk reduction, the use of different BTM across studies, the presence of numerous sources of pre-analytical variability of BTM (both technical and biological), and the lack of standardization of laboratory methods limit the clinical utility of BTM (Vasikaran et al. 2011).

In order to adopt international reference standards, in 2011 the IOF and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC),

on the basis of specific criteria for the selection of reference BTM standards, recommended the use of PINP to assess bone formation and of CTX to assess bone resorption (Vasikaran et al. 2011). According to this statement, the working group of the Committee of Scientific Advisors of the IOF later proposed in 2012 that a decrease in CTX and PINP less than the LSC at 95% confidence could be considered as an indicator of failure to respond to treatment with bisphosphonates (Díez-Pérez et al. 2012a).

However, specific international recommendations on the modality of assay and interpretation of BTM in clinical practice for the individual patient under treatment are lacking. Various national guidelines have recently expressed their opinion on the utility of BTM in the management of osteoporotic patients, some aiming for their routine application, while others being more cautious (Vasikaran et al. 2011). An attempt to elaborate an algorithm for the use of BTM in clinical practice was done in a Belgian consensus document which proposed to measure a BTM (serum CTX for antiresorptive therapy) at baseline and then after 3 months of therapy. If a significant decrease is not achieved, the clinician should check the treatment adherence and the method of drug administration, address potential problems detected, and then reassess the BTM after further 3 months (Fig. 3) (Bergmann et al. 2009). Conversely, if a

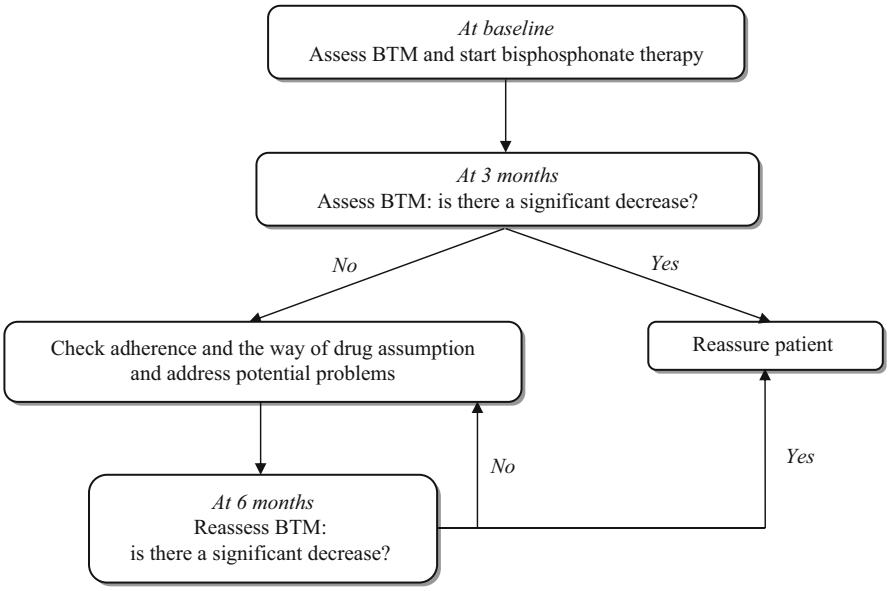

Fig. 3 An algorithm for the use of bone turnover markers (*BTM*) in clinical practice for monitoring bisphosphonate treatment efficacy for osteoporosis, suggested by the Belgian Bone Club: they proposed to measure BTM at baseline and then after 3 months of bisphosphonate therapy. If a significant decrease is not achieved, the clinician should check the treatment adherence and the method of drug administration, address potential problems detected, and then reassess the BTM after further 3 months. Conversely, if a significant change in BTM is obtained, the patient can be reassured (Bergmann et al. 2009). *BTM* bone turnover markers

significant change in BTM is obtained, the patient can be reassured and a positive feedback can be given already 3–6 months after the beginning of treatment (thus earlier than the demonstration of a BMD gain). This approach could have the advantage of maintaining or further improving adherence in the subsequent months of treatment (Delmas et al. 2007). However, other authors believe that this purpose alone does not justify the use of BTM and that the opportunity for the patient to discuss the therapy with a health-care professional is more beneficial to increase treatment adherence than the positive feedback itself given by the biochemical tests (Compston 2009).

If the changes in BTM following the initiation of osteoporosis treatment may be quite successfully used for predicting the fracture risk reduction, on the other hand, at the moment, there is no evidence to support the use of BTM to assess fracture risk after long-term bisphosphonate treatment. However, some authors consider useful the BTM for evaluating if bisphosphonates are still exerting their effects after their discontinuation, and they suggest to resume therapy when the BTM exceed the lower half of the premenopausal range (Adler et al. 2016). This approach is somewhat justified when considering that BTM in the lower range are associated with a reduced fracture risk (Vasikaran et al. 2011).

Genetic Markers

In the recent years, pharmacogenetic studies have provided several data on the genetic basis of individual response to osteoporosis treatments.

The vitamin D receptor (VDR) gene, which is located on the long arm of the chromosome 12, was one of the main genes that were investigated. VDR is a member of the steroid hormone nuclear receptor family which acts as a transcription factor after the interaction with its heterodimer partner, the retinoid X receptor. Several polymorphisms have been identified in the human VDR gene locus, of which the most studied are those identified by the restriction endonucleases ApaI, BsmI, FokI, and TaqI (Gennari et al. 2009). Among these polymorphisms, BsmI VDR genotypes were demonstrated to influence the efficacy of antiresorptive treatments in postmenopausal osteoporotic women. The bb BsmI VDR genotype (i.e., homozygous for the polymorphic allele) was associated with the highest therapeutic response to alendronate and hormone replacement therapy (Palomba et al. 2005). Another study in Southern Italy found that the FokI polymorphism of the VDR gene was associated with a better response to bisphosphonate treatment in postmenopausal osteoporosis (Conti et al. 2015).

On the basis of the mechanism of action of bisphosphonates, other pharmacogenetic studies examined the influence of genetic polymorphisms of genes encoding enzymes involved in the metabolic pathway inhibited by bisphosphonates (Fig. 1): the farnesyl pyrophosphate synthase (FPPS), the geranylgeranyl diphosphate synthase (GGPS), the mevalonate kinase (MVK), and the squalene synthase (FDFT1). In a Danish cohort of postmenopausal osteoporotic women, the homozygous CC genotype for rs2297480 FPPS single nucleotide polymorphism was associated with a decreased response of BTM to bisphosphonate

therapy when compared to the heterozygous AC and to the homozygous AA genotypes (Marini et al. 2008). Korean researchers found that the rs3840452 GGPS1 polymorphism was associated with the femoral neck BMD response rate to bisphosphonate therapy (Choi et al. 2010). Furthermore, in postmenopausal Chinese women, the rs10161126 single nucleotide polymorphism of MVK gene and the GTCCA haplotype in FDFT1 gene were associated with a better BMD response to alendronate therapy (Wang et al. 2015).

Finally, polymorphisms of the low-density lipoprotein receptor-related protein 5 (LRP5) gene, which encodes for an element of the Wnt pathway essential for the osteoblast differentiation and bone formation, were investigated. However, although the A1330V polymorphism of LRP5 gene was found to be associated with reduced BMD, available data on its influence on the response to bisphosphonates are conflicting. Whereas osteoporotic men homozygous for this polymorphism were found to respond to risedronate equally as well as the other genotype groups with respect to BMD (Kruk et al. 2009), Chinese postmenopausal women with homozygous genotype showed a higher possibility of poor BMD response at lumbar spine to alendronate treatment than those with other genotypes. Moreover, in this study, the trend of BTM reflected these different responses in BMD according to genotypes, since participants, who were homozygous for the polymorphism, had a smaller decrease in serum CTX and ALP levels than those with other genotypes after 12 months of treatment (Zhou et al. 2014).

A summary of these genetic factors found to be associated with the response to bisphosphonate treatment is presented in Table 4.

Overall, these studies provide an interesting point of view of the importance of the patient's genetic background to the response to osteoporosis therapies and of the future possibility of genotype-tailored osteoporosis therapies, in order to avoid that individuals with high probability of having an inadequate response to a treatment will take unnecessary medications.

However, further studies on larger samples are required and it must be considered that an important limitation of available pharmacogenetic studies is that the efficacy of osteoporosis treatment was not assessed as fracture risk reduction, but as BMD gain or BTM decrease which are only surrogate markers of the treatment efficacy.

Table 4 Genetic factors and polymorphisms associated with the response to bisphosphonate treatment. The sign "+" means a positive association, the sign "−" a negative association. See the text for further details. *VDR* vitamin D receptor, *FPPS* farnesyl pyrophosphate synthase, *GGPS1* geranylgeranyl diphosphate synthase, *MVK* mevalonate kinase, *FDFT1* squalene synthase, *LRP5* low-density lipoprotein receptor-related protein 5

Genetic factors	
BsmI VDR polymorphism	+
FokI VDR polymorphism	+
rs2297480 FPPS polymorphism	−
rs3840452 GGPS1 polymorphism	+
rs10161126 MVK polymorphism	+
GTCCA FDFT1 polymorphism	+
A1330V LRP5 polymorphism	−

Other Potential Markers

Several studies tried to determine other risk factors for predicting the antiresorptive treatment failure.

A multicentric, cross-sectional study of postmenopausal Spanish women on antiresorptives for osteoporosis (including not only bisphosphonates, but also raloxifene) found that the risk of inadequate response to antiresorptives (considered as the occurrence of a fragility fracture while on treatment) was significantly increased in patients with low levels of vitamin D, with low values of femoral fracture load, and with a fracture before treatment. These data suggest a worst microarchitectural deterioration could be a strong predictor of antiresorptive treatment failure (Díez-Pérez et al. 2012b). A subsequent study, based on the same cohort with the exclusion of patients on raloxifene treatment, assessed the association between the circulating levels of estradiol and sclerostin (a Wnt pathway inhibitor preferentially expressed by osteocytes) and the inadequate clinical efficacy of bisphosphonates. The authors found that increased circulating sclerostin levels and low estradiol levels were associated with the occurrence of a fragility fracture while on treatment. Moreover, serum sclerostin levels and a prior fragility fracture, adjusted by estradiol serum levels, were the only variables independently associated with the presence of an inadequate response on oral bisphosphonate treatment. However, important limitations of the present study are its retrospective design and the fact that the determinations of serum sclerostin and estradiol were performed during treatment rather than at baseline (Morales-Santana et al. 2015).

Using data from the Global Longitudinal Study of Osteoporosis in Women (GLOW), a large, prospective, observational cohort study of postmenopausal women in ten countries, the following variables were found to be independently associated with treatment failure: reduced quality of life (as measured by the SF-36 Health Survey (Brazier et al. 1992)), prior falls, and prior fracture (Díez-Pérez et al. 2014).

Finally, in a population-based cohort study in Spain and Denmark, significant predictors of multiple fragility fractures in patients with high adherence to oral bisphosphonate treatment were the older age in both populations and the history of fracture and dementia within one but not both populations (Hawley et al. 2016). At variance in an Italian study on postmenopausal women with primary osteoporosis, the current smoking was associated with an inadequate response to bisphosphonates despite a good compliance and normal vitamin D levels (Cairoli et al. 2014).

Conclusions

No osteoporosis treatments are able to eliminate fracture risk, but only to significantly reduce it. According to this assumption, an incident fragility fracture while on bisphosphonate treatment for at least 6 months cannot be considered a sign of

treatment failure, which is assumed when two or more fragility fractures occur in the course of therapy. The available evidence suggests that surrogate markers of an inadequate response to antiresorptives, such as bisphosphonates, are a decrease in BMD greater than the LSC at 95% confidence and a decrease in CTX and PINP lower than the LSC at 95% confidence (Díez-Pérez et al. 2012a). When one of these conditions occurs, the patient adherence to the drug and to the supplementation of calcium and vitamin D and the presence of a secondary cause of osteoporosis must be reviewed, since a poor compliance, an inadequate intake of calcium and vitamin D, and a secondary osteoporosis are the main reasons of a treatment failure (Lewiecki 2003; Fitzpatrick 2002).

If a good adherence is ascertained and a secondary osteoporosis is excluded, the working group of the Committee of Scientific Advisors of the IOF in 2012 recommended to consider a treatment change from bisphosphonates to a more potent drug when one of the following circumstances is fulfilled: (a) two or more fragility fractures, (b) one incident fracture and a significant decrease in BMD and/or no significant decrease in CTX or PINP, or (c) both a significant decrease in BMD and no significant decrease in CTX or PINP (Díez-Pérez et al. 2012a).

These criteria are based on the results of many studies which demonstrated that the changes in BMD and, in particular, the BTM modifications after the beginning of bisphosphonates independently correlate with the fracture risk reduction under treatment. Moreover, the BTM change, that is usually easy to measure, can be evaluated earlier than the BMD change, which requires about 18–24 months to be considered significant (Lee and Vasikaran 2012). Thus, BTM represent an attractive way to assess the treatment response. However, currently the availability of a wide number of BTM, their high pre-analytical and analytical variability, and the lack of standardization of laboratory methods limit the clinical utility of BTM (Vasikaran et al. 2011).

Further studies that apply international reference standard are needed to assess the capability for predicting fracture risk reduction under bisphosphonates of CTX and PINP, which have been defined by the IOF and the IFCC as the reference standard for bone resorption and bone formation, respectively (Vasikaran et al. 2011). The challenging goal is to establish universally accepted and reliable criteria based on the easy measurement of BTM able to early recognize the bisphosphonate failure before it becomes clinically evident.

The reasons which justify an inadequate response to antiresorptives in adherent patients with primary osteoporosis are not completely known. A hypothesis is that in these patients the bone microarchitecture is to such an extent altered that bisphosphonate therapy is not able to adequately work (Díez-Pérez et al. 2012b). Finally, other factors that may influence treatment efficacy come from genetics. The polymorphisms of the VDR gene and of genes involved in the mevalonate-to-cholesterol pathway inhibited by bisphosphonates and in the Wnt pathway were found to be associated with response to bisphosphonate. This suggests in future the interesting possibility of more personalized osteoporosis therapies (López-Delgado et al. 2016).

Potential Applications to Prognosis, Other Diseases, or Conditions

A rare but serious complication of long-term bisphosphonate treatment is the osteonecrosis of the jaw (ONJ), which is defined as the appearance of exposed bone in the maxillofacial region that persists for at least 8 weeks in the absence of previous radiotherapy in the craniofacial region. The risk of bisphosphonate-related ONJ appears to be very low in patients treated for osteoporosis (from 1/10.000 to 1/100.000 patient-treatment years), while it is significantly higher in oncologic patients treated intravenously with high doses for metastatic cancer (1–10%) (Khosla et al. 2007). Nevertheless, although the probability of this side effect is minimal in osteoporotic patients treated with bisphosphonates, the availability in clinical practice of a tool for the prediction of the individual risk of ONJ, especially if dental surgery is required, is appealing for the clinician. According to the assumption that the bisphosphonate-mediated suppression of bone turnover gives the main contribution in the pathophysiology of ONJ (Allen and Burr 2009), the markers of bone resorption have been suggested to be able to predict the ONJ risk in postmenopausal women receiving oral bisphosphonates for osteoporosis. In 2007 Marx and coauthors proposed the use of serum morning fasting CTX with this aim, considering values less than 100 pg/mL as high risk, between 100 pg/mL and 150 pg/mL as moderate risk, and greater than 150 pg/mL as minimal risk. Based on this conclusion, they suggested to defer dental surgery in the presence of CTX levels lower than 150 mg/dl, stopping temporarily bisphosphonate therapy if necessary to reach this CTX threshold (Marx et al. 2007). However, this recommendation has raised several concerns due to the lack of standardized laboratory protocols and of the estimate of the LSC of CTX levels, which takes into account both the analytical and biological variabilities. To date, the evidence does not support the use of CTX levels to predict the risk of ONJ and further studies are required on this topic (Baim and Miller 2009).

A second rare complication of long-term bisphosphonate treatment is the occurrence of an atypical femoral fracture (AFF). The diagnosis of AFF is based on subtrochanteric or femoral shaft location and the presence of $\geq$4 among minimal trauma, lateral cortex originating and transverse fracture, complete fractures extending through both cortices, periosteal or endosteal cortical thickening, and minimal comminution at most. Minor criteria are not required for the diagnosis but include increased cortical thickness of the diaphysis, bilaterality, a prodrome of thigh or groin pain, and delayed fracture healing (Dell et al. 2012). The age-adjusted AFF incidence is associated with the duration of bisphosphonate therapy, and it is estimated to rise from 1.8/100.000/year with a 2-year exposure to 113/100.000/year with 8–9.9-year exposure. Since the AFF pathogenesis seems to be related to a low bone turnover and in particular to low bone formation, the BTM have been studied for predicting the AFF occurrence. However, in the few patients studied, the correlation of bone histomorphometric parameters with BTM was poor and the urinary NTX was not consistently low (Odvina et al. 2005; Visekruna et al. 2008). Therefore, currently the BTM could not be used for predicting the AFF risk in patients treated long-term with bisphosphonates.

Summary Points

- Osteoporosis-approved therapies are able to reduce but not to eliminate fracture risk; thus the occurrence of a fragility fracture in the course of treatment does not necessarily mean a treatment failure, while the occurrence of a second fragility fracture after at least 6 months of therapy can be considered a sign that the drug has failed.
- Surrogate markers of bisphosphonate efficacy are the variations of bone mineral density (BMD) and of bone turnover markers (BTM) during therapy.
- A decrease in BMD greater than the least significant change (LSC) with a 95% level of confidence is considered as an indicator of failure to respond to bisphosphonates, although BMD variations explain only a limited part of their anti-fracture efficacy since treatment-induced changes in microarchitecture can significantly influence the fracture risk, independently of BMD.
- The short-term decrease in BTM under antiresorptive therapy is strongly related to the long-term BMD increase and to the fracture risk reduction. According to these data, a decrease in BTM less than the LSC with a 95% level of confidence is considered as an indicator of failure to respond to treatment with bisphosphonates.
- However, at the moment the clinical use of BTM is limited by their high pre-analytical and analytical variability and by the lack of standardized laboratory methods.
- Further studies based on international reference standard are needed to assess the ability of BTM to predict fracture risk reduction under bisphosphonates in order to elaborate clinical guidelines for the use of BTM to early recognize bisphosphonate failure before this condition becomes clinically evident.
- Pharmacogenetic studies have explored the genetic basis of individual response to osteoporosis treatments, and polymorphisms of the vitamin D receptor gene and of genes involved in the mevalonate-to-cholesterol pathway inhibited by bisphosphonates and in the Wnt pathway were found to be associated with the response to bisphosphonates.
- Markers of bone resorption have been suggested to predict the risk of osteonecrosis of the jaw and of atypical femoral fracture in postmenopausal women treated with bisphosphonates for osteoporosis; however, current evidence does not support their use for this aim and further studies are required on these topics.

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