THE ROLE OF BISPHOSPHONATES: POTENTIAL NOVEL PHARMACEUTICAL THERAPY FOR REDUCING CARDIOVASCULAR MORBIDITY AND MORTALITY

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Dedication

This dissertation is dedicated to my parents, Jen-Min and Min-Chu.

Abstract

Cardiovascular disease (CVD) is one of the most significant and costly public health problems in the U.S. and worldwide. In addition to the well-established risk factors, such as hypertension or diabetes, growing bodies of studies have demonstrated that vascular calcification is another strong and independent predictor of cardiovascular morbidity and mortality. The pathological process of vascular calcification includes many key modulators of bone and mineral metabolism. Hence, the question is raised regarding whether pharmacological agents for inhibiting bone loss can also provide a beneficial impact on inhibiting vascular calcification to lower CVD risks.

The primary objectives of this study were to: 1) examine the association between bisphosphonate use and the risk of a composite endpoint of congestive heart failure (CHF), myocardial infarction (MI) or stroke hospitalization, and 2) examine the impact of patient adherence to bisphosphonate therapy on the risk of CHF, MI or stroke hospitalization.

This study employed a retrospective cohort study design using Thomson Reuters MarketScan® Databases. Time dependent Cox proportional hazards models were used for analysis. 31,253 female patients with osteoporosis aged 45 years older were identified between January 1, 2001 and December 31, 2006. Among them, 16,510 were bisphosphonate users and 14,743 were non-bisphosphonate users. After adjustment for age and baseline comorbid conditions, bisphosphonate users were associated with 24% (95% CI=0.68-0.85) lower risk of the composite outcome than non-bisphosphonate users.

The age-stratified analysis showed that bisphosphonate users had a significant 45%, 42% and 30% lower risk of the composite outcome than non-bisphosphonate users among patients aged 45-54, 55-64 and ≥75 years, respectively. However, among patients aged 65-74 years, no difference in the risk of the composite outcome was observed. Adherent bisphosphonate users were associated with a decreased risk of the composite outcome as compared to non-adherent bisphosphonate users across all four MPR thresholds examined.

Overall, this study showed an association between bisphosphonate use, and reduced cardiovascular outcomes, but effect appeared to be age-dependent. Future prospective studies and/or observational studies that include laboratory, clinical and radiologic data to determine osteoporosis severity and degree of vascular calcification are needed to validate the results of this study.

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CHAPTER I. INTRODUCTION

Cardiovascular disease (CVD) is one of the most significant and costly public health problems in the U.S. and worldwide. It is estimated that more than one in three adults in the U.S. have one or more types of CVD. According to the 2012 American Heart Association, about 7.9, 9 and 7 million American adults have had a myocardial infarction (MI), heart failure and stroke, respectively (Roger et al., 2012). In 2008, CVD accounted for 16% of total healthcare expenditures in the U.S, consisting of $179 billion in direct costs and $118.5 billion in indirect costs (Roger et al., 2012). Of these figures, 96 and 19 billion are attributable to heart disease, including MI and heart failure, and stroke, respectively ("Medical Expenditure Panel Survey," 2008).

Risk factors for CVD, such as an abnormal level of blood cholesterol, hypertension, diabetes and cigarette smoking have been extensively studied (Lundberg, Stegmayr, Asplund, Eliasson & Huhtasaari, 1997; Greenland et al., 2003; Khot et al., 2003). In addition to these well-established risk factors, growing bodies of studies have demonstrated that vascular calcification is another strong and independent predictor of cardiovascular morbidity and mortality in the general population (Sangiorgi et al., 1998; Wilson et al., 2001; Schmermund et al., 2003; Tousoulis et al., 2012).

Vascular calcification exists in two distinct, but not mutually exclusive patterns: intimal (atherosclerotic) calcification and medial (arteriosclerotic) calcification. Intimal

calcification obstructs the artery lumen and is associated with stroke, myocardial infarction and angina pectoris (Sangiorgi et al., 1998). Medial calcification increases the stiffness of the elastic layer of the arterial wall, resulting in increased pulse pressure and leading to left ventricular hypertrophy, dysfunction and failure (Lehto, NIskanen, Suhonen, Rönnemaa & Laakso, 1996). Although the precise mechanisms of vascular calcification remain undefined, recent progress in basic molecular biology research has suggested that vascular calcification shares certain similarities with bone formation and mineralization, and its pathological process includes many key modulators of bone and mineral metabolism (Anderson, 1983; Giachelli et al., 1991; Waston et al., 1994; Abedin et al., 2004; Demer et al, 2008).

With the common underlying pathological mechanisms, the question is raised regarding whether pharmacological agents that inhibit bone loss can also provide a beneficial impact on inhibiting vascular calcification to lower CVD risks. Among the pharmacological agents that inhibit bone loss, bisphosphonates are the most widely prescribed and used medication in the U.S. Several studies have reported that some bisphosphonates, i.e., alendronate and ibandronate, inhibit experimentally induced artery calcification in rats through inhibiting osteoclast-mediated bone resorption, which is the same mechanism that inhibits bone loss (Price et al., 2001; Tamura et al., 2007; Saito et al., 2007). Despite the fact that bisphosphonates have shown a consistent effect on inhibiting vascular calcification in animal studies, the effect of bisphosphonates in humans has not been well studied. The clinical studies in this area are small and their

results are conflicting (Tankó et al., 2005; Luckish et al., 2008). No large-scale observational study has been conducted. Solid clinical evidence supporting the inhibitory effect of bisphosphonates on vascular calcification in humans is still lacking.

Additionally, although bisphosphonates have been shown to significantly inhibit bone loss in numerous clinical trials, their therapeutic benefits are often compromised by poor patient adherence in clinical practice due to difficulty in following the stringent dosing guidelines or adverse effects (Cramer, Amonkar, Hebborn & Altman, 2005; Siris et al., 2006; Briesacher, Andrade, Yood & Kahler, 2007; Penning-van Beest, Erkens, Olson & Herings, 2008; Gold, Trinh & Safi, 2009). Maintaining adherence to bisphosphonate therapy has been recommended to obtain optimal benefits. Similarly, maintaining adherence to cardiovascular therapy is also a critical strategy to achieve long-term control of CVD (Benner, 2002; Frishman, 2007; Munger, Van Tassell & LaFleur, 2007). Of the few clinical studies regarding the effect of bisphosphonates on inhibiting vascular calcification conducted thus far, none of them has examined the association between patient adherence and the cardiovascular effect of bisphosphonates. However, if patients want to have optimal benefits from bisphosphonate therapy, and healthcare practitioners want to manage their patients more effectively, understanding this issue is crucial.

To help better understanding bisphosphonates' effect on inhibiting vascular calcification in humans, the objectives of this study are to: 1) examine the association between bisphosphonate use and the risk of hospitalization for the three cardiovascular

diseases that have been linked to vascular calcification: congestive heart failure (CHF), myocardial infarction (MI) and stroke; and 2) estimate the impact of patient adherence to bisphosphonate therapy on the risk of CHF, MI and stroke hospitalization.

A. Objective and Aims

The overall goal of this study is to investigate if bisphosphonates can decrease the risk of cardiovascular diseases, CHF, MI or stroke that have been linked to vascular calcification. The long-term goals of this study are to: 1) contribute to the knowledge of novel pharmaceutical care strategies for treating vascular calcification and further reducing the cardiovascular morbidity and mortality in the general population; 2) to fill the knowledge gap remaining in the current animal studies and epidemiological studies done on humans; and 3) to stimulate future clinical trials in this area, which will assess the cause-effect relationship between vascular calcification and the use of bisphosphonates.

In keeping with the overall goal and specific objectives, this study evaluated three aims:

Aim 1: To investigate the overall effect of the three bisphosphonates of interest, alendronate, ibandronate and risedronate, on the risk of CHF, MI and stroke, the cardiovascular diseases that have been linked to vascular calcification.

Hypothesis 1: The risk of a composite endpoint, consisting of CHF, MI and stroke hospitalization, is lower in patients treated with bisphosphonates than

patients who are not treated with bisphosphonates, after controlling for confounders.

Aim 2: To investigate the effect of individual bisphosphonates on the risk of cardiovascular diseases that have been linked to vascular calcification.

Hypothesis 1: The risk of a composite endpoint, consisting of CHF, MI and stroke hospitalization is lower in patients treated with alendronate than patients who are not treated with bisphosphonates, after controlling for confounders.

Hypothesis 2: The risk of a composite endpoint, consisting of CHF, MI and stroke hospitalization is lower in patients treated with ibandronate than patients who are not treated with bisphosphonates, after controlling for confounders.

Hypothesis 3: The risk of a composite endpoint, consisting of CHF, MI and stroke hospitalization is lower in patients treated with risedronate than patients who are not treated with bisphosphonates, after controlling for confounders.

Aim 3: To investigate the influence of patient adherence to bisphosphonate therapy on the risk of cardiovascular diseases that have been linked to vascular calcification.

Hypothesis 1: The risk of a composite endpoint, consisting of CHF, MI and stroke hospitalization is lower in patients who are adherent to bisphosphonate therapy than patients being non-adherent, after controlling for confounders.

B. Study Design

This is a retrospective cohort study using pharmaceutical, inpatient, outpatient and enrollment claims data from the Thomson Reuters MarketScan® Databases. The MarketScan® Databases include nearly 150 million patients from 1995 to 2008. Among these patients, a large proportion are post-menopausal women (≥45 years of age) with osteoporosis. Along with the large sample size, the longitudinal integrity of the databases allows for long-term follow-up of many patients. Study patients were identified from the enrollees in the MarketScan® Databases from January 1, 2000 to December 31, 2007. In addition, the MarketScan® Databases allow access to detailed medical claims and prescription utilization among the enrollees. This information is essential for evaluation of the effect of bisphosphonates on the cardiovascular diseases of interest. To explore the study hypotheses, time dependent Cox proportional hazards modal was used.

C. Significance

Cardiovascular disease is one of the most significant causes of mobidities and mortality in the U.S. Considering the rising healthcare costs and their financial impact, it is important to understand what the future might hold for CVD. The progress in basic molecular biology study has demonstrated that vascular calcification sharing a common pathological mechanism with bone metabolism is a strong predictor of cardiovascular morbidity and mortality in addition to the well-established risk factors. Despite the progress in molecular biology research, it remains unclear whether medications that inhibit bone loss can provide beneficial impact on vascular calcification.

The main purpose of this study is to examine the impact of bisphosphonates on CVD that have been linked to vascular calcification. This study will help fill the knowledge gap remaining from the current animal studies and clinical studies. Up to the present, only a limited number of clinical studies have been conducted in this area and all of them had small sample size. Thus, results of these studies may not be generalizable to the general population of patients taking these drugs. A pharmacoepidemiologic study like this one can capitalize on the availability of large datasets and long follow-up periods to provide a more comprehensive understanding of the effects of bisphosphonates on CVD outcomes linked to vascular calcification.

Moreover, aging of the U.S. population and the emphasis on preventive care under healthcare reform has drawn a lot of research attention regarding cardiovascular morbidity and mortality. Recent studies have suggested that osteoporosis and CVD are not two distinct diseases. Patients with osteoporosis have been associated with higher risk of CVD because these two diseases share common risk factors (McFarlane, Muniyappa, Shin, Bahtiyar, & Sowers, 2004; Bevilacqua, Dominguez, Rosini, & Barbagallo, 2005). As the current primary therapeutic agents for osteoporosis, bisphosphonates may benefit two patient groups. This study will provide healthcare practitioners with new ideas and options when delivering pharmaceutical care to patients with both osteoporosis and CVD in order to use medications more effectively and safely, improve health outcomes and reduce healthcare expenditures.

CHAPTER II. LITERATURE REVIEW

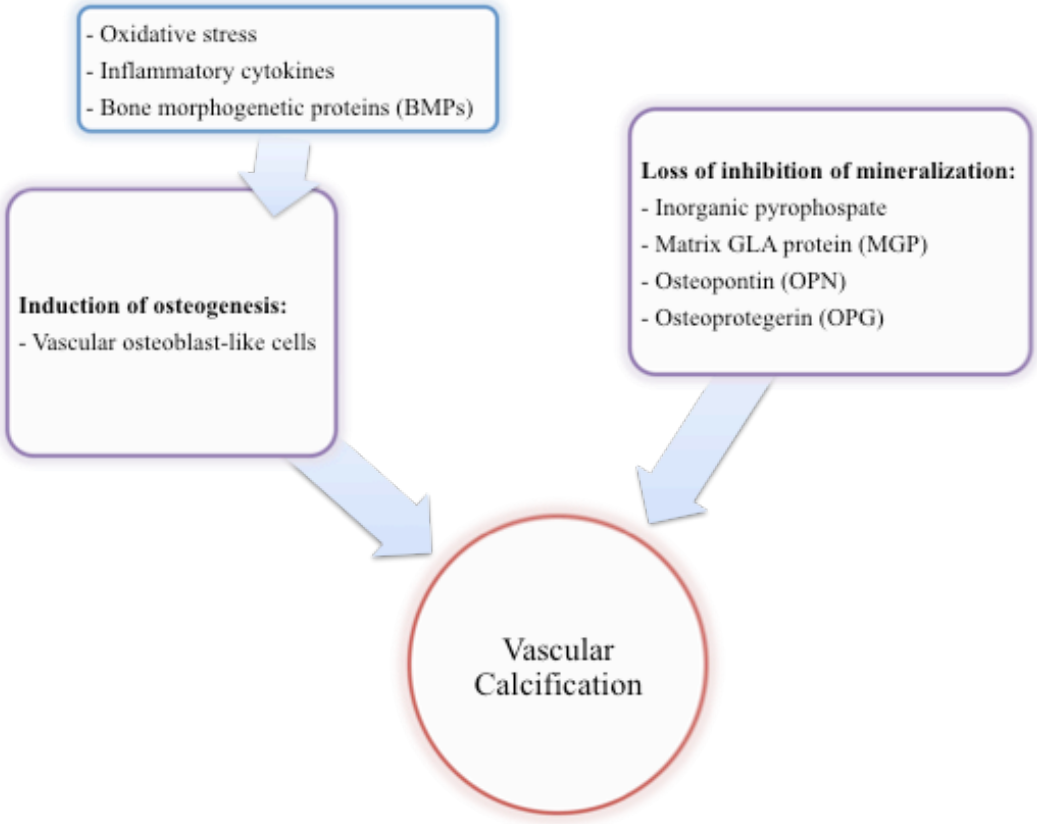
A. Pathogenic mechanism of vascular calcification

Ninety-nine percent of total body calcium is stored as small crystals called hydroxyapatite in bones. However, calcium salts are also found in various tissues other than bones; a situation which is broadly termed as extra-skeletal calcification (Moe & Chen, 2008). Some forms of extra-skeletal calcification occur in the setting of cellular necrosis that calcium can adhere to damaged proteins. Other abnormal forms of extra-skeletal calcification may develop at vascular sites, such as arterial walls, where cells can produce bone-forming transcription factors and proteins and therefore have osteoblastic potential. Vascular calcification, including calcification of general arteries, coronary arteries and cardiac valves, can lead to devastating and life-threatening cardiovascular outcomes depending on its extent and the organ affected.

Previously, vascular calcification was viewed as a passive, unregulated and degenerative process of aging. Although the precise mechanisms of vascular calcification remain undefined, recent progress in basic molecular biology and animal studies have suggested that vascular calcification is an active and tightly regulated process sharing many characteristics with bone formation (Anderson, 1983; Giachelli, Bae, Lombardi, Majesky & Schwartz, 1991; Waston et al., 1994). In addition, the pathological process of vascular calcification is regulated by many of the same key regulatory factors of bone cell differentiation and mineralization, such as matrix Gla protein, osteopontin, and

osteoprotegerin (Abedin, Tintut & Demer, 2004; Demer & Tintut, 2008). Currently two different but not mutually exclusive pathogenic mechanisms of vascular calcification have been proposed. One is induction of osteogenesis and the other is loss of inhibition of mineralization (Johnson, Leopold & Loscalzo, 2006). A diagram summarizing the two pathogenic mechanisms of vascular calcification is shown in Figure 1.

Figure 1: Pathogenic mechanisms of vascular calcification



1. Induction of osteogenesis

One explanation of the pathogenic mechanism of vascular calcification is that it is the consequence of a process similar to osteogenesis (bone formation) (Böstrom et al., 1993; Demer, 1995). Under normal circumstances, mesenchymal stem cells differentiate into adipocytes, chondrocytes, osteoblasts and vascular smooth muscle cells (VSMCs). However, some stimuli, such as oxidative stress, inflammatory cytokines, or bone morphogenetic proteins (BMPs) can induce VSMCs to undergo reversible differentiation into osteoblast-like cells (Giachelli, 2004; Abedin et al., 2004). Evidence suggests that the ability to undergo reversible differentiation of VSMCs may be critical in the development of calcification.

BMP is one of the stimuli that induce VSMCs to undergo osteoblastic differentiation. Of BMPs, BMP-2 is an important factor to trigger osteoblastic differentiation. Böstrom and colleagues (1993) have found increased expression of BMP-2 in calcified atherosclerotic lesions compared to normal arteries, suggesting that arterial calcification is regulated by BMP-2. Watson and colleagues (1994) have identified a special subpopulation of VSMCs, termed "calcifying vascular cells", which spontaneously undergo calcification in tissue culture. These calcifying vascular cells exhibit several characteristics of osteoblasts and can be modulated by BMP-2. In addition to the VSMCs, pericytes in the vessel wall may also play a role in vascular calcification. Pericytes share some properties with calcifying vascular cells and serve as a source of precursor cells that have the potential to become osteoblasts and chondrocytes (Doherty et al., 1998).

2. Loss of inhibition of mineralization

Human and animal studies have proved that several regulatory factors, including matrix Gla protein, osteopontin and osteoprotegerin exist that normally inhibit vascular calcification (Luo et al., 1997; Abedin et al., 2004; Min et al., 2000). Loss of these inhibitors or inadequate function of these inhibitors is associated with the presence of vascular calcification. These three inhibitors are discussed in more detail below.

(i) Matrix Gla Protein

Matrix Gla protein (MGP) is a mineral-binding extracellular matrix protein and is synthesized by vascular smooth muscle cells and chondrocytes. MGP regulates the formation of cartilage and the development of vascular calcification. Luo and colleagues (1997) studied MGP-deficient mice and found that in the absence of MGP, these mice not only expressed short stature, osteopenia or fractures, but also developed extensive arterial calcification. These findings suggest that MGP acts as an inhibitor of vascular and cartilage calcification.

In addition, it has been shown that vitamin K-dependent gamma-carboxylation is essential for normal MGP function (Sweatt, Sane, Hutson & Wallin, 2003). Schurgers and colleagues (2005) compared healthy and atherosclerotic arteries and found that fully carboxylated MGP was present in healthy arteries, whereas non-carboxylated MGP was expressed only in atherosclerotic arteries. The result suggests that non-carboxylated MGP is highly associated with vascular calcification. Therefore, conditions causing incomplete

gamma-carboxylation, such as vitamin K deficiency, may result in the development or progression of vascular calcification. The study done by Price and colleagues further supports this hypothesis. They found extensive calcification of arteries and heart valves in rats treated with warfarin, a vitamin K antagonist (Price, Faus & Williamson, 1998). Furthermore, a recent clinical study indicated that serum levels of MGP were lower in patients with coronary artery calcification (CAC) than without CAC. An inverse association was found between the serum level of MGP and the severity of CAC (Jono et al., 2004)

(ii) Osteopontin

Osteopontin (OPN) is a matrix protein that functions as an important inhibitor of vascular calcification. OPN interacts with the cell adhesion receptor on osteoclasts. This interaction helps anchor osteoclasts to bone surfaces and promotes bone resorption (Miyauchi et al., 1991). Steitz and colleagues (2002) found that this interaction also promotes resorption of ectopic calcification. They observed 4- to 5-times greater aortic valve calcification in OPN-deficient mice than in wild type mice, suggesting the inhibitory effect of OPN on vascular calcification. It has also been shown that OPN is highly expressed in atherosclerotic plaques (Giachelli et al., 1993). Comparing patients without coronary artery disease (CAD) to patients with CAD, Ohmori and colleagues (2003) found that plasma levels of OPN were significantly associated with the presence and extent of CAD. OPN levels have also been found to be positively correlated with aortic calcification in hemodialysis patients (Nitta et al., 2001). The high levels of OPN

expression are hypothesized to represent an adaptive mechanism aimed at inhibiting vascular calcification.

(iii) Osteoprotegerin

Osteoprotegerin (OPG) is a glycoprotein and identified as a member of the tumor necrosis factor-α (TNF-α) receptor superfamily. The discovery of OPG provides more evidence of the association between bone and vascular metabolism. OPG acts as a decoy receptor for RANKL (receptor activator of nuclear factor kappa B ligand) to prevent RANKL from interacting with its receptor RANK (receptor activator of nuclear factor kappa B) (Simonet et al., 1997). The interaction of RANKL with RANK is essential for osteoclast differentiation. Therefore, when OPG binds to RANKL, the interaction of RANKL with RANK is reduced and the osteoclast differentiation is inhibited. Bucay and colleagues (1998) investigated the role of OPG on osteoclast differentiation. They found that OPG-deficient mice developed severe osteoporosis as a result of over-expressed osteoclastic bone resorption. Unexpectedly, they found these OPG-deficient mice developed calcification in the aorta and renal arteries as well. This finding suggests that OPG may regulate vascular calcification through a mechanism related to bone resorption. Following up Bucay's study, Min and colleagues (2000) have shown that OPG is expressed in arteries of wild-type mice, but RANK and RANKL are not. On the contrary, RANK and RANKL were found in the calcified arteries of OPG-deficient mice. The study results suggest that the OPG/RANKL/RANK pathway may play an important role in the calcification process.

The inhibitory effect of OPG on vascular calcification was demonstrated in an animal model study conducted by Price and colleagues (Price, June, Buckley & Williamson, 2001). The study done by Jia and colleagues shows consist results. They found that the gene expression of OPG was reduced while the aortic calcium content was increased in rats (Jia, Li, Wang, Zhang, Liu, & Wang, 2012). In a human study involving 490 white women aged 65 and older, serum OPG levels were found to be associated with diabetes and cardiovascular mortality (Browner, Lui & Cummings, 2001). The finding suggests that OPG may be a marker of vascular calcification and the increased serum OPG levels may be a physiologically compensatory effect to prevent vascular calcification.

B. The association between vascular calcification and cardiovascular diseases

Epidemiological and clinical studies have suggested that the presence and extent of vascular calcification is a strong and independent predictor of subsequent cardiovascular morbidity and mortality in general populations (Sangiorgi et al., 1998; Wilson et al., 2001; Schmermund, Mönlenkamp & Erbel, 2003). Clinically, vascular calcification exists in two patterns: intimal (atherosclerotic) calcification and medial (arteriosclerotic) calcification. These two patterns of vascular calcification have distinct, but not mutually exclusive disease processes. They both contribute to progression of vascular calcification and aggravate arterial damage. Table 1 summarizes the location, risk factors and outcomes associated with each type of vascular calcification.

Table 1. Characteristics of intimal and medial vascular calcification

Type of Vascular Calcification	Location	Risk Factors	Associated Outcomes
Intimal calcification	-Coronary arteries -Large arteries, e.g. aorta	-Atherosclerosis -Hyperlipidemia -Osteoporosis -Hypertension -Inflammation	-Myocardial infarction -Stroke
Medial calcification	-Lower limb arteries, e.g. the femoral and tibial arteries -Aorta	-Type 2 DM -End-stage renal disease	-Congestive heart failure -Peripheral artery disease

1. Intimal calcification

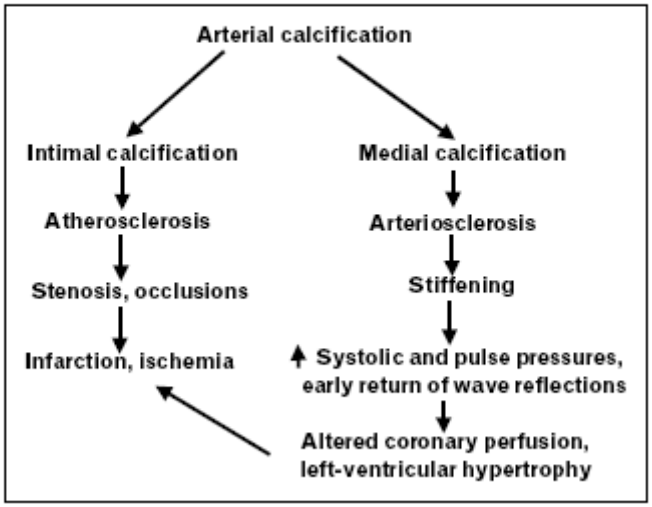
Intimal calcification occurs in the intimal layer of the artery and is mostly found in the coronary arteries and the aorta. In the general population, intimal calcification is associated with stroke, myocardial infarction and angina pectoris. Calcification in the intima represents a process of calcium deposition into atherosclerotic plaques, which may lead to erosions, plaque disruption and thrombosis. The extent of coronary calcification has been demonstrated to be positively correlated with the atherosclerotic plaque burden on autopsy and in imaging studies. Sangiorgi and colleagues (1998) used electron beam computed tomography (EBCT) to investigate the association between calcium deposition in the coronary artery and the burden of atherosclerotic plaques. They found that the more extensive the calcification, the more advanced the stage of atherosclerosis. Vliegenthart and colleagues (2005) found consistent results in the Rotterdam Coronary Calcification Study. Coronary calcium scores were obtained from

1795 subjects without a history of coronary heart disease. The study results show that higher coronary calcification score is significantly associated with increasing risk of coronary heart disease.

2. Medial calcification

Medial calcification, also known as Mönckeberg's arteriosclerosis, occurs in the medial layer of the artery. In contrast to intimal calcification, medial calcification does not obstruct the artery lumen. However, it increases the stiffness of the elastic layer of the arterial wall, resulting in increased pulse pressure and leading to left ventricular hypertrophy, dysfunction and failure (Lehto, Niskanen, Suhonen, Rönnemaa & Laakso, 1996). Medial calcification is typically found in the muscle-type conduit arteries, such as femoral and tibial arteries, but it is also commonly seen in the aorta (Mackey, Venkitachalam & Sutton-Tyrrell, 2007). Medial calcification was initially viewed as clinically insignificant because it was non-occlusive and considered part of the aging process. However, recent studies have indicated that medial calcification, which occurs with particularly high frequency in patients with type 2 diabetes mellitus and end-stage renal disease, is a strong predictor of cardiovascular disease as well (London et al., 2003; Lehto, NIskanen, Suhonen, Rönnemaa & Laakso, 1996). A schematic representation of the clinical impacts of intimal and medial calcification is shown in Figure 2 below.

Figure 2: Schematic representation of the clinical impacts of intimal and medial calcification



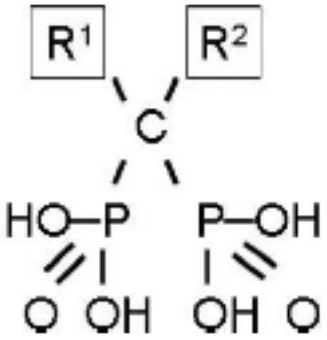
Note. Adapted from "Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia," by London G.M., Marchais S.J., Guerin A.P., & Metivier F., 2005, *Current Opinion in Nephrology and Hypertension, 14*(6), 525-531.

C. Mechanism of action of bisphosphonates

Since the introduction of bisphosphonates into clinical practice, they have become the treatment of choice for an array of bone disorders in which excessive osteoclast-mediated bone resorption underlies disease pathology, including Paget's disease, malignancies metastatic to bone, hypercalcemia of malignancy and osteoporosis (Russell, 2006). Structurally, bisphosphonates are synthetic pyrophosphate analogs. The core structure of bisphosphonates is made up of a central nonhydrolyzable carbon flanked with two phosphate groups and two side chains designated R^1 and R^2 (Watts & Diab, 2010). As

shown in Figure 3, the P-C-P structure provides bisphosphonates with a high affinity for hydroxyapatite crystals on bone surfaces. The R^1 side chain further increases the binding affinity of bisphosphonates to calcium and the R^2 side chain determines the antiresorptive potency of bisphosphonates. Modification of the R^2 side chain has widened the relative antiresorptive potency in newer compared to older bisphosphonate compounds (Drake, Clarke & Khosla, 2008). Table 2 summarizes the structure and relative potencies of some bisphosphonates in clinical use.

Figure 3: General bisphosphonate structure



Note. From "Long-term use of bisphosphonates in osteoporosis," by Watts, N.B. & Diab, D.L., 2010, *The Journal of Clinical Endocrinology and Metabolism, 95*(4), 1555-1565.

Table 2. Structure and relative potency of bisphosphonates commonly used in the U.S.

Compound	R^1	R^2	Relative potency
Pamidronate	OH	$(CH_2)_2NH_2$	100
Alendronate	OH	$(CH_2)_3NH_2$	500
Ibandronate	OH	$(CH_2)_2NCH_3(CH_2)_4CH_3$	1000
Risedronate	OH	CH_2-3-pyridinyl	2000
Zoledronate	OH	$CH_2C_3N_2H_3$	10,000

Note. From "Bisphosphonates: Mechanism of action and role in clinical practice," by Drake, M. T., Clarke, B. L., & Khosla, S., 2008, *Mayo Clinic Proceedings.Mayo Clinic, 83*(9), 1032-1045.

The most widely used bisphosphonates in current clinical practice, alendronate, risedronate, ibandronate and zoledronate, all have one or more nitrogen molecules in their R^2 side chains. These nitrogen-containing bisphosphonates inhibit osteoclast-mediated bone resorption by inhibiting the activity of farnesyl diphosphate synthase, a key regulatory enzyme in the mevalonic acid pathway. The mevalonic acid pathway is the biosynthetic pathway for the production of cholesterol, other sterols and isoprenoid lipids, including farnesyl diphosphate. Farnesyl diphosphate is critical for the posttranslational modification (prenylation) of GTP-binding proteins, which regulate osteoclast formation, function and survival. Therefore, inhibition of farnesyl diphosphate synthase interferes with prenylation of GTP-binding proteins and ultimately leads to reduced osteoclast-mediated bone resorption and accelerated osteoclast apoptosis (Rogers, 1999; Drake et al.,

2008; Watt & Diab, 2010).

D. The role of bisphosphonates in inhibiting vascular calcification

The effects of bisphosphonates on vascular calcification have been evaluated in recent animal studies. Price and colleagues have conducted a series of animal studies to investigate the factors associated with vascular calcification and found that vascular calcification is related to bone resorption. Based on their findings, Price and colleagues carried out another series of animal studies to examine if bisphosphonates that inhibit bone resorption would inhibit vascular calcification as well. In the first study, vascular calcification was induced in rats by treatment with high doses of warfarin alone. More extensive vascular calcification was induced by treatment with warfarin plus high doses of vitamin D. They found that calcification of arteries and heart valves by warfarin or warfarin plus vitamin D was completely inhibited in rats receiving either alendronate 0.1 mg/kg/day or ibandronate 0.05 mg/kg/day. These doses have been demonstrated in animals to inhibit bone resorption without impairing normal bone mineralization. The study results support the capability of bisphosphonates to inhibit vascular calcification (Price, Faus & Williamson, 2001).

In the second study, Price and colleagues examined the effect of ibandronate on inhibition of soft tissue calcification induced by toxic doses of vitamin D. A total of 28 rats were used in the study. Sixteen of them received toxic doses of vitamin D (12.5 mg/kg) alone and twelve of them received the same dose of vitamin D together with

ibandronate 0.25 mg/kg/day. Pronounced calcification was observed in the aorta, the carotid, femoral, mesenteric, hepatic, and renal arteries, kidney and lung in rats treated with vitamin D alone using Alizarin red staining, whereas no evidence of calcification in any of these tissues was found in rats treated with vitamin D together with ibandronate. They further analyzed tissue levels of calcium and phosphate in the aorta, trachea, lung and kidney to obtain a quantitative measure of treatment effect. Compared to rats given vitamin D plus ibandronate, the tissue levels of calcium and phosphate was significantly greater in rats given vitamin D alone ($p<0.001$). These results suggested that ibandronate, a bone resorption inhibitor, could inhibit vascular calcification as well as calcification in various soft tissues (Price, Buckley & Williamson, 2001).

Evidence from other studies also supports that bisphosphonates inhibit vascular calcification. Tamura and colleagues have evaluated the effect of etidronate on inhibition of aortic calcification in rats with renal failure. After treating with calcitriol (vitamin D_3) to induce aortic calcification, rats were given etidronate 5 mg/kg, 10 mg/kg (these doses do not affect bone metabolism) or no treatment. They found that compared with the rats receiving no treatment, the treatment of 5 mg/kg and 10 mg/kg etidronate significantly inhibited aortic calcification by 46% and 74%, respectively ($p<0.01$) (Tamura et al., 2007).

In the study done by Saito and colleagues, pamidronate was reported to inhibit arterial calcification induced by inorganic phosphate in bovine aortic smooth muscle cells.

Bovine aortic smooth muscle cells were cultured in medium containing pamidronate 10^{-10}, 10^{-8} or 10^{-6} mmol and inorganic phosphate 3 mmol. When treated with pamidronate 10^{-6} mmol, aortic calcification was significantly reduced by 87.5% compared with treatment with inorganic phosphate alone. They also found that the inhibition effect of pamidronate on aortic calcification is monotonically increased with increasing doses (Saito, Wachi, Sato, Sugitani & Seyama, 2007). Although the precise mechanism by which bisphosphonates inhibit vascular calcification is still unclear, these animal studies provide evidence that bisphosphonates can inhibit vascular calcification.

Despite consistent results shown in animal studies, limited clinical studies have been done to evaluate the effects of bisphosphonate on vascular calcification in humans and their results are conflicting. Tankó and colleagues conducted a post hoc analysis on the effects of oral and intravenous ibandronate on aortic calcification in 474 Danish postmenopausal women aged 55 to 80 years from two 3-year randomized, placebo-controlled clinical trials originally designed to evaluate the efficacy and safety of ibandroante. All subjects had osteoporosis manifested by low bone mineral density (BMD) measurements at lumbar spine (*T* scores between -2.0 and -5.0) and one to four vertebral fractures. In the oral ibandronate trial, subjects received placebo, ibandronate 2.5 mg daily or ibandronate 20 mg every other day in every 3-month period. In the intravenous ibandronate trial, subjects were given placebo or ibandronate 0.5 or 1.0 mg every 3 months. Aortic calcification was assessed by lateral X-ray. The study results indicated that over the 3-year study period, every treatment dose group showed a

significant increase in BMD measurements at both lumbar spine and hip as compared to placebo. However, none of the treatment groups showed a significant decreases in the progression of aortic calcification over 3 years (Tankó, Qin, Alexandersen, Bagger & Christiansen, 2005). On the contrary, risedronate showed beneficial vascular effects in a prospective study by Luckish and colleauges. Sixty-eight postmenopausal women with osteoporosis (defined as BMD *T* score ≤ -2.5 at spine or femoral neck) were recruited and treated with oral risedronate 35 mg per week for 6 months. Results indicated that during the 6-month treatment period, risedronate significantly improved both large and small artery elasticity measured by arterial pulse wave. Risedronate also reduced systemic vascular resistance calculated as mean arterial pressure divided by cardiac output. Large artery elasticity index increased from 9.9±3.3 to 11.5±3.2 ml/mmHg*100, $p<0.0001$; small artery elasticity index increased from 2.6±1.1 to 3.3±1.2 ml/mmHg*100, $p<0.0001$; and systemic vascular resistance decreased from 1876.1±457.7 to 1646.1±260.2 dyne/sec/cm^{-5}, $p=0.013$ (Luckish et al., 2008). The conflicting findings from these studies suggest that the effects of bisphosphonates on vascular calcification in humans are still indefinite. Additional evidence supporting (or not supporting) the effect of bisphosphonates on vascular calcification in humans is needed.

E. Utilization of bisphosphonates

1. Bisphosponate use in osteoporosis

Bisphosphonates are currently the most widely prescribed and used medications in the U.S. for various forms of osteoporosis. The Food and Drug Administration (FDA) has

approved four bisphosphonates, alendronate (Fosamax®), risedronate (Actonel®), ibandronate (Boniva®) and zoledronic acid (Reclast®), for osteoporosis treatment and prevention. Etidronate is used in Europe and Canada for osteoporosis, but it is only approved in the U.S. for heterotopic ossification, hypercalcemia of malignancy and Paget's disease. Pamidronate is indicated for bone metastasis, hypercalcemia of malignancy and Paget's disease in the U.S.

Bisphosphonates have a relatively good safety profile. The most common adverse effects associated with all bisphosphonate therapies include esophagitis, upper gastrointestinal irritation and mild-to-moderate musculoskeletal pain (de Groen, 1996; Wysowski & Chang, 2005). However, it has been more than 10 years since bisphosphonates became available on the market. With increased prescribing of bisphosphonates over time, other less common adverse effects have been reported. Osteonecrosis of the jaw (ONJ) defined as "the presence of exposed bone in the maxillofacial region that does not heal within eight weeks after identification by a health care provider" has been reported as a potential complication in patients receiving bisphosphonates, especially intravenous (IV) bisphosphonates. The incidence of ONJ seems to be low in patients receiving oral bisphosphonates for osteoporosis (estimated to be ≤ 1 in 100,000 patient-treatment years), whereas it is higher in patients receiving IV bisphosphonates (pamidronate and zoledronic acid) for malignancy (ranging from 1 to 10 in 100 patient-treatment years) (Khosla et al., 2007).

Another rare but serious adverse event related to bisphosphonate use is atrial fibrillation. It was first reported in the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON) study. Although the overall incidence of cardiovascular adverse events was similar between zoledronic acid and placebo, the incidence of serious atrial fibrillation was significantly higher among patients in the zoledronic acid than placebo group (1.3% in zoledronic acid vs. 0.5% in placebo, $p<0.001$) (Black et al., 2007). Overall, the risk of serious adverse effects associated with bisphosphonate use is low based on currently available data.

A brief description of each of the three bisphosphonates under study (alendronate, ibandronate and risedronate), evidence for fracture prevention and typical dosage regimens are provided in the subsequent sections.

1) Alendronate: Alendronate is indicated for the treatment (10 mg/day or 70 mg/week) and prevention (5 mg/day or 35 mg/week) of postmenopausal osteoporosis. The main goal for the treatment of postmenopausal osteoporosis is to reduce fracture rate and consequently reduce the impact of fracture on patients' quality of life. Being one of the leading pharmacological interventions for postmenopausal osteoporosis, the anti-fracture efficacy of alendronate has been demonstrated in the Fracture Intervention Trial (FIT), a randomized, double blind placebo-controlled trial. FIT had two arms: the vertebral fracture arm and the clinical fracture arm. In the vertebral fracture arm, 2027 women aged 55 to 81

years with low bone mineral density (BMD) and at least one vertebral fracture at baseline were randomly assigned to alendronate (n=1022) or placebo (n=1005) and followed up for 36 months. Compared with placebo, alendronate significantly reduced the risks of vertebral and non-vertebral fractures (hazard ratio (HR) for vertebral fracture=0.45, 95% CI: 0.27-0.72; HR for hip fracture=0.49, 95% CI: 0.23-0.99; HR for wrist fracture=0.52, 95% CI: 0.31-0.87) (Black et al., 1996). In the clinical fracture arm, a total of 4432 women aged 55 to 80 years with low BMD but no history of vertebral fracture were randomly assigned to alendronate (n=2214) or placebo (n=2218). Alendronate effect depended on subjects' BMD at baseline. In women with a BMD T score $\leq$ -2.5, alendronate significantly reduced risk of vertebral and hip fractures by 50% (95% CI: 0.31-0.82) and 56% (95% CI: 0.18-0.97), respectively. However, there was no significant reduction on fracture risk among those with a BMD T score > -2.5 (Cummings et al., 1998). Except for postmenopausal osteoporosis, the other FDA-labeled indications for alendronate include osteoporosis in men, glucocorticoid-induced osteoporosis and Paget's disease.

2) Risedronate: Risedronate is also indicated for the treatment (5 mg/day or 35 mg/week) and prevention (5 mg/day or 35 mg/week) of postmenopausal osteoporosis as well as osteoporosis in men, glucocorticoid-induced osteoporosis and Paget's disease. The Vertebral Efficacy With Risedronate Therapy (VERT) Study assessed the effect of risedronate on reducing vertebral and non-vertebral

fractures in postmenopausal women. A total of 2458 women younger than 85 years with at least one vertebral fracture at baseline were enrolled and randomly assigned to receive placebo (n=820) or 5 mg risedronate (n=821). Compared with placebo, risedronate yielded increases in BMD at lumber spine, femoral neck, and femoral trochanter of 5.4%, 1.6% and 3.3% respectively in the 3 years of follow-up. The relative risk of new vertebral fracture in the risedronate group versus placebo group was 0.59 (95% CI: 0.43-0.82) while the relative risk of non-vertebral fracture was 0.6 (95% CI: 0.39-0.94) (Harris et al., 1999). Study results provided evidence that risedronate significantly reduces the risks of both vertebral and non-vertebral fractures in women with established postmenopausal osteoporosis.

3) Ibandronate: Ibandronate is approved by the FDA for the treatment (2.5 mg/day or 150 mg/month) and prevention (2.5 mg/day or 150 mg/month) of postmenopausal osteoporosis. In an effort to improve patient adherence to bisphosphonate therapy, ibandronate was formulated as a once monthly formulation. Both daily and monthly ibandronates have been demonstrated to effectively increase BMD and reduce the incidence of vertebral fracture in postmenopausal women at risk of osteoporosis. In the Oral Ibandronate Osteoporosis Vertebral Fracture Trial in North American and Europe (BONE), a randomized, double-blind, placebo-controlled study, Chesnut and colleagues (2004) evaluated the anti-fracture efficacy of oral ibandronate administered daily

(2.5 mg/day) or intermittently (20 mg every other day for 12 doses every 3 months) in 2946 postmenopausal women with a BMD T score ≤ -2 at the lumbar spine and at least one vertebral fracture. Over 3 years of follow-up, both daily and intermittent ibandronate therapies significantly reduced the risk of incident vertebral fractures by 62% ($p=0.0001$) and 50% ($p=0.0006$), respectively, compared with placebo. However, neither of them showed significant effect on reducing the risk of non-vertebral fractures. The effect of monthly ibandronate was assessed in the Monthly Oral Ibandronate in Ladies (MOBILE) study, a randomized, double blind, non-inferiority, phase III trial. A total of 1609 women aged 55 to 80 years with a BMD T score between -2.5 and -5 were enrolled and randomly assigned to one of the four treatment groups: 2.5 mg daily, single dose of 50 mg/day administered on 2 consecutive days in one month (50+50 mg once monthly), 100 mg once monthly and 150 mg once monthly. Increases in BMD at the lumbar spine were observed in all treatment groups: 5.0%, 5.3%, 5.6% and 6.6% for 2.5 mg daily, 50+50 mg once monthly, 100 mg once monthly and 150 mg once monthly, respectively. Furthermore, 150 mg once monthly treatment showed a significant increase in the lumbar spine BMD ($p<0.0001$) as compared to 2.5 mg daily treatment. The findings suggest that all monthly ibandronate regimens are at least as effective as the daily regimen (Reginster et al., 2006).

In summary, alendronate and risedronate have been demonstrated to significantly reduce the risk of both vertebral and non-vertebral fractures. However, only ibandronate

shows significant effects on reducing vertebral fractures. The indications, dosages and FDA approval dates of the three study bisphosphonates are summarized in Table 3.

Table 3. Indications, dosages and FDA approval dates of study bisphosphonates

Bisphosphonate	FDA-labeled indication	Dosage*	FDA approval date
Alendronate (Fosamax®)	- Postmenopausal osteoporosis - Postmenopausal osteoporosis; prophylaxis - Osteoporosis, male - Osteoporosis due to corticosteroid - Paget's disease	Prevention: 5 mg/day orally or 35 mg/wk orally Treatment: 10 mg/day orally or 70 mg/wk orally	Sept. 1995 (oral daily) Oct. 2000 (oral weekly)
Risedronate (Actonel®)	- Postmenopausal osteoporosis - Postmenopausal osteoporosis; prophylaxis - Osteoporosis, male - Osteoporosis due to corticosteroid - Osteoporosis due to corticosteroid; prophylaxis - Paget's disease	Prevention and treatment: 5 mg/day orally or 35 mg/wk orally	April 2000 (oral daily) May 2002 (oral weekly)
Ibandronate (Boniva®)	- Postmenopausal osteoporosis - Postmenopausal osteoporosis; prophylaxis	Prevention: 150 mg/mo orally Treatment: 2.5 mg/day orally or 150 mg/mo orally or 3 mg/IV every 3 mo	March 2005 (oral monthly) Jan. 2006 (IV every 3 mo)

* Table shows only dosages for prevention and treatment of postmenopausal osteoporosis

Source: The Food and Drug Administration. Retrieved November 15, 2010, from the World Wide Web: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm

2. Adherence to bisphosphonate therapy

Bisphosphonates have been shown to significantly reduce the risks of vertebral and

non-vertebral fractures in postmenopausal women with osteoporosis from numerous clinical trials (Black et al., 1996; Reginster et al., 2000; Stakkestad et al., 2008). Although their efficacy has been demonstrated after 1 to 3 years of continuous treatment in clinical trials, long-term use of bisphosphonates is required to achieve their optimal benefits. However, the therapeutic benefits of bisphosphonates are often compromised by patients' poor adherence in clinical practice. The main reasons for poor adherence to bisphosphonate therapy include difficulty in following the stringent dosing guidelines, adverse events or lack of apparent improvement in disease symptoms. Among them, the stringent dosing guidelines are the most significant reason that contributes to patients' poor adherence to bisphosphonates (Cramer & Silverman, 2006).

Properly taking a bisphosphonate is not as simple as taking many other oral medications. Patients have to be fasting overnight prior to taking a bisphosphonate, take with 8 oz of water alone, and stay upright and fasting for 30-60 minutes after taking a bisphosphonate. Any food or beverage other than water interferes with the bioavailability of bisphosphonates. Therefore, many patients find it is inconvenient to incorporate this complex dosing instruction into daily routines, consequently resulting in inadequate adherence to bisphosphonate therapy. A number of studies have assessed adherence to bisphosphonates and the consequences of poor adherence with bisphosphonate. The study results indicated that long-term use of bisphosphonates in clinical settings might still be sub-optimal (Cramer, Amonkar, Hebborn & Altman, 2005; Siris et al., 2006; Briesacher, Andrade, Yood & Kahler, 2007; Penning-van Beest, Erkens, Olson & Herings, 2008;

Gold, Trinh & Safi, 2009).

Poor adherence to bisphosphonate therapy has been reported in two retrospective studies using pharmaceutical and medical claims data from MedStat MarketScan databases. The mean medication possession ratio (MPR), defined as the ratio of the total number of days supply within the follow-up period to the length of follow-up, was used as the measure of adherence. Siris and colleagues evaluated adherence to bisphosphonate therapy in postmenopausal women. A total of 35,537 women aged 45 years and older with an index prescription claim for alendronate or risedronate between July 1, 1999 and December 31, 2001 were included in the study and followed up for 24 months. Patients with a MPR value ≥ 0.8 were defined as adherent. Over 24 months, only 43% of women had an MPR value ≥ 0.8. They conducted an additional analysis in a cohort subset of women with specified diagnosis of postmenopausal osteoporosis (n=6391). Similarly, 47% of patients were adherent to bisphosphonate therapy in this sub-cohort. Compared to non-adherent patients, adherent patients had significantly lower risks of vertebral and non-vertebral fractures in the overall cohort (HR for vertebral fractures= 0.6, $p<0.001$; HR for non-vertebral fractures= 0.8, $p<0.001$) (Siris et al., 2006). Briesacher and colleagues examined adherence to bisphosphonates over time from 2000 to 2004. The study included a total of 17,988 patients aged 40 years and older, with a diagnosis of osteoporosis and new to alendronate or risedronate. They found that adherence to bisphosphonates decreased gradually over time. After 1 year of follow-up, 43%, 23% and 34% of patients reached high adherence (MPR: 80%-100%), moderate adherence (MPR:

40%-79%), and low adherence (MPR: 0-39%) respectively. After 3 years of follow-up, the percentages of patients that achieved high and moderate adherence dropped to 31% and 17%, respectively, but the percentage of patients having low adherence increased to 52%. In addition, patients with a MPR value ≥ 80% had a non-significant, but reduced risk of fractures compared with patients with a MPR value between 0-19% (OR=0.75, p=0.1) (Briesacher et al, 2007).

Comparable results were seen in another retrospective study using data from PHARMO Record Linkage System (PHARMO RLS), which includes pharmacy and hospitalization data of more than two million residents of the Netherlands. The study population consisted of 8,822 new female alendronate or risedronate users aged 45 years and older. Adherence was measured by MPR over 90-day intervals. Patients with a MPR value < 80% were defined as non-adherent. Results showed a monotonically increasing trend in the percentages of patients with a MPR value < 80% over time. The percentage of non-adherent patients increased from 34% after 6 months of follow-up, to 42% after one year, 51% after two years and 60% after three years of follow up. Non-adherent patients were associated with 1.4 times significantly higher risks of fracture than adherent patients (95% CI: 1.04-1.91) (Penning-van Beest et al., 2008).

It has been suggested that reducing the frequency and complexity of dosing regimens may improve patient adherence to bisphophonate therapy. The development of weekly or monthly bisphosphonate dosing is an attempt to improve patient adherence. Carmer and

colleagues compared patient adherence to daily and weekly bisphosphonates using administrative claims data from 30 different healthcare plans in the U.S. Of the 2,741 osteoporotic postmenopausal women who were new to bisphosphonate treatment, 2,010 were prescribed daily alendronate or risedronate, and 731 were prescribed weekly alendronate. The mean MPR for the overall cohort was 61% in the 12-month follow-up period, and was significantly lower for once-daily users (58%) than for once-weekly users (69%) ($p \leq 0.0001$). Similarly, when adherence was evaluated in two age groups (> 65 years and ≤ 65 years), better adherence was seen in weekly bisphosphonate users in two age groups. In patients older than 65 years, the mean MPR was 55% for daily bisphosphonate while it was 63% for weekly bisphosphonate ($p=0.002$). In addition, younger patients (≤ 65 years) had better adherence to both dosing regimens than older patients (> 65 years). The mean MPRs were 60% for daily bisphosphonate and 72% for weekly bisphosphonate in younger patients. The study results suggest that although adherence to both dosing regimens is less than satisfactory, weekly bisphosphonate is associated with better adherence (Carmer et al., 2005).

However, the results from a recent study do not show superior patient adherence with monthly bisphosphonate dosing. Gold and colleagues compared patient adherence to weekly risedronate and monthly ibandronate over one year using the Intercontinental Marketing Services (IMS) longitudinal prescription database. Adherence was measured by MPR in three patient cohorts: overall patient sample (current users of weekly risedronate or monthly ibandronate), patients new to bisphosphonate therapy, and patients

new to bisphosphonate therapy after initial market availability for each drug. In the overall patient sample, 228,969 and 3,520 patients received risedronate and ibandronate, respectively. Adherence was significantly different between the two drugs in the overall sample (80% for risedronate vs. 75% for ibandronate, $p<0.0001$). However, there was no significant difference between adherence to the two drugs in patients new to bisphosphonate therapy. The mean MRP was 73% for risedronate while it was 74% for ibandronate ($p=0.6$). Similarly, no significant difference in adherence between products was shown in patients new to therapy after initial market availability (75% for risedronate vs. 74% for ibandronate, $p=0.09$) (Gold et al., 2009).

F. Summary of the literature

Although vascular calcification has been demonstrated to be a strong and independent predictor of cardiovascular morbidity and mortality in the general population (Sangiorgi et al., 1998; Wilson et al., 2001; Schmermund et al., 2003), the precise mechanisms of vascular calcification remain undefined. However, recent progress in basic molecular biology research has drawn attention to the findings, which suggest that vascular calcification shares certain similarities with bone formation and mineralization, and the pathological process of vascular calcification includes many key modulators of bone and mineral metabolism (Anderson, 1983; Giachelli et al., 1991; Waston et al., 1994; Abedin et al., 2004; Demer et al, 2008).

With the common underlying mechanisms mentioned above, the question regarding whether pharmacological agents that inhibit bone loss can also provide a beneficial impact on inhibiting vascular calcification is raised. Among the pharmacological agents that inhibit bone loss, bisphosphonates that inhibit bone loss through inhibiting osteoclast-mediated bone resorption are the most widely prescribed and used medication in the U.S. Several studies have reported that some bisphosphonates, i.e., alendronate, etidronate and ibandronate, inhibit experimentally induced artery calcification in rats (Price et al., 2001; Tamura et al., 2007; Saito et al., 2007). However, despite the consistent results shown in the animal studies, results from limited epidemiological studies in humans have been conflicting (Tankó et al., 2005; Luckish et al., 2008). Solid clinical evidence supporting the effect of bisphosphonates on inhibiting vascular calcification in humans is still lacking and there are still major gaps in knowledge.

First, none of the studies that examined the cardiovascular effects of bisphosphonates were conducted in a large population sample with a long follow-up period. Bisphosphonates have been shown to have high affinity to bone and are retained in bone for several months. Therefore, a longer follow-up time and larger sample size may be necessary to detect potential cardiovascular effects of bisphosphonates. Second, none of the epidemiological studies have included alendronate. Alendronate has been shown to significantly inhibit bone resorption and is one of the most prescribed bisphosphonates for osteoporosis. It might have significant impact on cardiovascular outcomes. Third, none of the cardiovascular studies have taken adherence to bisphosphonate therapy into

consideration. Adherence has been shown to have a significant impact on the anti-fracture efficacy of bisphosphonates, but it has been demonstrated that patient adherence to bisphosphonates is low. Therefore, patient adherence might impact the cardiovascular effects of bisphosphonates.

In summary, bisphosphonates have been shown to inhibit vascular calcification in animal studies. However, the results from human studies are inconsistent, and the effect of alendronate and the consequences of adherence to bisphosphonates on cardiovascular effects remain unknown. Further examination of the potential cardiovascular effects of bisphosphonates is needed to fill the gap of knowledge remaining from existing animal studies and human epidemiological studies and to stimulate future clinical trials in this area which may provide a proved cause-effect association between vascular calcification and the use of bisphosphonates.

CHAPTER III. CONCEPTUAL FRAMEWORK AND STUDY HYPOTHESES

A. Conceptual Framework

Vascular calcification is a frequent pathological condition in the aging population. Studies have shown that vascular calcification shares certain characteristics with bone formation and mineralization. Given the presently available evidence, bisphosphonates might be a potential pharmacological agent for inhibiting vascular calcification. In order to understand the effect of bisphosphonates on inhibition of vascular calcification, a conceptual framework that primarily rests on a series of animal studies (Price, Faus & Williamson, 1998; Price, Faus & Williamson, 2000; Bucay et al., 1998; Price, Faus & Williamson, 2001) was employed.

Matrix Gla protein (MGP) is a vitamin K-dependent calcium binding protein. It is expressed by a variety of tissues and cells, but predominantly found in bone and calcified cartilage. Studies have demonstrated that MGP functions as a calcification inhibitor *in vivo*. MGP gene knockout mice have been shown to develop extensive calcification of arteries and heart valves. To better understand this phenomenon, Price and colleagues induced arterial calcification in MGP-deficient rats by feeding them high doses of warfarin, a vitamin K antagonist and found the same pattern of vascular calcification seen in MGP gene knockout mice (Price, Faus & Williamson, 1998). In addition to indicating that vitamin K modification is critical to normal MGP function, they observed another

interesting phenomenon-warfarin failed to induce vascular calcification in rats fed a diet that prohibited bone development. This observation suggests that the susceptibility of rats to warfarin-induced vascular calcification is related to bone development.

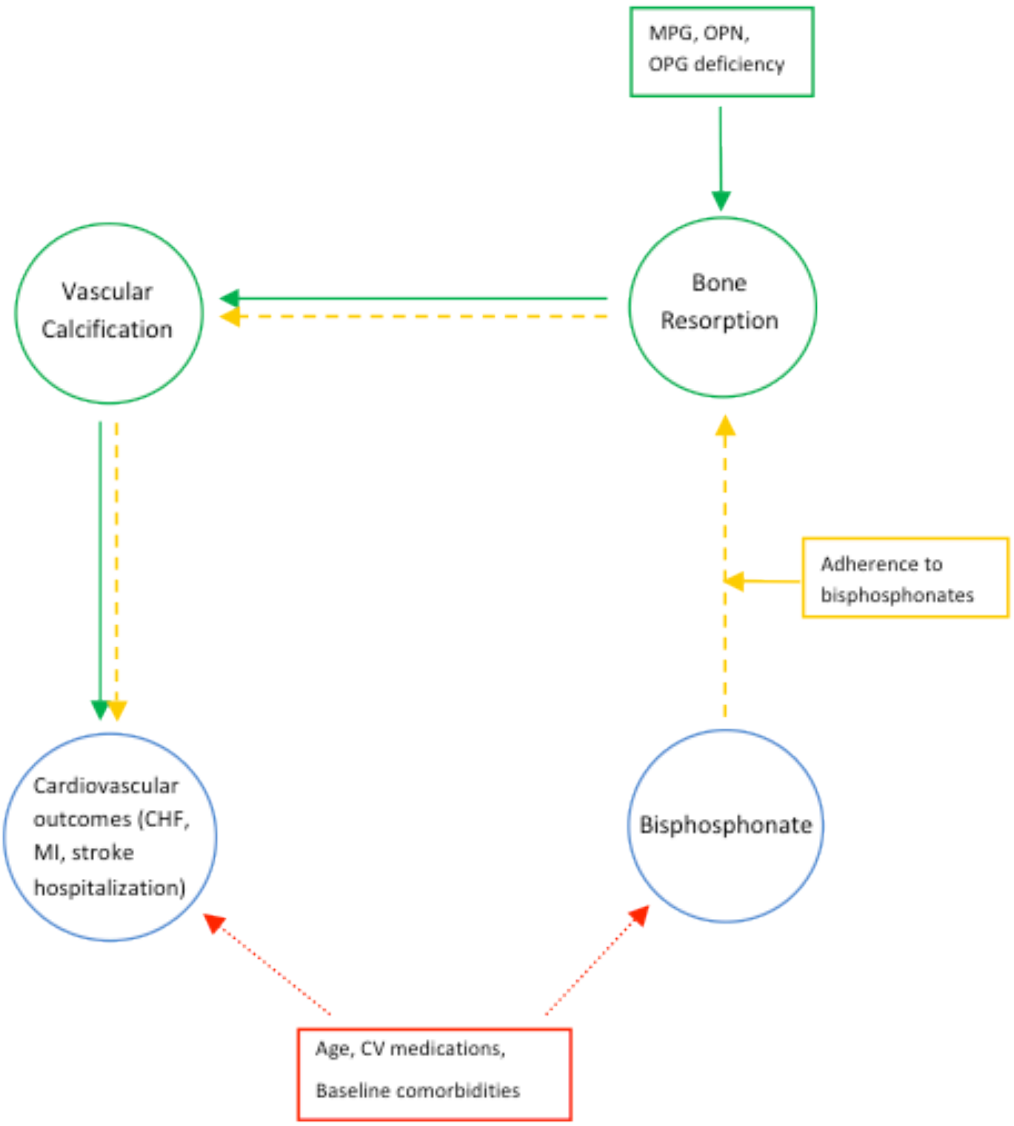
Based on their findings, Price and colleagues conducted a second study by concurrently treating rats with warfarin and vitamin D. Aggravated arterial calcification was observed in these rats. Because vitamin D is known as a potent bone resorption stimulant and warfarin induced arterial calcification by inhibiting MGP to act as a calcification inhibitor without altering serum calcium level, the synergistic effect of warfarin and vitamin D on vascular calcification may be best explained by the hypothesis that vascular calcification is related to bone resorption (Price, Faus & Williamson, 2000). This hypothesis is further supported by Bucay and colleagues (1998) in a study of osteoprotegerin (OPG) gene knockout mice. OPG is a secreted protein that inhibits the development of osteoclasts. They found that targeted deletion of OPG in mice resulted in severe and early-onset osteoporosis due to excessive bone resorption. Moreover, these OPG-deficient mice exhibited extensive calcification of aorta and renal arteries. These findings suggest a possible pathological association between bone resorption and vascular calcification.

Based on the hypothesis that vascular calcification is linked to bone resorption, Price and colleagues (2001) examined two of the nitrogen-containing bisphosphonates, alendronate and ibandronate at usual doses used for inhibiting bone resorption, as

inhibitors of vascular calcification. Both drugs completely inhibited warfarin-induced calcification in arteries and heart valves in rats. The study not only provides support for the hypothesis that vascular calcification may be linked to bone resorption, but also shows the potential ability of bisphosphonates for inhibiting vascular calcification.

Therefore, built on these studies, my study hypotheses are based on evidence that vascular calcification is associated with bone resorption. Bisphosphonates, which inhibit bone resorption, may inhibit vascular calcification and ultimately reduce cardiovascular morbidity and mortality. The conceptual framework is presented in Figure 5.

Figure 4: Conceptual Framework



Solid line: increase; Dash line: decrease

Variables in the red box: confounding factors

Variables in the green box/circle: variables can't be measured in the model

B. Study hypotheses

The study hypotheses were as follows:

1. Sub-study I hypotheses

H_{A-1}: Patients treated with bisphosphonates have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients who are not treated with bisphosphonates. The sub-hypotheses include:

H_{A-2}: After controlling for age, comorbid conditions and concomitant medications at baseline, patients treated with bisphosphonates have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients who are not treated with bisphosphonates.

2. Sub-study II hypotheses

Given that all of the bisphosphonates under study work through the same mechanism, the important confounders/covariates ($p<0.1$) determined from sub-study I were used in the hypotheses of both sub-studies II & III.

H_{B-1}: After controlling for age, comorbid conditions and concomitant medications at baseline, patients treated with **alendronate** have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients who are not treated with bisphosphonate.

H_{B-2}: After controlling for age, comorbid conditions and concomitant medications at baseline, patients treated with **ibandronate** have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients who are not treated with bisphosphonate.

H_{B-3}: After controlling for age, comorbid conditions and concomitant medications at baseline, patients treated with **risedronate** have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients who are not treated with bisphosphonate.

3. Sub-study III

H_{c-1}: Among patients treated with bisphosphonates, after controlling for age, comorbid conditions and concomitant medications at baseline, patients with higher adherence to bisphosphonate therapy have lower rates of a composite endpoint of CHF, MI or stroke hospitalization than patients with lower adherence to bisphosphonate therapy.

CHAPTER IV. METHODS

This study was conducted using a longitudinal retrospective observational cohort study design, incorporating pharmaceutical, inpatient, outpatient and enrollment claims data from the Thomson Reuters MarketScan® Databases.

A. Database description

The MarketScan® Databases include person-specific healthcare services records across inpatient, outpatient, prescription drug and carve-out services from a selection of large employers, health plans, and government and public organizations. The MarketScan® Databases links paid claims and encounter data to patient demographic and enrollment information across healthcare sites and types of providers. More than 500 million claim records are available in the databases over the past 10 years. These data represent the healthcare utilization of insured employees and their dependents. The insured employees include currently active employees, early retirees with Comprehensive Omnibus Budget Reconciliation Act (COBRA) continues and Medicare-eligible retirees having employ-provided Medicare Supplemental plans (Thomson Reuters, 2008). The MarketScan® Databases collectively refers to the following databases: Commercial Claims and Encounters Database, Medicare Supplemental and Coordination of Benefit (COB) Database, Health and Productivity Management Database, Benefit Plan Design Database, and Medicaid Database. For the purpose of this study, the Commercial Claims and Encounter Database and Medicare Supplemental and COB Database were combined for analysis. The combined database includes the Annual Enrollment Summary,

Enrollment Detail, Inpatient Admissions, Inpatient Services, Outpatient Services, and the Outpatient Pharmaceutical Claims Tables, which are briefly described below:

(1) *Annual Enrollment Summary and Detail Tables*: The Enrollment Tables comprise person level enrollment records with demographic and plan information on all enrollees in the MarketScan® Commercial Claims and Encounters and Medicare Supplemental and COB Databases. The Annual Enrollment Summary Table contains a single record per person per period of continuous enrollment, while the Enrollment Detail Table contains one record per person per month of enrollment for an individual enrollee. All medical and pharmaceutical claims can be linked to enrollment data by unique person-level enrollee identifiers (ENROLID).

(2) *Inpatient Admission Table*: The Inpatient Admission Table is composed of records that summarize information about a hospital admission. In addition to the summarized payment information for all services related to a hospital admission, the Inpatient Admission Table contains information for the principle and up to 14 secondary diagnoses and procedures found on the service records to make up the admission.

(3) *Inpatient Services Table*: The Inpatient Services Table contains physician and facility encounters and services associated with an inpatient admission. A Cases and Services Link (CASEID) identifier exists on both the Inpatient Admissions and the Inpatient Services Tables to link the individual service records that each admission comprises.

(4) *Outpatient Services Table*: The Outpatient Services Table includes information of paid claims and encounter data associated with services that were provided in a doctor's office, hospital outpatient facility, emergency room or other outpatient

facility. Each service record contains information on two diagnoses recorded with the International Classification of Diseases, Ninth Edition, Clinical Modifications (ICD-9-CM) classification system. One procedure recorded with ICD-9 procedure code, Current Procedural Terminology (CPT) codes or Health Care Financing Agency (HCFA) Common Procedure Coding System (HCPCS) codes, date and place of service, provider type and amounts paid by health plan and patients are available on the Outpatient Services Table as well.

(5) *Outpatient Pharmaceutical Claims Table*: The Outpatient Pharmaceutical Claims data are available for a large portion of the individuals represented in the inpatient and outpatient medical services tables. The outpatient pharmaceutical data are linked by ENROLID to the medical data. The Outpatient Pharmaceutical Claims Table contains the National Drug Code (NDC), fill date, days supply, and patient identifiers allowing for patient specific longitudinal tracking of medication refill patterns and changes in medications.

B. Study subjects and periods

The study subjects were drawn from enrollees in the combined MarketScan® Commercial Claims and Encounter Database and Medicare Supplemental and COB Database for the period from January 1, 2000 to December 31, 2007. Specific study period and subjects for each sub-study are described below.

1. Sub-studies I & II

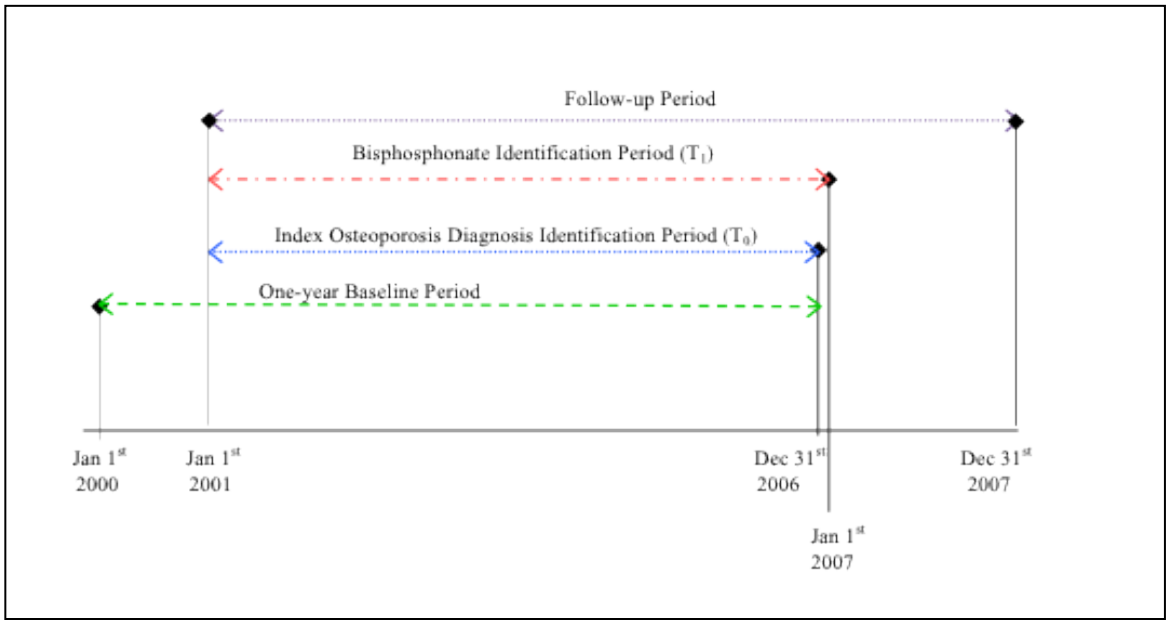
The purpose of sub-study I was to examine the overall effects of three bisphosphonates, alendronate, ibandronate and risedronate, on a composite endpoint of congestive heart failure (CHF), myocardial infarction (MI) or stroke hospitalization, the cardiovascular events that have been linked to vascular calcification. The purpose of sub-study II was to examine the individual effect of each bisphosphonate on a composite endpoint of CHF, MI or stroke hospitalization. The study period for both sub-studies I & II was from January 1, 2000 to December 31, 2007.

To be included in the study, the patient had to have either one inpatient claim or two outpatient claims on different dates within 12 months with primary diagnosis of osteoporosis between January 1, 2001 and December 31, 2006 (index osteoporosis diagnosis identification period, T_0) (Hebert et al., 1999). The first inpatient claim or the first outpatient claim with primary diagnosis of osteoporosis within T_0 was identified as the index osteoporosis diagnosis and the service date was defined as the index osteoporosis diagnosis date. A 12-month time period prior to the index osteoporosis diagnosis date (baseline period) was examined for baseline patient characteristics. The first prescription for a bisphosphonate filled between the index osteoporosis diagnosis date and January 1, 2007 (bisphosphonates identification period, T_1) was identified as the index prescription and the service date was defined as the index prescription date.

Patients who had received a prescription for a bisphosphonate during T_1 were defined

as bisphosphonate users and patients who had never received any prescription for a bisphosphonate during T_1 were defined as non-bisphosphonate users. All patients were followed from the index osteoporosis diagnosis date to the first cardiovascular event under investigation, end of enrollment, death or December 31, 2007, whichever came first. Figure 6 below outlines the study time period for sub-studies I & II.

Figure 5: Sub-studies I & II time periods



Patients who met the following criteria were included in the sub-studies I & II:

(1) Had an index diagnosis for osteoporosis

(2) Were female

(3) Aged 45 or older at the index osteoporosis diagnosis date

(4) Were continuously enrolled at least 12 months prior to the index osteoporosis diagnosis date

(5) Had no diagnosis of osteoporosis during the 12-month baseline period

(6) Had not taken any bisphosphonate during the 12-month baseline period

Patients who met the following criteria were excluded from sub-studies I & II:

(1) Had CHF, MI or stroke hospitalization during the 12-month baseline period

(2) Were diagnosed with cancer, Paget's disease, HIV infection, had a kidney transplant or had chronic kidney disease or were undergoing dialysis during the 12-month baseline period or follow-up period

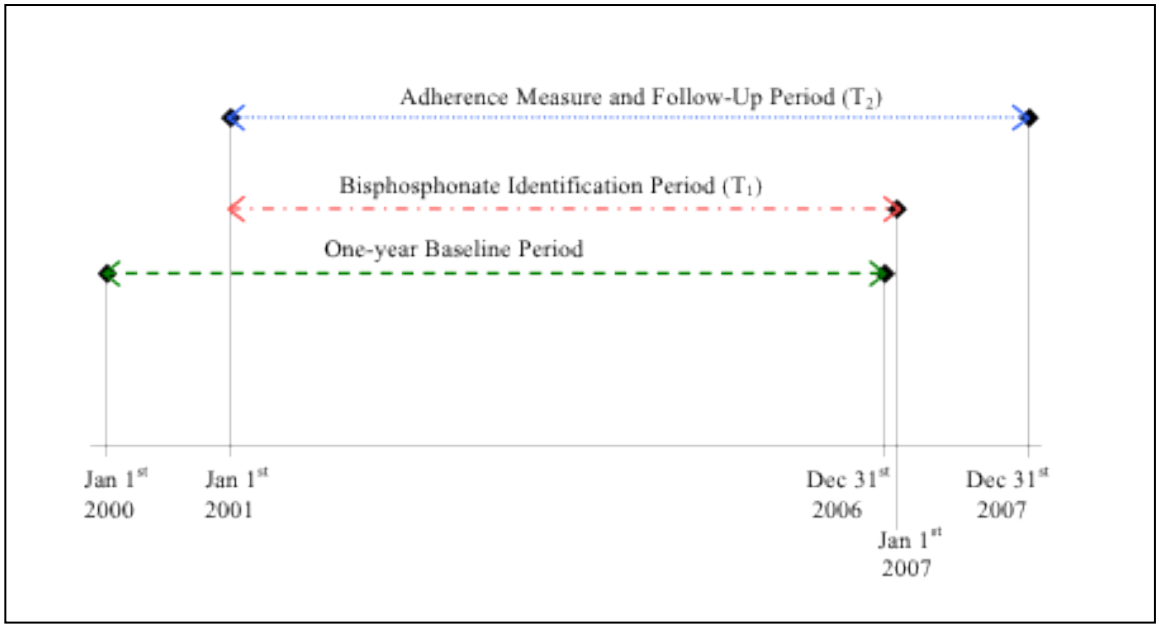
(3) Were having both bisphosphonates use and diagnosis for Paget's disease, hypercalcemia or bone malignancy during the 12-month baseline period.

2. Sub-study III

The objective of sub-study III was to examine the impact of adherence to bisphosphonate therapy on a composite endpoint of CHF, MI or stroke hospitalization, the cardiovascular events that have been linked to vascular calcification. The study period for sub-study III was from January 1, 2000 to December 31, 2007. The definitions of index osteoporosis diagnosis date, index prescription date and the 12-month baseline period were the same as sub-studies I & II. However, only patients who had a prescription filled for a bisphosphonate between the index osteoporosis diagnosis date and January 1, 2007 and met all other inclusion and exclusion criteria for sub-studies I & II were included in sub-study III. Patients with any outcome event prior to the index prescription date were excluded from the analysis. Patients were followed from the index

prescription date to the first cardiovascular events under investigation, end of enrollment, death or December 31, 2007, whichever came first. Adherence to bisphosphonates was measured for every patient in the period from the index prescription date to the end of follow-up (T_2). A sensitivity analysis was conducted on the following criterion: adherence to bisphosphonates was measured for every patient in the 12-month period after the index prescription date. Figure 7 below outlines the time period for sub-study III.

Figure 6: Sub-study III time period



C. Study variables

All variables in the study are summarized in Table 4 and operational definitions are provided following the table.

Table 4. Study variables

Variables	Type/Value	Source	Use
Bisphosphonate use	Dichotomous 1=yes; 0=no	Pharmacy claims	Independent variable in sub-study I
Bisohosphonate use	Categorical 0=no use; 1=alendronate; 2=ibandronate; 3=risedronate	Pharmacy claims	Independent variable in sub-study II
Adherence to bisphosphonates	Dichotomous 1=adherent (MPR in T_2 ≥80%, 70%, 60% or 50%); 0=non-adherent (MPR in T_2 <80%, 70%, 60% or 50%)	Pharmacy claims	Independent variable in sub-study III
A composite hospitalization for CHF, MI or stroke	Continuous (rate)	Inpatient claims	Dependent variable in sub-studies I, II & III
Age	Continuous (year)	Enrollment claims	Covariates in sub-studies I, II & III
Baseline atherosclerotic heart disease; CHF; Cerebral vascular accident /Transient ischemic attack; Peripheral vascular disease; Other cardiac diseases; Chronic obstructive pulmonary disease; GI diseases; Dysrhythmia; Diabetes; Anemia; Hypertension; Dyslipidemia	Dichotomous 1=yes; 0=no (for each of the comorbid condition)	Inpatient and outpatient claims	
Use of statins	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of non-statin lipid lowering agents	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of ACE inhibitors	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of ARBs	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of beta blockers	Dichotomous	Pharmacy	

	1=yes; 0=no	claims	
Use of calcium channel blockers	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of diuretics	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of oral anti-diabetic agents	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of insulin	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of estrogen/ hormone therapy	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of calcitonin	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of SERMs	Dichotomous 1=yes; 0=no	Pharmacy claims	
Use of teriparatide	Dichotomous 1=yes; 0=no	Pharmacy claims	

CHF: congestive heart failure, MI: myocardial infarction, GI disease: gastrointestinal disease, ACE inhibitor: angiotensin-converting enzyme inhibitor, ARB: angiotensin II receptor blocker, SERM: selective estrogen receptor modulator

Bisphosphonate use

Bisphosphonate use was the independent variable in sub-studies I & II. It was defined as receiving any prescription for bisphosphonates in T_1. Bisphosphonates available during the study period included alendronate, ibandronate, and risedronate. National Drug Codes (NDCs) were used to identify bisphosphonate prescriptions. A binary variable was created as follows for sub-study I: 0=No, 1=Yes. A categorical variable was created as follows for sub-study II: 0=No, 1=alendronate, 2=ibandronate, 3=risedronate. Patients who switched from one bisphosphonate to another were still considered as bisphosphonate users. Discontinuation of bisphosphonate use was defined as having a treatment gap greater than 30 days between two prescriptions refills (Steiner and Prochazka, 1997).

Adherence to bisphosphonates

Adherence to bisphosphonates was the independent variable in sub-study III. The International Society for Pharmacoeconomics and Outcomes Research (ISPOR) has reached consensus and defined medication adherence as "the extent to which a patient acts in accordance with the prescribed interval and dose of a dosing regimen" (Cramer et al., 2008). One of the most common methods to estimate medication adherence using retrospective databases is to calculate the medication possession ratio (MPR), which is operationalized as the number of days of medication supplied within the refill interval, divided by the number of days in refill interval (Steiner & Prochazka, 1997; Peterson et al., 2007). In this study, the ISPOR definition of medication adherence was adopted and MPR was used to measure adherence to bisphosphonates. The MPR of bisphosphonates in T_2 was calculated as the ratio of total days supply of bisphosphonate prescriptions in T_2 to total days of T_2. For the sensitivity analysis, MPR was calculated as the ratio of total days supply of bisphosphonate prescription in the 12-month period after index prescription date to 365 days. If the date of run-out-of the last bisphosphonate prescription exceeded the last date of follow-up period, the days supply of the last bisphosphonate prescription was truncated at the last date of follow-up period. A higher MPR indicates better adherence. Multiple cut-points of MPR (80%, 70%, 60%, and 50%) were used to assess the association between adherence and outcomes. A switch from one bisphosphonate to another was considered continued on bisphosphonate therapy. A binary variable indicating status of adherence to bisphosphonates was constructed as

follows: 1=Adherent (MPR ≥ 80%, 70%, 60%, or 50%), 0=Non-adherent (MPR < 80%, 70%, 60%, or 50%).

$$\text{MPR} = \frac{\text{total days supply of bisphosphonate prescription in } T_2}{\text{total days of } T_2} \times 100\%$$

Sensitivity analysis for MPR

$$MPR = \frac{\text{total days supply of bisphosphonate prescription in 12 months after index prescription date}}{365} \text{x} 100\%$$

A composite hospitalization of CHF, MI or stroke

Due to the limitation of the databases, vascular calcification could not be directly measured in the study. However, recent studies have indicated that CHF, MI and stroke are the solid cardiovascular events that have been linked to vascular calcification (Sangiorgi et al., 1998; Wilson et al., 2001; Schmermund et al., 2003; London et al., 2005). Hence, instead of using vascular calcification itself as the dependent variable, a composite endpoint of CHF, MI or stroke hospitalization was selected as the outcome measures for all three sub-studies. Hospitalization for CHF, MI or stroke was defined as having at least one inpatient claim with primary diagnosis for CHF, MI or stroke during the follow-up period. ICD-9-CM codes in Appendix 1 were used to identify CHF, MI and stroke hospitalizations.

Age

There is a known positive relationship between age and the likelihood of having cardiovascular diseases. Therefore, age (years) was included as the covariate for all-three sub-studies. It was defined as the patient's age at the index osteoporosis diagnosis date. For this study, age was calculated as the year of index osteoporosis diagnosis date minus the year of birth.

Baseline comorbidities

Baseline comorbidities may influence the risk of future cardiovascular outcomes. For this study, comorbidity data were the covariates for all three sub-studies and assessed using individual disease states related to the outcome, including atherosclerotic heart disease, CHF, cerebral vascular accident and transient ischemic attack, peripheral vascular disease, other cardiac diseases, chronic obstructive pulmonary disease (COPD), gastrointestinal (GI) diseases, dysrhythmia, diabetes, anemia, hypertension, and dyslipidemia (Kannel & McGee, 1979; O'Kelly et al., 2012; Stokes et al., 1989;). Patients with these disease conditions were identified using ICD-9-CM codes specified in Appendix 2. All inpatient and outpatient claims during the 12-month baseline period were examined for each patient and binary variables were created to depict the presence of these disease conditions at baseline (1=Yes; 0=No). To determine if a patient had a certain disease condition, the patient had to have either one inpatient claim or at least two outpatient claims on different dates within 12 months with an ICD-9-CM code for the diseases of interest (Hebert et al., 1999).

Concomitant drug therapy

Concomitant drug therapy may modify or confound the cardiovascular effect of bisphosphonates. Four types of concomitant drug therapy were evaluated in the study as covariates, including lipid-lowering drugs (LLDs), anti-hypertensive medications, anti-diabetic medications and other medications for treating osteoporosis. LLDs, anti-hypertensive medications and anti-diabetic medications have been found to decrease the risk of cardiovascular disease (Nissen et al., 2004; Pignone, Phillips & Mulrow, 2000; Tzoulaki et al., 2009; Holme et al., 2010). Other medications for treating osteoporosis may influence the effects of bisphosphonates on both bones and cardiovascular outcomes. The classes of LLDs included statins and non-statin LLDs (bile acid sequestrants, cholesterol absorption inhibitors, fibrates, niacin, and omega 3 fatty acids). The classes of anti-hypertensive medications included angiotensin converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers (ARBs), beta blockers, calcium channel blockers and diuretics. Anti-diabetic medications available during the study period were alpha-glucosidase inhibitors, biguanide, dipeptidyl peptidase 4 (DPP-4) inhibitors, incretin mimetic agents, insulin, meglitinides, amylin analogs, sulfonylureas, and thiazolidinedione. For this study, anti-diabetic medications were categorized into two groups, oral anti-diabetic agents and insulin. Except for bisphosphonates, other anti-osteoporositic medications evaluted were estrogen/hormone therapy, raloxifene, calcitonin and teriparatide. The use of concomitant medications was defined as receiving at least one prescription of these medications during the 12-month baseline period.

NDCs were used to identify any prescription for these medications. A binary variable was created as follows: 1=Yes; 0=No.

D. Statistical analysis

Statistical analyses including descriptive analyses, bivariate analyses and multivariable analyses were conducted in this study. All statistic analyses were conducted using SAS® version 9.1 (SAS Institute., Cary, NC). All p-values were two sided and $p<0.05$ was considered statistically significant.

1. Descriptive analysis

All study variables were analyzed descriptively to examine differences between bisphophonate users and non-users. Means, medians, standard deviations and percentiles were calculated for continuous variables. Numbers and percents were provided for dichotomous and polychotomous variables. These results were stratified by the use of bisphosphonates in sub-studies I & II and adherence to bisphosphonates in sub-study III. Bivariate comparisons of baseline characteristics between cohorts were conducted with t-tests or chi-square test.

2. Cox proportional hazards regression

Time-to-event analyses using Cox proportional hazards (PH) regression model was used to estimate the hazard of a composite endpoint of CHF, MI or stroke hospitalization,

comparing patients treated with bisphosphonates to patients not being treated with bisphosphonates in sub-studies I & II. In sub-study III, Cox PH regression model was used as well for examining the influence of patient adherence to bisphosphonates on the risk of a composite endpoint of CHF, MI or stroke hospitalization.

The Cox PH model models the hazard at time *t* for an individual with a given set of exploratory variables. The general form of Cox PH model is shown below (Cox, 1972):

$$h(t,X) = h_0 t \exp\left[\sum_{i=1}^{p} \beta_i X_i\right]$$

h_0(t)=baseline hazard

X=($X_1, X_2, ..., X_p$), a given set of explanatory variables

An important feature of Cox PH model assumption is that only the baseline hazard is a function of time. X_i is time-independent meaning that the value of any variable for a given subject does not change over time. However, it is possible to consider X_i to be time-dependent, which means that the value of X_i for a given subject changes over time. Accordingly, the Cox PH model can be extended to incorporate both time-independent and time-dependent variables. The extended Cox PH model is (Kleinbaum & Klein, 2005):

$$h(t,X(t)) = h_0(t)\exp[\sum_{i=1}^{p_1} \beta_i X_i + \sum_{j=1}^{p_2} \delta_j X_j(t)]$$

$X(t)=(X_1, X_2, ...,X_{p1}, X_1(t), X_2(t), ... X_{p2}(t))$,

$X_1, X_2, ...,X_{p1}$: time-independent explanatory variables

$X_1(t), X_2(t), \ldots X_{p2}(t)$: time-dependent explanatory variables

The formula for the hazard ratio at time *t* that derives from the extended Cox PH model is shown below.

$$HR(t) = \frac{h(t,X^*(t))}{h(t,X(t))} = \exp[\sum_{i=1}^{p_1} \beta_i (X^*_i - X_i) + \sum_{j=1}^{p_2} \beta_j (X^*_j(t) - X_j(t))]]$$

The independent variable in sub-study I was the overall use of bisphosphonates at time *t*. Because patients were alternately on and off drug during the follow-up, the use of bisphosphonate was considered to be time-dependent. This variable was defined to take on the value 0 or 1 at time *t* when the patient was off drug or on drug, respectively. Therefore, if a patient did not receive any bisphosphonate throughout the study, the value of this variable was 0 at all times. If a patient had received a bisphosphonate during follow-up, the value of this variable was 0 at the beginning of follow-up and continued to be 0 until the time when receiving a bisphosphonate. Then, the value of this variable changed to 1 and remained 1 until the next change on the status of bisphosphonate use. Similarly, the independent variable in sub-study II was the use of different bisphosphonates at time *t*. The value of this variable depended on the status of bisphosphonate use (on or off drug) and the type of bisphosphonates received. When a patient was off drug, this variable was defined to take on the value 0. When a patient received alendronate, ibandronate or risedronate, the value changed to 1, 2, or 3, respectively. Because it was possible that patients switched from one bisphosphonate to

another during the follow-up period, the value of this variable changed between 0 and 3 according to the bisphosphonate they received.

The specific model fitted for sub-study I is:

log(HR of a composite endpoint of CHF, MI or stroke hospitalization)=β_1*Δ(the overall use of bisphosphonates)+β_2*Δ(age)+β_3*Δ(baseline comorbidities)+β_4*Δ(use of LLDs, anti-hypertensive drugs, anti-diabetic drugs and drugs for treating osteoporosis)

The specific model fitted for sub-study II is:

log(HR of a composite endpoint of CHF, MI or stroke hospitalization)=β_1*Δ(the individual use of bisphosphonates)+β_2*Δ(age)+β_3*Δ(baseline comorbidities)+β_4*Δ(use of LLDs, anti-hypertensive drugs, anti-diabetic drugs and drugs for treating osteoporosis)

The specific model fitted for sub-study III is:

log(HR of a composite endpoint of CHF, MI or stroke hospitalization)=β_1*Δ(adherence to bisphosphonates)+β_2*Δ(age)+β_3*Δ(baseline comorbidities)+β_4*Δ(use of LLDs, anti-hypertensive drugs, anti-diabetic drugs and drugs for treating osteoporosis)

3. Immortal time bias adjustment

Immortal time is defined as a period of follow-up time, during which the study outcome cannot occur. It typically occurs in observational studies of drug effects, where initiation with a medication is established after cohort entry and throughout follow-up

period. Given the definition of exposure, a study subject is event-free and becomes "immortal" during the period between cohort entry and the first exposure to the drug under study (Suissa, 2007; Suissa 2008; Shariff, Cuerden, Jain, & Garg, 2008).

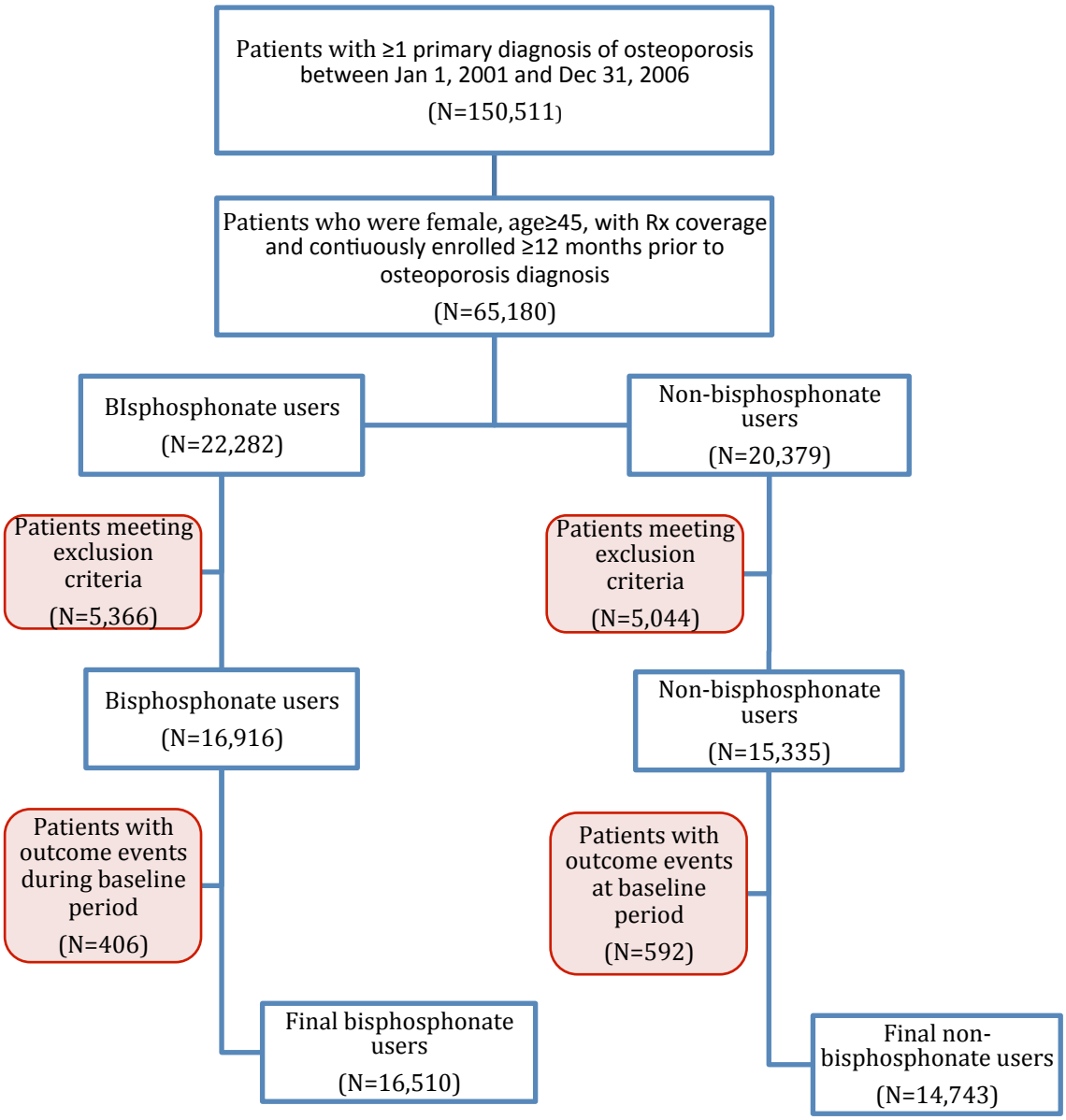
In this study, the exposure to bisphosphonate therapy was made over time after receiving osteoporosis diagnosis (cohort entry). To be able to receive a prescription for bisphosphonate, patients have to survive during the period between T_0 (cohort entry) and T_1 (the first prescription of bisphosphonates). As a result, this period is inevitably event-free for patients who have received bisphosphonate therapy. By defining these patients who have received bisphosphonate therapy as exposed, we would misclassify the period between T_0 and T_1 as exposed. To correct immortal time bias, a time-dependent definition of exposure was used. The period between T_0 and T_1 was classified as unexposed and the follow-up time with drug use after T_1 was classified as exposed (Liu, Weinhandl, Gilbertson, Collins, & St. Peter, 2012).

CHAPTER V. RESULTS

A. Data extraction and cohort construction

The MarketScan® databases were used to identify patients meeting the pre-specified inclusion and exclusion criteria. 150,511 patients with at least one primary diagnosis for osteoporosis between January 1, 2001 and December 31, 2006 were identified. Of these patients, 65,180 patients were female, age 45 years or older, with prescription drug coverage and continuously enrolled at least 12 months prior to the osteoporosis diagnosis date. After excluding patients who had received bisphosphonate use at baseline, filled their first bisphosphonate prescription after January 1, 2007 or had bisphosphonate days supply >180 or ≤0. 20,379 non-bisphosphonate users and 22,282 bisphosphonate users were eligible for analysis. After examining the study eligibility of each patient based on the predefined inclusion and exclusion criteria, a total of 31,253 patients, including 14,743 non-bisphosphonate users and 16,510 bisphosphonate users, were included in the final study cohort. Figure 8 shows how the final study cohort was constructed.

Figure 7: Cohort Development



B. Descriptive Statistics

1. Demographics

Bisphosphonate users were younger than non-bisphosphonate users. The average age at osteoporosis diagnosis was 66.4 years for bisphosphonate users and 68.6 years for non-bisphosphonate users. The age of bisphosphonate users at initiation of bisphosphonate treatment was also examined. There was an average time gap 3 months between osteoporosis diagnosis and bisphosphonate initiation. The average age at bisphosphonate initiation was 66.7 years. Descriptive statistics for the age of study patients are summarized in Table 5.

Table 5. Mean age of study patients

Cohort	N	Age (mean±SD)
Non-bisphosphonate user[a]	14743	68.56±12.5
Bisphosphonate user[a]	16510	66.39±11.11[c]
Bisphosphonate user[b]	16510	66.71±11.13[c]

a: Age at osteoporosis diagnosis
b: Age at treatment initiation
c: Statistically significant different from non-bisphosphonate users ($p<0.0001$)

2. Comorbidities and concomitant medication use

(i) Comorbidities and concomitant medication use in study patients at baseline

Hypertension was the most common comorbidity at baseline (12 months prior to osteoporosis diagnosis) in both patient cohorts (20.6% in non-bisphosphonate users vs. 18.9% in bisphosphonate users; $p<0.0001$), followed by dyslipidemia (10.3% in non-bisphosphonate users vs. 10.4% in bisphosphonate users, $p=0.88$). Other comorbidites with prevalence ≥ 5% in both cohorts included COPD and dysrhythmia. Except for dyslipidemia, the presence of all comorbidities examined was significantly higher in non-

bisphosphonate users than bisphosphonate users. The presence of comorbidities at baseline is summarized in Table 6.

Table 6. Comorbidities of study patients at baseline

Comorbidity	Non-Bisphosphonate user (N=14,743)		Bisphosphonate user (N=16,510)		P value
	n	%	n	%	
Atherosclerotic heart disease	701	4.8	587	3.6	<0.0001
CHF	245	1.7	179	1.1	<0.0001
CVA/TIA	408	2.8	351	2.1	0.0002
PVD	490	3.3	379	2.3	<0.0001
Other cardiac diseases	405	2.8	392	2.4	0.04
COPD	1098	7.5	1078	6.5	0.002
GI diseases	243	1.7	179	1.1	<0.0001
Liver disease	68	0.5	47	0.3	0.01
Dysrhythmia	1060	7.2	913	5.5	<0.0001
Diabetes	866	5.9	763	4.6	<0.0001
Anemia	627	4.3	507	3.1	<0.0001
Hypertension	3041	20.6	3113	18.9	<0.0001
Dyslipidemia	1523	10.3	1714	10.4	0.88

Abbreviation: CHF: congestive heart failure, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease

In general, the use of concomitant medications was less in bisphosphonate users than non-bisphosphonate users at baseline. Among the five classes of anti-hypertensive medications examined, a significantly smaller proportion of bisphosphonate users had received angiotensin receptor blockers (ARB), beta-blockers, and diuretics compared to non-bisphosphonate users. The use of anti-diabetic medications was also less common in bisphosphonate users. Compared to non-bisphosphonate users, more bisphosphonate users had received statins, but fewer had received non-statin lipid lowering agents. Among the 4 classes of osteoporosis medications examined, use of estrogen and hormones therapy was higher while selective estrogen receptor modulator (SERM),

teriparatide was lower in bisphosphonate users as compared to non-bisphosphonate users. The use of concomitant medications at baseline is summarized in Table 7.

Table 7. Use of concomitant medications at baseline

Medication	Non-BP user (N=14,743)		BP user (N=16,510)		P value
	n	%	n	%	
Anti-hypertensive medications					
ACEI	2015	13.7	2332	14.1	0.24
ARB	1488	10.1	1367	8.3	<0.0001
Beta-blocker	2656	18.0	2678	16.2	<0.0001
CCB	2053	13.9	2309	14.0	0.88
Diuretics	3493	23.7	3479	21.1	<0.0001
Lipid lowering agents					
Statins	3149	21.7	3895	23.6	<0.0001
Non-statin LLA	833	5.7	817	5.0	0.006
Anti-diabetic medications					
OAD	841	5.7	801	4.9	0.0007
Insulin	220	1.5	185	1.1	0.004
Other osteoporosis medications					
Estrogen/hormone therapy	2371	16.1	3414	20.7	<0.0001
Calcitonin	1268	8.6	1091	6.6	<0.0001
SERM	1558	10.6	1430	8.7	<0.0001
Teriparatide	151	1.0	74	0.5	<0.0001

Abbreviation: BP: bisphosphonate, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CCB: calcium channel blocker, LLA: lipid lowering agent, OAD: oral anti-diabetic medication, SERM: selective estrogen receptor modulator

(ii) Comorbidities and concomitant medication use of bisphosphonate users at treatment initiation

Because of the time-dependent nature of treatment, the treatment of some bisphosphonate users did not initiate at the beginning of follow-up period. For these patients, their clinical characteristics may change during the time window from the beginning of follow-up to treatment initiation. The longer the time window, the higher the likelihood patients' characteristics will change. Therefore, it is not appropriate to only

measure the clinical characteristics of bisphosphonate users at either baseline or at treatment initiation. In this study, the clinical characteristics of bisphosphonate users was assessed and reported at both baseline and treatment initiation.

A higher percentage of bisphosphonate users had presence of comorbidities at treatment initiation as compared to non-bisphosphonate users. Except for estrogen and hormone therapy, the use of concomitant medications was also higher at treatment initiation in bisphosphonate users than non-bisphosphonate users at baseline. By treatment initiation, bisphosphonate users had accumulated additional comorbidities and their comorbidity and concomitant medication profiles shifted to be more similar to non-bisphosphonate users. The presence of comorbidities and the use of concomitant medications at treatment initiation are presented in Appendix 3 and 4.

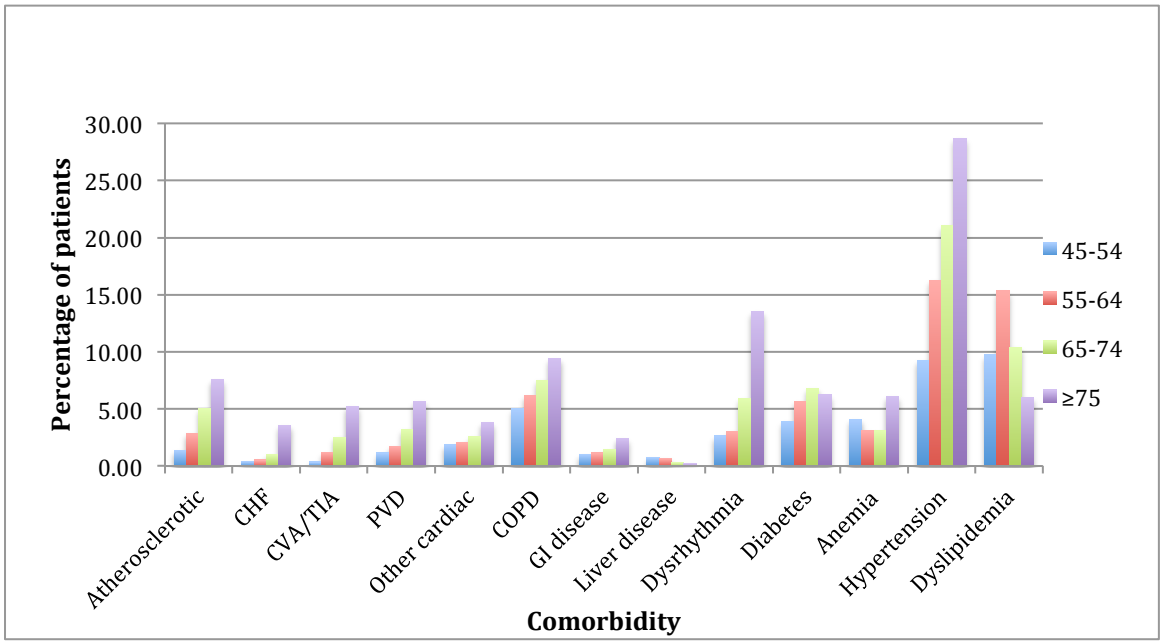
(iii) Comorbidities and concomitant medication use in study patients stratified by age

The examination of comorbidities and the use of concomitant medications were further stratified by age (45-54, 55-64, 65-74, ≥75 years). Comorbidities and concomitant medication use at baseline and at treatment initiation were reported for non-bisphosphonate users and bisphosphonate users, respectively.

For most of the comorbidities examined, their presence increased with age for both bisphosphonate users and non-bisphosphonate users. On the contrary, the presence of

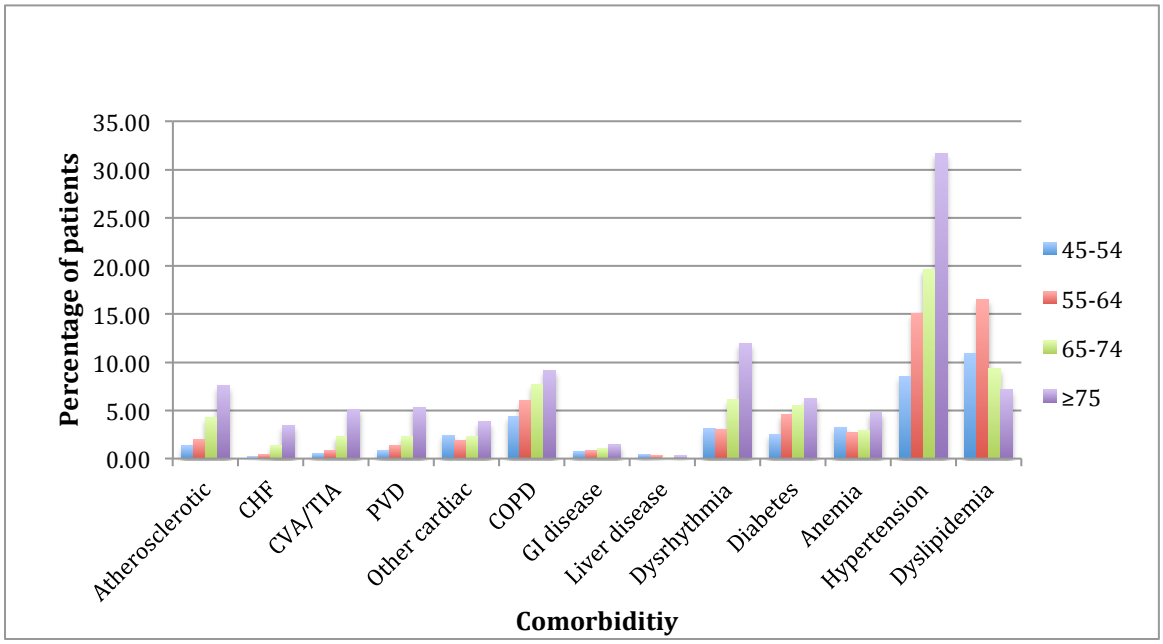
liver disease decreased with age in both patient cohorts. The presence of dyslipidemia was highest among patients age 55-64 years in both patient cohorts (Figures 9 and 10). Compared to non-bisphosphonate users in each of the age categories, the presence of comorbidities tended to be presence less prevalent among bisphosphonate users, but there were some exceptions. Among patients age 75 years and older, the presence of hypertension and dyslipidemia was significantly higher in bisphosphonate users than non-bisphosphonate users. The presence of comorbidites by age categories is presented in Table 8.

Figure 8. Comorbidities of non-bisphosphonate users at baseline stratified by age



Abbreviation: Atherosclerotic: atherosclerotic heart disease, CHF: congestive heart failure, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, Other cardiac: other cardiac disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease

Figure 9. Comorbidities of bisphosphonate users at treatment initiation stratified by age



Abbreviation: Atherosclerotic: atherosclerotic heart disease, CHF: congestive heart failure, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, Other cardiac: other cardiac disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease

Table 8. Comorbidities of study patients stratified by age

Comorbidity[§]	Age 45-54 years					Age 55-64 years				
	Non-BSP user (N=1936)		BSP user (N=2368)		P value	Non-BSP user (N=4507)		BSP user (N=5623)		P value
	n	%	n	%		n	%	n	%	
AHD	27	1.4	31	1.3	0.81	129	2.9	114	2.0	0.006
CHF	8	0.4	4	0.2	0.13	24	0.5	25	0.4	0.53
CVA/TIA	7	0.4	13	0.6	0.37	55	1.2	50	0.9	0.10
PVD	22	1.1	20	0.8	0.33	79	1.8	79	1.4	0.16
Other cardiac diseases	36	1.9	58	2.5	0.19	94	2.1	108	1.9	0.56
COPD	97	5.0	103	4.4	0.31	280	6.2	341	6.1	0.76
GI diseases	20	1.0	18	0.8	0.34	52	1.2	48	0.9	0.13
Liver disease	15	0.8	11	0.5	0.19	31	0.7	19	0.3	0.01
Dysrhythmia	51	2.6	73	3.1	0.38	137	3.0	169	3.0	0.92
Diabetes	76	3.9	59	2.5	0.007	255	5.7	256	4.6	0.01
Anemia	79	4.1	77	3.3	0.15	141	3.1	152	2.7	0.20
Hypertension	179	9.3	203	8.6	0.44	733	16.3	849	15.1	0.11
Dyslipidemia	189	9.8	259	10.9	0.21	692	15.4	928	16.5	0.12
Comorbidity	**Age 65-74 years**					**Age ≥ 75 years**				
	Non-BSP user (N=3306)		BSP user (N=4003)		P value	Non-BSP user (N=4994)		BSP user (N=4516)		P value
	n	%	n	%		n	%	n	%	
AHD	166	5.0	171	4.3	0.13	379	7.6	342	7.6	0.98
CHF	34	1.0	54	1.4	0.21	179	3.6	153	3.4	0.60
CVA/TIA	84	2.5	91	2.3	0.46	262	5.3	232	5.1	0.81
PVD	106	3.2	90	2.3	0.01	283	5.7	239	5.3	0.42
Other cardiac diseases	85	2.6	91	2.3	0.41	190	3.8	173	3.8	0.95
COPD	249	7.5	307	7.7	0.83	472	9.5	413	9.2	0.61
GI diseases	49	1.5	43	1.1	0.12	122	2.4	65	1.4	0.0004
Liver disease	11	0.3	6	0.2	0.11	11	0.2	13	0.3	0.51
Dysrhythmia	196	5.9	244	6.1	0.77	676	13.5	538	11.9	0.02
Diabetes	224	6.8	222	5.6	0.03	311	6.2	280	6.2	0.96
Anemia	102	3.1	118	3.0	0.73	305	6.1	217	4.8	0.005
Hypertension	696	21.1	786	19.6	0.13	1433	28.7	1429	31.6	0.002
Dyslipidemia	343	10.4	375	9.4	0.15	299	6.0	324	7.2S	0.02

Abbreviation: BSP: bisphosphonate, AHD: atherosclerotic heart disease, CHF: congestive heart failure, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease

[§]Comorbidities were examined at baseline and at treatment initiation for non-bisphosphonate users and bisphosphonate users, respectively.

The overall pattern of concomitant medication use was similar for both bisphosphonate users and non-bisphosphonate users. Increasing use of anti-hypertensive medications, calcitonin and teriparatide with age was observed in both cohorts. For both bisphosphonate users and non-bisphosphonate users, the use of non-statin lipid lowering agents (LLAs), insulin and selective estrogen receptor modulators (SERMs) increased with age among patients younger than 75 years, while the usage dropped in patients age 75 years and older. When making comparisons between bisphosphonate users and non-bisphosphonate users in different age categories, different use patterns were observed for some medications. Among patients younger than 65 years, a higher percentage of non-bisphosphonate users had received anti-hypertensive medications, whereas among patients age 65 years and older, the use of anti-hypertensive medications was higher in bisphosphonate users. The use of statins was slightly higher in non-bisphosphonate users among patients age 45-54 years compared to bisphosphonate users. However, among patients age 55 years and older, the use of statins was significantly higher in bisphosphonate users than non-bisphosphonate users. The use of concomitant medications by age categories is summarized in Table 9.

Table 9. Use of concomitant medications in study patients stratified by age

Medication[§]	Age 45-54 years					Age 55-64 years				
	Non-BSP user (N=1936)		BSP user (N=2368)		P value	Non-BSP user (N=4507)		BSP user (N=5623)		P value
	n	%	n	%		n	%	n	%	
Anti-hypertensive medication										
ACEI	132	6.8	138	5.8	0.18	490	10.9	603	10.7	0.81
ARB	83	4.3	84	3.6	0.21	378	8.4	366	6.5	0.0003
Beta-blocker	153	7.9	198	8.4	0.58	603	13.4	626	11.1	0.0006
CCB	116	6.0	123	5.2	0.26	366	8.1	468	8.3	0.71
Diuretics	237	12.2	242	10.2	0.04	846	18.8	934	16.6	0.005
Lipid lowering agent										
Statins	212	11.0	250	10.6	0.68	873	19.4	1195	21.3	0.02
Non-statin										
LLA	66	3.4	69	2.9	0.35	280	6.2	287	5.1	0.02
Anti-diabetic medication										
OAD	67	3.5	65	2.7	0.18	238	5.3	230	4.1	0.005
Insulin	23	1.2	23	1.0	0.49	60	1.3	63	1.1	0.34
Other osteoporosis medication										
EHT	368	19.0	537	22.7	0.003	996	22.1	1367	24.3	0.009
Calcitonin	56	2.9	67	2.8	0.90	228	5.1	264	4.7	0.40
SERM	87	4.5	122	5.2	0.32	449	10.0	556	9.9	0.90
Teriparatide	13	0.7	9	0.4	0.18	45	1.0	40	0.7	0.12
Medication	**Age 65-74 years**					**Age ≥ 75 years**				
	Non-BSP user (N=3306)		**BSP user (N=4003)**		**P value**	**Non-BSP user (N=4994)**		**BSP user (N=4516)**		**P value**
	n	**%**	**n**	**%**		**n**	**%**	**n**	**%**	
Anti-hypertensive medication										
ACEI	452	13.7	646	16.1	0.003	941	18.8	1023	22.7	<0.0001
ARB	366	11.1	387	9.7	0.05	661	13.2	621	13.8	0.46
Beta-blocker	645	19.5	772	19.3	0.81	1255	25.1	1200	26.6	0.11
CCB	508	15.4	647	16.2	0.35	1063	21.3	1124	24.9	<0.0001
Diuretics	784	23.7	962	24.0	0.75	1626	32.6	1518	33.6	0.27
Lipid lowering agent										
Statins	916	27.7	1229	30.7	0.005	1148	23.0	1424	31.5	<0.0001
Non-statin										
LLA	235	7.1	265	6.6	0.41	252	5.1	272	6.0	0.04
Anti-diabetic medication										
OAD	228	6.9	248	6.2	0.23	308	6.2	283	6.3	0.84
Insulin	57	1.7	54	1.4	0.19	80	1.6	56	1.2	0.14
Other osteoporosis medication										
EHT	549	16.6	793	19.8	0.0004	458	9.2	490	10.9	0.006
Calcitonin	304	9.2	344	8.6	0.37	680	13.6	509	11.3	0.0006
SERM	474	14.3	499	12.5	0.02	548	11.0	457	10.1	0.18
Teriparatide	34	1.0	42	1.1	0.93	59	1.2	60	1.3	0.52

Abbreviation: BSP: bisphosphonate, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CCB: calcium channel blocker, LLA: lipid lowering agent, OAD: oral anti-diabetic medication, EHT: estrogen/hormone therapy, SERM: selective estrogen receptor modulator
[§]Medication use was examined at baseline for non-bisphosphonate users and at treatment initiation for bisphosphonate users.

3. Index bisphosphonate and outcome events associated with each bisphosphonate

Alendronate was the most common index bisphosphonate received by the study bisphosphonate users. 57.4% of the bisphosphonate users received alendronate, and 33.0% received risedronate. Ibandronate was the least common index bisphosphonate received by 9.6% of the bisphosphonate users. Index bisphosphonate use and the number of outcome events associated with each bisphosphonate is summarized in Table 10.

Table 10. Index bisphosphonate use and associated outcome events[*]

Bisphosphonate	Number of patients (N=16,510)		Number of events
	n	%	n
Alendronate	9478	57.4	221
Ibandronate	1580	9.6	43
Risedronate	5452	33.0	142

*Outcome event was defined as the composite endpoint of CHF, MI or stroke hospitalization, which was defined as having at least one inpatient claim with primary diagnosis for CHF, MI or stroke during the follow-up period.

4. Follow-up time and time to treatment

Non-bisphoshonate users had shorter mean follow-up time than bisphosphonate users. The mean follow-up time was 735 days for non-bisphosphonate users and 939 days for bisphosphonate users. In both patient cohorts, younger patients had longer follow-up time compared to older patients. 341 bisphosphonate users had an outcome event and were censored before treatment initiation. Of the remaining bisphosphonate users, there was an average gap of 121 days between osteoporosis diagnosis and treatment initiation. The

descriptive statistics for follow-up time and time to treatment are summarized in Tables 11 and 12, respectively.

Table 11. Follow-up time of study patients

Patient cohorts	N	Length of follow-up, days					
		Mean ± SD	Min	25 percentile	50 percentile	75 percentile	Max
Non-BSP user	14743	735±488	1	392	636	1001	2555
45-54 years	1936	792±554	4	400	655	1072	2547
55-64 years	4507	753±485	1	410	652	1023	2548
65-74 years	3306	769±481	2	427	690	1041	2539
≥75 years	4994	673±462	2	342	587	922	2555
BSP user	16150	939±552					
45-54 years	2368	967±588	1	522	855	1293	2555
55-64 years	5623	956±556	4	522	861	1331	2545
65-74 years	4003	934±555	3	526	860	1314	2552
≥75 years	4516	906±518	1	514	847	1307	2555

Abbreviation: BSP: bisphosphonate

Table 12. Time to treatment of bisphosphonate users

Bisphosphonate users	N	Time to treatment, days					
		Mean ± SD	Min	25 percentile	50 percentile	75 percentile	Max
Overall	16169	121±226	0	16	38	100	2101
Age category							
45-54 years	2338	95±177	0	15	35	81	1728
55-64 years	5521	116±219	0	16	38	94	2067
65-74 years	3889	125±233	0	17	39	101	2101
≥75 years	4421	137±247	0	16	41	121	2075

C. Analysis of Sub-Study I

1. Analysis of hypothesis H_{A-1}

Hypothesis H_{A-1} evaluated the crude relationship between the use of bisphosphonates and the risk of the composite endpoint of CHF, MI or stroke hospitalization. The unadjusted analysis showed that compared to non-bisphosphonate users, bisphosphonate

users had a 36% lower risk of the outcome (Hazard Ratio (HR)=0.64, 95% Confidence Interval (CI)=0.58-0.71, p<0.0001). The results from the unadjusted model are presented in Table 13.

Table 13. Unadjusted model for hypothesis H_{A-1}

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use				
Yes	-0.44	0.06	0.64 (0.58, 0.71)	<0.0001
No *(reference)*				

2. Analysis of hypothesis H_{A-2}

Hypothesis H_{A-2} examined the association between bisphosphonate use and the risk of being hospitalized for CHF, MI or stroke after adjusting for age, baseline comorbidities and baseline concomitant medications. The initial assessment included a model that adjusted for all of the covariates. The results of the fully adjusted model indicated that hazard ratio was shifted toward the null compared to the unadjusted model, but the results were still statistically significant. After adjustment, bisphoshonate users had a 26% lower risk of the composite outcome than non-bisphosphonate users (HR=0.74, 95% CI=0.66-083, p<0.0001). The fully adjusted model also indicated that several covariates, including baseline cardiovascular comorbidities, use of statins and use of anti-hypertensive medications significantly confounded the relationship between bisphosphonate use and the outcome. The results of the fully adjusted model are presented in Table 14.

Table 14. Full model, adjusted for all covariates for hypothesis H_{A-2}

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.30	0.06	0.74 (0.66, 0.83)	<0.0001
Age	0.06	0.002	1.07 (1.06, 1.07)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.66	0.09	1.94 (1.64, 2.29)	<0.0001
PVD	0.24	0.09	1.28 (1.06, 1.54)	0.009
Other cardiac disease	0.14	0.11	1.15 (0.94, 1.42)	0.17
COPD	0.63	0.07	1.89 (1.65, 2.15)	<0.0001
GI disease	0.08	0.16	1.08 (0.79, 1.48)	0.63
Liver disease	-0.29	0.47	0.75 (0.30, 1.90)	0.55
Dysrhythmia	0.54	0.06	1.72 (1.52, 1.96)	<0.0001
DM	0.13	0.12	1.14 (0.91, 1.43)	0.25
Anemia	0.25	0.09	1.29 (1.07, 1.55)	0.008
HTN	-0.02	0.06	0.98 (0.88, 1.10)	0.73
Dyslipidemia	-0.10	0.08	0.91 (0.77, 1.07)	0.26
Baseline LLA use *(referent: no use)*				
Statins	0.25	0.05	1.29 (1.16, 1.43)	<0.0001
Non-statin LLA	0.24	0.09	1.27 (1.06, 1.52)	0.01
Baseline anti-hypertensive medication use *(referent: no use)*				
ACEI	0.28	0.06	1.33 (1.18, 1.49)	<0.0001
ARB	0.09	0.07	1.09 (0.95, 1.25)	0.20
Beta blocker	0.29	0.05	1.34 (1.20, 1.49)	<0.0001
CCB	0.23	0.06	1.25 (1.12, 1.40)	<0.0001
Diuretics	0.28	0.05	1.32 (1.19, 1.47)	<0.0001
Baseline anti-diabetic medication use *(referent: no use)*				
Insulin	0.24	0.12	1.27 (1.00, 1.62)	0.05
OAD	0.30	0.17	1.35 (0.98, 1.88)	0.07
Baseline osteoporosis medication use *(referent: no use)*				
EHT	0.008	0.07	1.01 (0.89, 1.15)	0.90
Calcitonin	0.12	0.07	1.13 (0.99, 1.29)	0.07
SERM	-0.18	0.08	0.84 (0.72, 0.98)	0.03
Teriparatide	-0.25	0.25	0.78 (0.48, 1.28)	0.32

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension, LLA: lipid lowering agent, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CCB: calcium channel blocker, LLA: lipid lowering agent, OAD: oral anti-diabetic medication, EHT: estrogen/hormone therapy, SERM: selective estrogen receptor modulator

The results from the fully adjusted model suggested that concomitant medications resulted in model over adjustment. Therefore, two sensitivity analyses were conducted to examine if the results were sensitive to different sets of covariates. One sensitivity analysis was adjusted for age and comorbidities (sensitivity analysis 1), and the other was adjusted for age and concomitant medications (sensitivity analysis 2). The results of sensitivity analysis 1 showed that bisphosphonate user was associated a 24% lower risk of the composite outcome than non-bisphosphonate user (HR=0.76, 95% CI=0.68-0.85, $p<0.0001$) (Table 15). The results of sensitivity analysis 2 suggested that bisphosphonate user was associated with a 29% decreased risk of the composite outcome compared to non-bisphosphonate user (HR=0.71, 95% CI=0.64-0.80, $p<0.0001$) (Table 16). Both sensitivity analyses showed similar results to the full model indicating that concomitant medication use did not provide additional information when added to comorbidities in explaining the relationship between bisphosphonate use and the risk of outcome. Therefore, for the rest of the analyses, models were adjusted only for comorbidities.

Table 15. Sensitivity analysis 1: adjusted for age and baseline comorbidities

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.27	0.06	0.76 (0.68, 0.85)	<0.0001
Age	0.07	0.002	1.07 (1.07, 1.08)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.73	0.09	2.07 (1.74, 2.45)	<0.0001
PVD	0.25	0.10	1.28 (1.06, 1.55)	0.01
Other cardiac disease	0.20	0.11	1.22 (0.99, 1.50)	0.06
COPD	0.67	0.07	1.95 (1.71, 2.22)	<0.0001
GI disease	0.009	0.16	1.01 (0.73, 1.39)	0.96
Liver disease	-0.35	0.49	0.70 (0.27, 1.85)	0.48
Dysrhythmia	0.67	0.06	1.95 (1.72, 2.21)	<0.0001
DM	0.59	0.08	1.81 (1.55, 2.11)	<0.0001
Anemia	0.26	0.10	1.29 (1.07, 1.56)	0.008
HTN	0.24	0.05	1.28 (1.15, 1.41)	<0.0001
Dyslipidemia	0.02	0.08	1.02 (0.87, 1.20)	0.81

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension

Table 16. Sensitivity analysis 2: adjusted for age and concomitant medications

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.34	0.06	0.71 (0.64, 0.80)	<0.0001
Age	0.07	0.002	1.07 (1.07, 1.08)	<0.0001
Baseline LLA use *(referent: no use)*				
Statins	0.24	0.05	1.27 (1.15, 1.40)	<0.0001
Non-statin LLA	0.25	0.09	1.28 (1.07, 1.53)	0.007
Baseline anti-hypertensive medication use *(referent: no use)*				
ACEI	0.31	0.06	1.36 (1.21, 1.52)	<0.0001
ARB	0.11	0.07	1.12 (0.98, 1.28)	0.11
Beta blocker	0.39	0.05	1.48 (1.34, 1.64)	<0.0001
CCB	0.28	0.06	1.33 (1.19, 1.48)	<0.0001
Diuretics	0.32	0.05	1.38 (1.24, 1.53)	<0.0001
Baseline anti-diabetic medication use *(referent: no use)*				
Insulin	0.41	0.16	1.51 (1.10, 2.08)	0.01
OAD	0.29	0.09	1.33 (1.12, 1.59)	0.001
Baseline osteoporosis medication use *(referent: no use)*				
EHT	-0.004	0.07	1.00 (0.88, 1.13)	0.95
Calcitonin	0.15	0.07	1.16 (1.01, 1.33)	0.03
SERM	-0.23	0.08	0.79 (0.68, 0.93)	0.004
Teriparatide	-0.17	0.25	0.84 (0.52, 1.38)	0.50

Abbreviation: LLA: lipid lowering agent, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CCB: calcium channel blocker, LLA: lipid lowering agent, OAD: oral anti-diabetic medication, EHT: estrogen/hormone therapy, SERM: selective estrogen receptor modulator

3. Analysis of hypothesis H_{A-1} stratified by age

The analysis for hypothesis H_{A-1} was further stratified by age categories to examine if the relationship between bisphosphonate use and the risk of the composite outcome differed in different age categories. The crude stratified model indicated that the risk of the composite outcome was lower in bisphosphonate users compared to non-bisphosphonate users in all of the age categories. The greatest difference in the risk of the composite outcomes between bisphosphonate users and non-bisphosphonate users was seen in patients age 45-54 years (HR=0.48, 95% CI=0.24-0.95, p=0.04), and the smallest

difference was seen in patient age 65-74 years (HR=0.95, 95% CI=0.77-1.18, p=0.64). The results of crude stratified model are presented in Table 17.

Table 17. Unadjusted model for hypothesis H_{A-1} stratified by age

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Age 45-54 years	-0.73	0.35	0.48 (0.24, 0.95)	0.04
Age 55-64 years	-0.66	0.17	0.52 (0.37, 0.72)	<0.0001
Age 65-74 years	-0.05	0.11	0.95 (0.77, 1.18)	0.64
Age ≥ 75 years	-0.41	0.07	0.66 (0.58, 0.76)	<0.0001

4. Analysis of hypothesis H_{A-2} stratified by age

The analysis for hypothesis H_{A-2} was also stratified by age. The stratified model showed that after adjustment for age and baseline comorbidities, bisphosphonate users had a 45%, 42% and 30% lower risk of the composite outcome than non-bisphosphonate users among patients age 45-54, 55-64 and ≥75 years, respectively. However, among patients age 65-74 years, there was no difference between bisphosphonate users and non-bisphosphonate users in the risk of the composite outcome after adjusting for age and baseline comorbidities. Several significant covariates had positive parameter estimates indicating that they were associated with an increased risk of the composite outcome compared to the reference group when all other variables were held constant. The models showed baseline cerebral vascular accident or transient ischemic attack (CVA/TIA), COPD, diabetes and hypertension were associated with significantly higher risk of the composite outcome in all four age categories. The results of the fully adjusted model stratified by age are presented in Table 18 to Table 21.

Table 18. Adjusted model for hypothesis H_{A-2} stratified by age (45-54 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.61	0.34	0.55 (0.28, 1.07)	0.08
Baseline comorbidity *(referent: no history)*				
CVA/TIA	2.42	0.68	11.21 (2.98, 42.13)	0.0003
PVD	1.08	0.64	2.94 (0.84, 10.34)	0.09
Other cardiac disease	-1.35	0.91	0.26 (0.04, 1.54)	0.14
COPD	2.12	0.30	8.37 (4.68, 14.97)	<0.0001
GI disease	-0.04	0.63	0.96 (0.28, 3.29)	0.95
Liver disease	0.05	1.29	1.05 (0.08, 13.17)	0.97
Dysrhythmia	0.76	0.43	2.14 (0.92, 5.01)	0.08
DM	1.37	0.37	3.93 (1.89, 8.18)	0.0002
Anemia	0.76	0.52	2.14 (0.78, 5.92)	0.14
HTN	0.80	0.35	2.22 (1.12, 4.40)	0.02
Dyslipidemia	-0.28	0.42	0.75 (0.33, 1.71)	0.50

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension

Table 19. Adjusted model for hypothesis H_{A-2} stratified by age (55-64 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.55	0.17	0.58 (0.41, 0.81)	0.001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	1.01	0.36	2.73 (1.34, 5.58)	0.006
PVD	1.12	0.29	3.06 (1.75, 5.36)	<0.0001
Other cardiac disease	0.36	0.35	1.43 (0.73, 2.82)	0.30
COPD	0.73	0.20	2.08 (1.40, 3.11)	0.0003
GI disease	0.30	0.53	1.35 (0.48, 3.78)	0.57
Liver disease	-0.01	0.86	0.99 (0.18, 5.34)	0.99
Dysrhythmia	0.99	0.24	2.69 (1.70, 4.28)	<0.0001
DM	0.77	0.22	2.17 (1.40, 3.36)	0.0005
Anemia	0.33	0.30	1.40 (0.78, 2.51)	0.27
HTN	0.63	0.16	1.88 (1.37, 2.58)	0.0001
Dyslipidemia	-0.0009	0.19	1.00 (0.69, 1.45)	0.99

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension

Table 20. Adjusted model for hypothesis H_{A-2} stratified by age (65-74 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	0.003	0.11	1.00 (0.81, 1.25)	0.98
Baseline comorbidity *(referent: no history)*				
CVA/TIA	1.15	0.20	3.17 (2.15, 4.66)	<0.0001
PVD	0.71	0.22	2.04 (1.33, 3.13)	0.001
Other cardiac disease	1.02	0.21	2.78 (1.85, 4.18)	<0.0001
COPD	0.74	0.15	2.11 (1.56, 2.83)	<0.0001
GI disease	0.50	0.33	1.64 (0.87, 3.11)	0.13
Liver disease	0.17	1.05	1.18 (0.15, 9.22)	0.87
Dysrhythmia	0.47	0.17	1.60 (1.14, 2.23)	0.006
DM	0.71	0.16	2.03 (1.48, 2.80)	<0.0001
Anemia	0.27	0.24	1.31 (0.82, 2.09)	0.26
HTN	0.30	0.11	1.35 (1.08, 1.69)	0.008
Dyslipidemia	0.10	0.16	1.10 (0.80, 1.52)	0.56

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension

Table 21. Adjusted model for hypothesis H_{A-2} stratified by age (≥ 75 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*	-0.36	0.07	0.70 (0.60, 0.80)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.63	0.10	1.88 (1.55, 2.28)	<0.0001
PVD	0.03	0.12	1.03 (0.82, 1.30)	0.79
Other cardiac disease	0.06	0.13	1.07 (0.83, 1.37)	0.62
COPD	0.48	0.08	1.62 (1.37, 1.90)	<0.0001
GI disease	0.06	0.18	1.06 (0.75, 1.51)	0.73
Liver disease	-1.46	1.05	0.23 (0.03, 1.81)	0.16
Dysrhythmia	0.75	0.07	2.11 (1.84, 2.42)	<0.0001
DM	0.35	0.10	1.42 (1.16, 1.75)	0.0006
Anemia	0.30	0.11	1.35 (1.09, 1.68)	0.007
HTN	0.17	0.06	1.18 (1.05, 1.33)	0.005
Dyslipidemia	-0.18	0.12	0.83 (0.66, 1.05)	0.13

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease, DM: diabetes, HTN: hypertension

D. Analysis of Sub-Study II

1. Analysis of hypothesis H_{B-1}, H_{B-2}, and H_{B-3}

The variables with p-values less than 0.1 were identified from the fully adjusted model of sub-study I. The model of sub-study II was refitted with these variables. Hypothesis H_{B-1}, H_{B-2} and H_{B-3} examined the relationship between individual bisphosphonate use and the risk of the composite endpoint of CHF, MI or stroke hospitalization. The results from the adjusted model suggested that compared to non-bisphosphonate users, all three bisphosphonates showed protective effect for lowering the risk of the composite outcome. Compared to non-bisphosphonate users, patients who received risedronate were associated with the greatest decrease in the risk of the composite outcomes after adjustment for the covariates. Risedronate users had a 38% decreased risk of the composite outcome compared to non-bisphosphonate users (HR=0.62, 95% CI=0.51-0.76, $p<0.0001$). All of the covariates had positive parameter estimates suggesting that the presence of these covariates was associated with increased risk of the composite outcome after adjusting for everything else. The results are summarized in Table 22.

Table 22. Adjusted model for hypothesis H_{B-1}, H_{B-2}, and H_{B-3}

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Alendronate	-0.21	0.07	0.81 (0.72, 0.93)	0.002
Ibandronate	-0.15	0.15	0.86 (0.65, 1.15)	0.31
Risedronate	-0.48	0.10	0.62 (0.51, 0.76)	<0.0001
Baseline comorbidity *(referent: no history)*				
Age	0.07	0.002	1.07 (1.07, 1.08)	<0.0001
CVA/TIA	0.74	0.09	2.09 (1.77, 2.47)	<0.0001
PVD	0.26	0.10	1.30 (1.07, 1.57)	0.007
COPD	0.67	0.07	1.96 (1.72, 2.24)	<0.0001
Dysrhythmia	0.69	0.06	2.00 (1.77, 2.26)	<0.0001
DM	0.60	0.08	1.82 (1.56, 2.13)	<0.0001
Anemia	0.26	0.09	1.30 (1.09, 1.56)	0.005
HTN	0.25	0.05	1.28 (1.16, 1.42)	<0.0001

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

2. Analysis of hypothesis H_{B-1}, H_{B-2} and H_{B-3} stratified by age

The analysis for hypothesis H_{B-1}, H_{B-2}, and H_{B-3} was further stratified by age. The results of the adjusted stratified model showed that compared to non-bisphosphonate users, a 38%, 48% and 25% lower risk of the composite outcome was observed in alendronate users age 45-54, 55-64 and ≥75 years, respectively. On the contrary, among patients age 65-74 years, alendronate users were associated with an 8% increased risk of the composite outcome compared to non-bisphosphonate users after controlling for baseline comorbidities. Similar relationship was observed between the use of ibandronate and the risk of the composite outcome after controlling for baseline comorbidities. Patients with use of ibandronate had lower risk of the composite outcome among patients age 55-64 and ≥75 years, but higher risk of the composite outcome among patients age 65-74 years. Due to the small sample size of ibandronate users age 45-54 years, no

difference in HR was observed. Risedronate use was associated with lower risk of the composite outcome compared to non-bisphosphonate users after adjustment for baseline comorbidities in all of the four age categories. All of the covariates included in the model were associated with increased risk of the composite outcome. The results are presented in Table 23 to Table 26.

Table 23. Adjusted model for hypothesis H_{B-1}, H_{B-2}, and H_{B-3} stratified by age (45-54 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Alendronate	-0.47	0.40	0.62 (0.28, 1.36)	0.24
Ibandronate	-13.89	0.22	0.00 (0.00, 0.00)	<0.0001
Risedronate	-0.63	0.62	0.53 (0.16, 1.78)	0.30
Baseline comorbidity *(referent: no history)*				
CVA/TIA	2.36	0.64	10.63 (3.03, 37.33)	0.0002
PVD	1.06	0.64	2.87 (0.82, 10.12)	0.10
COPD	2.07	0.30	7.92 (4.42, 14.19)	<0.0001
Dysrhythmia	0.53	0.42	1.71 (0.75, 3.90)	0.21
DM	1.34	0.36	3.82 (1.87, 7.78)	0.0002
Anemia	0.63	0.49	1.88 (0.72, 4.91)	0.20
HTN	0.69	0.34	2.00 (1.02, 3.92)	0.04

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 24. Adjusted model for hypothesis H_{B-1}, H_{B-2}, and H_{B-3} stratified by age (55-64 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Alendronate	-0.65	0.22	0.52 (0.34, 0.80)	0.003
Ibandronate	-0.37	0.42	0.69 (0.30, 1.56)	0.37
Risedronate	-0.44	0.28	0.65 (0.37, 1.12)	0.12
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.99	0.36	2.70 (1.34, 5.41)	0.005
PVD	1.14	0.28	3.12 (1.79, 5.42)	<0.0001
COPD	0.75	0.20	2.11 (1.43, 3.14)	0.0002
Dysrhythmia	1.06	0.23	2.88 (1.83, 4.54)	<0.0001
DM	0.80	0.22	2.22 (1.45, 3.42)	0.0003
Anemia	0.35	0.29	1.42 (0.80, 2.52)	0.23
HTN	0.63	0.16	1.87 (1.37, 2.55)	<0.0001

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 25. Adjusted model for hypothesis H_{B-1}, H_{B-2}, and H_{B-3} stratified by age (65-74 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Alendronate	0.08	0.13	1.08 (0.84, 1.40)	0.54
Ibandronate	0.22	0.24	1.25 (0.78, 2.02)	0.36
Risedronate	-0.35	0.22	0.71 (0.46, 1.09)	0.11
Baseline comorbidity *(referent: no history)*				
CVA/TIA	1.10	0.20	3.00 (2.03, 4.43)	<0.0001
PVD	0.75	0.22	2.13 (1.38, 3.27)	0.001
COPD	0.74	0.15	2.10 (1.55, 2.85)	<0.0001
Dysrhythmia	0.64	0.16	1.90 (1.40, 2.60)	<0.0001
DM	0.76	0.16	2.14 (1.56, 2.94)	<0.0001
Anemia	0.36	0.22	1.43 (0.93, 2.21)	0.11
HTN	0.34	0.11	1.40 (1.12, 1.75)	0.003

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 26. Adjusted model for hypothesis H_{B-1}, H_{B-2}, and H_{B-3} stratified by age (≥ 75 years)

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
Bisphosphonate use *(referent: no use)*				
Alendronate	-0.28	0.08	0.75 (0.64, 0.89)	0.0008
Ibandronate	-0.32	0.21	0.72 (0.48, 1.09)	0.12
Risedronate	-0.57	0.13	0.56 (0.43, 0.73)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.62	0.10	1.87 (1.54, 2.26)	<0.0001
PVD	0.04	0.12	1.04 (0.83, 1.31)	0.73
COPD	0.48	0.08	1.61 (1.37, 1.90)	<0.0001
Dysrhythmia	0.76	0.07	2.13 (1.86, 2.44)	<0.0001
DM	0.35	0.10	1.35 (1.16, 1.75)	0.0006
Anemia	0.30	0.11	1.35 (1.10, 1.67)	0.005
HTN	0.17	0.06	1.18 (1.05, 1.33)	0.006

Abbreviation: CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

E. Analysis of Sub-Study III

1. Descriptive statistics of medication possession ratio (MPR)

Sub-study III examined the impact of adherence to bisphosphonate therapy on the risk of CHF, MI or stroke hospitalization. Adherence to bisphosphonate therapy was measured by MPR, which was calculated in the 12 months after treatment initiation. Of 16,510 bisphosphonate users, 3,159 were removed from the analysis because they did not have at least 12 months follow-up time after treatment initiation. A total of 13,351 bisphosphonate users were included in the final analysis of sub-study III. The mean MPR within 12 months after treatment initiation for these patients was 69.5%. Over half of the bisphosphonate users (53.6%) were adherent defined as having MPR ≥80%. The proportion of adherent patients increased with decreasing MPR threshold. 59.5%, 65.3%

and 68.7% of the bisphosphonate users were considered adherent when the cut-off point for MPR was 70%, 60% and 50%, respectively (Table 27).

Table 27. MPR in 12 months after treatment initiation

	N	MPR (Mean±SD)	MPR threshold ≥80% n(%)	≥70% n(%)	≥60% n(%)	≥50% n(%)
BSP users	13,351	69.5±34.0	7,160(53.6)	7,948(59.5)	8,714(65.3)	9,127(68.7)

Abbreviation: MPR: medication possession ratio, BSP: bisphosphonate

2. Analysis of hypothesis H_{c-1}: unadjusted model

Hypothesis H_{c-1} examined if higher adherence to bisphosphonate therapy was associated with lower risk of CHF, MI or stroke hospitalization among bisphosphonate users. The initial assessment was to examine the relationship of adherence to bisphosphonate therapy and the risk of the composite outcome using an unadjusted model. The results of the unadjusted model showed that bisphosphonate users who were adherent to bisphosphonate therapy had lower risk of the composite outcome compared to bisphosphonate users who were not adherent regardless which MPR threshold was used for measuring adherence. However, higher MPR was not necessarily associated with lower risk of the composite outcome. Bisphosphonate users with MPR ≥80% had a 26% lower risk of the outcome compared to bisphosphonate users with MPR <80% (HR=0.74, 95% CI=0.62-0.88, p=0.0007), while bisphosphonate users with MPR ≥50% were associated with a 27% decreased risk of the composite outcome compared to bisphosphonate users with MPR <50% (HR=0.73, 95% CI=0.61-0.88, p=0.0009). The results of the unadjusted model are presented in Table 28.

Table 28. Unadjusted model for hypothesis H_{c-1}

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
MPR				
≥80%	-0.31	0.09	0.74 (0.62, 0.88)	0.0007
<80% *(reference)*				
MPR				
≥70%	-0.39	0.09	0.68 (0.57, 0.81)	<0.0001
<70% *(reference)*				
MPR				
≥60%	-0.34	0.09	0.71 (0.59, 0.85)	0.0001
<60% *(reference)*				
MPR				
≥50%	-0.31	0.09	0.73 (0.61, 0.88)	0.0009
<50% *(reference)*				

Abbreviation: MPR: medication possession ratio

3. Analysis of hypothesis H_{c-1}: adjusted model

The adjusted model assessed whether the relationship between adherence to bisphosphonate therapy and the risk of the composite outcome changed after adjustment for age and baseline comorbidities. The results suggested that after adjustment for age and baseline comorbidities, bisphosphonate users who were adherent to bisphosphonate therapy still had significantly lower risk of the composite outcome compared to non-adherent bisphosphonate users at each of the MPR threshold (Figure 11). Similar to the results of the unadjusted model, better adherence to bisphosphonate therapy was not necessarily associated with lower risk of the composite outcome. Among the four MPR thresholds examined, patients with MPR ≥ 70% was associated with the largest decrease in the risk of the composite outcome compared to patients with MPR <70% (HR=0.74, 95% CI=0.62-0.88, p=0.0006). All of the covariates, except for baseline anemia were

associated with increased risk of the composite outcome. The results of the adjusted model for hypothesis H_{c-1} are presented in Table 29 to Table 32.

Figure 10. Hazard ratio of the risk of CHF, MI or stroke hospitalization at different MPR threshold

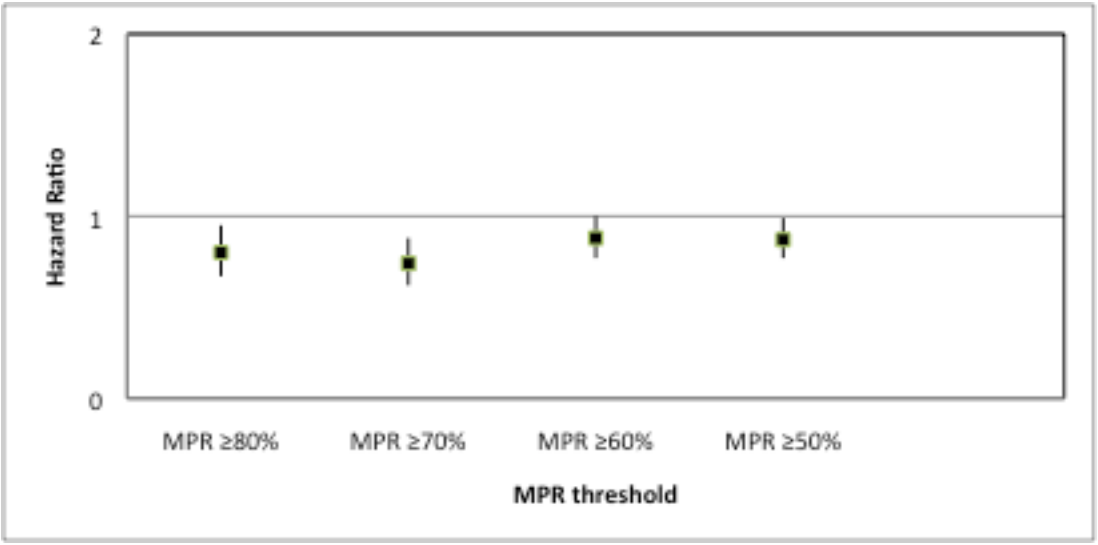


Table 29. Adjusted model for hypothesis H_{c-1}: MPR ≥80% vs. MPR <80%

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
MPR				
≥80%	-0.23	0.09	0.80 (0.67, 0.95)	0.01
<80% *(reference)*	-	-	-	-
Age	0.08	0.004	1.08 (1.07, 1.09)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.69	0.18	2.00 (1.40, 2.86)	0.0001
PVD	0.29	0.20	1.34 (0.90, 1.98)	0.15
COPD	0.53	0.14	1.69 (1.29, 2.21)	0.0001
Dysrhythmia	0.64	0.13	1.89 (1.47, 2.43)	<0.0001
DM	0.53	0.16	1.70 (1.24, 2.32)	0.0009
Anemia	-0.22	0.23	0.80 (0.51, 1.27)	0.35
HTN	0.02	0.10	1.03 (0.84, 1.26)	0.82

Abbreviation: MPR: medication possession ratio, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 30. Adjusted model for hypothesis H_{c-1}: MPR ≥70% vs. MPR <70%

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
MPR				
≥70%	-0.30	0.09	0.74 (0.62, 0.88)	0.0006
<70% *(reference)*	-	-	-	-
Age	0.08	0.004	1.08 (1.07, 1.09)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.70	0.18	2.01 (1.41, 2.87)	0.0001
PVD	0.29	0.20	1.34 (0.91, 1.99)	0.14
COPD	0.52	0.14	1.68 (1.29, 2.20)	0.0001
Dysrhythmia	0.63	0.13	1.88 (1.46, 2.41)	<0.0001
DM	0.52	0.16	1.68 (1.23, 2.30)	0.001
Anemia	-0.21	0.23	0.81 (0.51, 1.28)	0.37
HTN	0.03	0.10	1.03 (0.84, 1.26)	0.79

Abbreviation: MPR: medication possession ratio, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 31. Adjusted model for hypothesis H_{c-1}: MPR ≥60% vs. MPR <60%

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
MPR				
≥60%	-0.26	0.09	0.77 (0.64, 0.92)	0.004
<60% *(reference)*	-	-	-	-
Age	0.08	0.004	1.08 (1.07, 1.09)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.70	0.18	2.02 (1.41, 2.88)	0.0001
PVD	0.29	0.20	1.34 (0.91, 1.98)	0.14
COPD	0.53	0.14	1.69 (1.30, 2.21)	0.0001
Dysrhythmia	0.63	0.13	1.88 (1.46, 2.41)	<0.0001
DM	0.53	0.16	1.71 (1.25, 2.33)	0.0008
Anemia	-0.21	0.24	0.81 (0.51, 1.28)	0.37
HTN	0.02	0.10	1.03 (0.84, 1.26)	0.81

Abbreviation: MPR: medication possession ratio, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

Table 32. Adjusted model for hypothesis H_{c-1}: MPR ≥50% vs. MPR <50%

Variable	Parameter Estimate	Standard Error	HR (95% CI)	p value
MPR				
≥50%	-0.24	0.09	0.79 (0.66, 0.95)	0.01
<50% *(reference)*	-	-	-	-
Age	0.08	0.004	1.08 (1.07, 1.09)	<0.0001
Baseline comorbidity *(referent: no history)*				
CVA/TIA	0.70	0.18	2.01 (1.41, 2.87)	0.0001
PVD	0.30	0.20	1.35 (0.91, 2.00)	0.13
COPD	0.53	0.14	1.69 (1.29, 2.21)	0.0001
Dysrhythmia	0.63	0.13	1.89 (1.47, 2.42)	<0.0001
DM	0.54	0.16	1.72 (1.26, 2.34)	0.0007
Anemia	-0.21	0.23	0.81 (0.51, 1.29)	0.38
HTN	0.02	0.10	1.03 (0.84, 1.26)	0.81

Abbreviation: MPR: medication possession ratio, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, DM: diabetes, HTN: hypertension

CHAPTER VI. DISCUSSION AND CONCLUSIONS

A. Discussion

Vascular calcification is now understood as a result of an active and tightly regulated process involving bone formation and mineralization. Extensive research has shown that vascular calcification is independently associated with increased risks of cardiovascular morbidity and mortality (Schmermund et al., 2003; Arad, Goodman, Roth, Newstein & Guerci, 2005; Bastos et al., 2012; Harbaugh et al., 2012; Lee et al., 2012). Therefore, any medication that can slow or reverse the progression of vascular calcification could provide a new way to treat patients with cardiovascular diseases. According to animal studies, bisphosphonates have consistently shown beneficial effects on inhibiting vascular calcification in rats through inhibiting bone resorption (Price et al., 2001; Tamura et al., 2007; Saito et al., 2007). However, the results of clinical studies on humans in this area are conflicting (Tankó et al., 2005; Luckish et al., 2008; Elmariah et al., 2010). This study examined the association between bisphosphonate use and the risk of cardiovascular diseases that have been linked to vascular calcification in post-menopausal women. The findings of this study help to fill the gap of knowledge remaining from animal studies and clinical and epidemiological evidence in humans.

1. Sub-study I

In this study of post-menopausal women with osteoporosis, a 24% lower risk of CHF, MI or stroke hospitalization was observed in those taking bisphosphonates from the primary analysis of sub-study I. This finding is in accordance with the results from

animal studies suggesting that bisphosphonates may reduce cardiovascular risks through the inhibition of vascular calcification. In a retrospective cohort study, Steinbuch and colleagues assessed mortality in patients who had enrolled in the North American Postmenopausal Osteoporosis Studies. They observed a non-significant decreasing trend in cardiovascular mortality, which was mainly attributed to decrease in stroke mortality in patients in risedronate group compared to placebo group (Steinbuch et al., 2002). Luckish and colleagues (2008) examined the vascular impact of risedronate in a small open-label prospective study of 68 post-menopausal women with osteoporosis. They reported that risedronate significantly improved large and small artery elasticity after 6 months of treatment. The finding of this study supports these previous reports. In addition, based on the evidence that CHF, MI and stroke are linked to vascular calcification (Sangiorgi et al., 1998; Wilson et al., 2001; Schmermund et al., 2003), the finding of this study suggests that the observed beneficial effect of bisphosphonates on cardiovascular morbidity and mortality might be due to modulation of vascular calcification.

Results from the stratification analysis of sub-study I show an interesting finding in patients aged 65 to 74 years. In contrast to the association of bisphosphonate use with a decreased risk of CHF, MI or stroke hospitalization seen in patients aged younger than 65 and patients aged 75 and older, there was no difference observed in the risk of CHF, MI or stroke hospitalization between use and no use of bisphosphonates in patients aged 65 to 74 years (HR=1.00, 95% CI: 0.81-1.25). The reasons for this association are unclear. However, there are some possible explanations. First, because of the high affinity to bone,

the doses of bisphosphonates may influence how bisphosphonates are distributed to bone and extra-skeletal soft tissues and further influence calcium flux in the body. Results from an animal study suggest that bisphosphonates inhibit artery calcification at doses inhibiting bone resorption (Price et al., 2001). For treatment of osteoporosis in humans, bisphosphonates are typically used at doses that would inhibit bone resorption. However, bisphosphonates may only present a protective effect on vascular calcification after reaching a certain dose threshold. In a study using nationwide registry data from Denmark, Vestergaard and colleagues reported an inverse dose-response association between alendronate and the risk of acute MI and atherosclerosis of the coronary arteries (Vestergaard, 2012). Compared to patients who did not use alendronate, a decreased risk of acute MI and atherosclerosis of the coronary arteries was observed in patients using alendronate at high doses (≥ 1 unit of average daily dose in the study), but not at low doses (≤ 0.66 unit of average daily dose in the study). Their findings suggest that alendronate may only exert a protective effect on vascular calcification at high doses. Bisphosphonate dosing may be influenced by the severity of osteoporosis and severity of osteoporosis may be less in patients aged 65-74 compared to those that were younger or older.

Decline in bone mass in women typically accelerates at menopause and continues to progress with age. Therefore, compared to patients in the other three age categories in this study, patients aged 75 years and older are more likely to have more severe osteoporosis after controlling for other risk factors. Both the National Osteoporosis

Foundation Clinician's Guide for Prevention and Treatment of Osteoporosis (2008) and the U.S. Preventive Service Task Force (2011) recommend routine screening for osteoporosis in women age 65 years and older regardless of clinical risk factors and in younger post-menopausal women at increased risk of osteoporosis fractures. Therefore, patients who get diagnosed with osteoporosis at ages younger than 65 may have more severe osteoporosis compared to patients who get diagnosed through guideline-recommended routine screening starting at age 65 years. In this study, patients aged younger than 65 and aged 75 and older may represent a group of patients with more severe osteoporosis and more likely be treated with higher doses. Based on Vestergaard's study (2012), if bisphosphonate dose impacts vascular calcification effect, the effect would be expected to be most obvious in patients with more severe osteoporosis because they are more likely to be treated at higher doses. Further studies will be needed to check if bisphosphonate doses are different in patients aged younger than 65 and aged 65 and older.

In addition, this study uses the MarketScan Databases, which consist of medical and pharmacy claims data for people with employer-sponsored health insurance. For Medicare-eligible retirees, only those who receive supplemental health care and/or prescription drug coverage through employer-sponsored retiree health coverage are included in the MarketScan Databases. This database limitation may lead to selection bias because claims for prescription drugs and other health care utilization may not be found in MarketScan Databases in patients who have both employer-sponsored retiree

health coverage and Medicare. Patients' comorbidities were examined for evidence that patients aged 65 and older in MarketScan Databases were not vastly different from patients within the same age range in the general population. Similar to the general population, comorbidities increased as age increased. However, there may still be some issues affecting the completeness of follow-up data in patients aged 65 and older as compared to patients age younger than 65.

2. Sub-study II

The hypothesis that bisphosphonates may reduce cardiovascular risk through the inhibition of vascular calcification is supported by the finding from sub-study I. In sub-study II, the effect of each individual bisphosphonate was examined. In the primary analysis of sub-study II, the lowest risk of CHF, MI or stroke hospitalization was seen in patients receiving risedronate, followed by alendronate and ibandronate. This difference may be related to the potency of the three bisphosphonates studied. The relative potency of these medications as inhibitors of bone resorption are: risedronate > alendronate > ibandronate (Drake et al., 2008). In accordance with the relative potency, risedronate was associated with the lowest risk of CHF, MI or stroke hospitalization. However, alrendronate showed greater protective effect than ibandronate. This may be due to the small numbers of patients in the ibandronate group. The bisphosphonates identification period in this study was from January 1, 2001 to January 1, 2007. Ibandronate was approved by the FDA in March 2005. Typically, it takes about 3 to 6 months for a new medication to be adopted into clinical practice. It was only in 2006 that ibandronate was

being prescribed fairly routinuely. In fact, 57% and 33% of the study bisphosphonate users received alendronate and risedronate, respectively, as their index bisphosphonate. Only 10% of the study bisphosphonate users received ibadronate as their index bisphosphonate. Further studies with larger sample size are needed to confirm the finding. Another possible explanation is the pharmacologic mechanism of the three bisphosphonates. Alendronate and risedronate have been demonstrated to significantly reduce the risk of both vertebral and non-vertebral fractures in clinical trials, whereas ibandronate only shows significant effects on reducing vertebral fractures. Although all three bisphosphonates prevent bone loss through inhibiting bone resorption, there may be some differences in their pharmacologic mechanism, which may have influence on their effect on vascular calcification.

3. Sub-study III

In sub-study III, the association between adherence to bisphosphonates and the risk of CHF, MI or stroke hospitalization was examined. Adherence was defined by MPR, and multiple cut-points of MPR (80%, 70%, 60%, and 50%) were used to assess the association between adherence and outcomes. A significant lower risk of CHF, MI or stroke hospitalization was observed in bisphosphonate users who were adherent to bisphosphonate at each of the MPR cut-points. Vestergaard and colleagues reported similar findings as this study. They found a non-significant lower risk of acute MI and a significant lower risk of atherosclerosis in alendronate users who were adherent to the drug as compared to non-adherent alendronate users (Vestergaard et al., 2012). As

previously discussed for sub-study I, the doses of bisphosphonates may play a role in the effect on vascular calcification. The finding of sub-study III supports this hypothesis. During the 12-month follow-up period of sub-study III, the adherent bisphosphonate users were exposed to higher cumulative doses than non-adherent bisphosphonate users, which may explain why adherent bisphosphonate users were associated with a lower risk of CHF, MI or stroke hospitalization.

Surprisingly, instead of in patients with MPR ≥ 80%, the largest decrease in the risk of the composite outcome was observed in patients with MPR ≥ 70%. Bisphosphonates' high affinity to bones may provide an explanation. Because bones uptake the majority of the absorbed bisphosphonates, soft tissues, such as coronary arteries, are only exposed to high serum concentrations of bisphosphonates for a short period of time. Therefore, the vascular effect of bisphosphonates may be due to lower sustained serum concentrations of bisphosphonates from the gradual release from bone. If this is the case then bisphosphonate effect on vascular calcification may not be as sensitive to adherence as is bisphosphonate effect on bones.

B. Limitation

A retrospective claims data analysis was used to address the research hypotheses. While the MarketScan database provides valuable information on medication use and reflects real world practice, this study is limited in some aspects and should be discussed.

First, the study is limited in its ability to infer causal relationship due to the non-randomized nature of observational study. Therefore, no causal relationship can be proven between the use of bisphosphonate and the risk of the composite end point of CHF, MI or stroke hospitalization. Similarly, this study cannot prove the causal relationship between adherence to bisphosphonate therapy and the risk of the composite outcome.

Second, the MarketScan database does not include laboratory values. Therefore, disease severity based on laboratory tests is not available in this study. According to the established evidence that vascular calcification is linked to CHF, MI and stroke, hospitalization for CHF, MI or stroke was used as a proxy for patients with presence of vascular calcification, but the extent of vascular calcification was not assessable. Information such as vascular and valvular electron-beam computed tomography (CT) score could have helped support the association seen in this study.

Third, the administrative claims data can only be used to assess patients' medication acquisition behaviors instead of their medication consumption behaviors. The analysis of the study used filling a bisphosphonate prescription as the marker for actual use of bisphosphonate. However, whether patients actually took their medications as prescribed could not be measured in the study. It has been shown that in an older population, filling a prescription is highly predictive of medication consumption (Grymonpre, Cheang, Fraser, Metge, & Sitar, 2006). The difference between filling the prescription and actual

consumption of medication should still be taken into consideration when interpreting the results. Despite this limitation, pharmacy claims data are still believed to be more objective in measuring adherence compared to other measures (Wang et al., 2004).

C. Conclusions and recommendations for future studies

This study examined the use of bisphosphonates and the risk of a composite cardiovascular outcome of CHF, MI or stroke hospitalization. Results from a series of animal studies were applied in this study as the conceptual framework. This study showed that post-menopausal female patients with bisphosphonate use were significantly associated with a lower risk of CHF, MI or stroke hospitalization as compared to non-bisphosphonate users. As one of the first studies that examined the effect of bisphosphonates in different age categories, the study showed that bisphosphonates presented a beneficial effect on reducing risk of CHF, MI or stroke hospitalization in patients younger than 65 and older than 75 years of age. However, no protective effect of bisphosphonate was observed in patients aged 65 and 74 years. When the bisphosphonates were examined individually, patients who received risedronate were associated with the lowest risk of CHF, MI or stroke hospitalization as compared to patients who received alendronate or ibandronate. This study also examined the link between adherence to bisphosphonate therapy and the cardiovascular outcomes. The results suggested that adherent bisphosphonate users had lower risk of CHF, MI or stroke hospitalization than non-adherent bisphosphonate users.

Given the retrospective nature and data limitations of this study, the results of this study need to be validated in future prospective studies and/or observational studies that include laboratory, clinical and radiologic data to determine osteoporosis severity and degree of vascular calcification. In addition, studies are needed to further clarify the effect of cumulative bisphosphonate dose on calcification-related cardiovascular events. Bisphosphonate therapy for osteoporosis is considered a long-term chronic therapy. Given the fairly short mean follow-up time of this study, these study findings should be considered as the short-term effect of bisphosphonates. Therefore, future studies with longer follow-up time are needed to determine the long-term effect of bisphosphonates on vascular calcification and related outcomes as well as the potential for increased risk of other outcomes, such as osteonecrosis of the jaw. In addition, longer follow-up time will also allow for examination of the association between long-term adherence to bisphosphonate therapy and calcification-related events.

In conclusion, this study suggests an association between short-term bisphosphonate use and decreased risk of CHF, MI or stroke hospitalization in post-menopausal women younger than 65 years and 75 years and older. Adherent bisphosphonate users were associated with a decreased risk of CHF, MI or stroke hospitalization as compared to non-adherent bisphosphonate users. The findings of this study provide clinicians with new ideas and options when delivering care to patients with both osteoporosis and cardiovascular disease. Given the evidence between vascular calcification and cardiovascular morbidity and mortality and the potential beneficial effect of

bisphosphonates on vascular calcification, further examination is warranted for these agents in post-menopausal women who have both osteoporosis and cardiovascular diseases.

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APPENDIX

Appendix 1. Definition and ICD-9-CM codes used to identify CHF, MI and stroke hospitalization

Disease	ICD-9-CM code	Description
CHF	402.01	Hypertensive heart disease: Malignant, with heart failure
	402.11	Hypertensive heart disease: Benign, with heart failure
	402.91	Hypertensive heart disease: Unspecified, with heart failure
	404.01	Hypertensive heart and renal disease: Malignant, with heart failure
	404.11	Hypertensive heart and renal disease: Benign, with heart failure
	404.91	Hypertensive heart and renal disease: Unspecified, with heart failure
	428.0	Congestive heart failure, unspecified
	428.1	Left heart failure
	428.2	Systolic heart failure
	428.3	Diastolic heart failure
	428.4	Combined systolic and diastolic heart failure
	428.9	Heart failure, unspecified
MI	410.0	Acute myocardial infarction of anterolateral wall
	410.1	Acute myocardial infarction of other anterior wall
	410.2	Acute myocardial infarction of inferolateral wall
	410.3	Acute myocardial infarction of inferoposterior wall
	410.4	Acute myocardial infarction of other inferior wall
	410.5	Acute myocardial infarction of other lateral wall
	410.6	Acute myocardial infarction: True posterior wall infarction
	410.7	Acute myocardial infarction: Subendocardial infarction
	410.8	Acute myocardial infarction of other specified sites
	410.9	Acute myocardial infarction: Unspecified site
	414.0	Coronary atherosclerosis
	414.8	Other specified forms of chronic ischemic heart disease
	414.9	Chronic ischemic heart disease, unspecified
Stroke (ischemic stroke)	433.0	Occlusion and stenosis of precerebral arteries: Basilar artery
	433.1	Occlusion and stenosis of precerebral arteries: Caroid artery

	433.2	Occlusion and stenosis of precerebral arteries: Vetebral artery
	433.3	Occlusion and stenosis of precerebral arteries: Multiple and bilateral
	433.8	Occlusion and stenosis of precerebral arteries: Other specified precerebral artery
	433.9	Occlusion and stenosis of precerebral arteries, unspecified
	434.0	Occlusion of cerebral arteries: Cerebral thrombosis
	434.1	Occlusion of cerebral arteries: Cerebral embolism
	434.9	Occlusion of cerebral arteries, unspecified
	437.0	Cerebral atherosclerosis
	437.1	Other generalized ischemic cerebrovascular disease

Appendix 2. ICD-9-CM codes used to identify baseline comorbidities

Comorbidity	ICD-9-CM code
Atherosclerotic heart disease	410-414, V45.81, V45.82
Congestive heart failure	398.91, 425, 428, 402.x1, 404.x1, 404.x3
Cerebral vascular accident/Transient ischemic attack	430-438
Peripheral vascular disease	440-444, 447, 451-453, 557
Other cardiac diseases	420-423, 429, 785.0-785.3, V42.1, V42.2, V43.3, V45.0, V53.3
Chronic obstructive pulmonary disease	490-494, 496, 510
GI diseases	456.0-456.2, 530.7, 531-534, 569.84, 569.85, 578
Liver disease	570, 571, 572.1, 572.4, 573.1-573.3, V42.7
Dysrhythmia	426-427, 424-424.9, V42.2, V43.3, V45.0, V53.3
Diabetes	250, 357.2, 362.0, 366.41
Anemia	280-285
Hypertension	362.11, 401.xx-405.xx, 437.2
Dyslipidemia	272.0-272.4, 272.9

Appendix 3. Comorbidities of study patients

Comorbidity	Non-BSP users at baseline (N=14,743)		BSP users at treatment initiation (N=16,510)		p-value
	n	%	n	%	
Atherosclerotic heart disease	701	4.8	658	4.0	0.0009
CHF	245	1.7	236	1.4	0.10
CVA/TIA	408	2.8	386	2.3	0.02
PVD	490	3.3	428	2.6	0.0001
Other cardiac diseases	405	2.8	430	2.6	0.44
COPD	1098	7.5	1164	7.1	0.18
GI diseases	243	1.7	174	1.1	<0.0001
Liver disease	68	0.5	49	0.3	0.02
Dysrhythmia	1060	7.2	1024	6.2	0.0005
Diabetes	866	5.9	817	5.0	0.0003
Anemia	627	4.3	564	3.4	0.0001
Hypertension	3041	20.6	3267	19.8	0.07
Dyslipidemia	1523	10.3	1886	11.4	0.002

Abbreviation: BSP: bisphosphonate, CHF: congestive heart failure, CVA/TIA: cerebral vascular accident/transient ischemic attack, PVD: peripheral vascular disease, COPD: chronic obstructive pulmonary disease, GI disease: gastric intestinal disease

Appendix 4. The use of concomitant medications of study patients

Medication	Non-BP users at baseline (N=14,743)		BP users at treatment initiation (N=16,510)		p-value
	n	%	n	%	
Anti-hypertensive medications					
ACEI	2015	13.7	2410	14.6	0.02
ARB	1488	10.1	1458	8.8	0.0001
Beta-blockers	2656	18.0	2796	16.9	0.01
CCB	2053	13.9	2362	14.3	0.33
Diuretics	3493	23.7	3656	22.1	0.001
Lipid lowering agents					
Statins	3149	21.4	4098	24.8	<0.0001
Non-statin LLAs	833	5.7	893	5.4	0.35
Anti-diabetic medications					
OAD	841	5.7	826	5.0	0.006
Insulin	220	1.5	196	1.2	0.02
Other osteoporosis medications					
EHT	2371	16.08	3187	19.30	<0.0001
Calcitonin	1268	8.60	1184	7.17	<0.0001
SERM	1558	10.57	1634	9.90	0.05
Teriparatide	151	1.02	151	0.91	0.32

Abbreviation: BP: bisphosphonate, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, CCB: calcium channel blocker, LLA: lipid lowering agent, OAD: oral anti-diabetic medication, EHT: estrogen/hormone therapy, SERM: selective estrogen receptor modulator

Appendix 5. Letter of Approval from the Institution Review Board (IRB)

TO : stpet002@umn.edu, chen1064@umn.edu,

The IRB: Human Subjects Committee determined that the referenced study is exempt from review under federal guidelines 45 CFR Part 46.101(b) category #4 EXISTING DATA; RECORDS REVIEW; PATHOLOGICAL SPECIMENS.

Study Number: 1003E80012

Principal Investigator: Yen-Wen, Cindy Chen

Title(s):
The role of bisphosphonates: potential novel pharmaceutical therapy for reducing cardiovascular morbidity and mortality

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This e-mail confirmation is your official University of Minnesota RSPP notification of exemption from full committee review. You will not receive a hard copy or letter. This secure electronic notification between password protected authentications has been deemed by the University of Minnesota to constitute a legal signature.

The study number above is assigned to your research. That number and the title of your study must be used in all communication with the IRB office.

If you requested a waiver of HIPAA Authorization and received this e-mail, the waiver was granted. Please note that under a waiver of the HIPAA Authorization, the HIPAA regulation [164.528] states that the subject has the right to request and receive an accounting of Disclosures of PHI made by the covered entity in the six years prior to the date on which the accounting is requested.

If you are accessing a limited Data Set and received this email, receipt of the Data Use Agreement is acknowledged.

This exemption is valid for five years from the date of this correspondence and will be filed inactive at that time. You will receive a notification prior to inactivation. If this research will extend beyond five years, you must submit a new application to the IRB before the study?s expiration date.

Upon receipt of this email, you may begin your research. If you have questions, please call the IRB office at (612) 626-5654.

You may go to the View Completed section of eResearch Central at http://eresearch.umn.edu/ to view further details on your study.

The IRB wishes you success with this research.

We have created a short survey that will only take a couple of minutes to complete. The questions are basic, but will give us guidance on what areas are showing improvement and what areas we need to focus on:

https://umsurvey.umn.edu/index.php?sid=36122&lang=um

Appendix 6. Letter of Agreement of Access to MedStat database From Minneapolis Medical Research Foundation (MMRF)

March 30, 2010

Yen-Wen Chen

407 7th Street SE, Apt 201

Minneapolis, MN 55414

Dear Yen-Wen,

The purpose of this letter of agreement is to outline the terms and conditions under which you, Yen-Wen, shall be granted access to the "MEDSTAT database" (hereinafter database) for the purpose of researching and writing a dissertation in the pursuit of a Ph.D. degree from the University of Minnesota, entitled "The role of bisphosphonates: potential novel pharmaceutical therapy for reducing cardiovascular morbidity and mortality." You agree that your access to the database shall be exclusively for the purpose explicitly outlined in this agreement, and for no other purposes. This access is contingent upon your continuing relationship with the MMRF, and you agree and acknowledge that such access may be terminated by MMRF without cause at any time. You acknowledge and agree that the MMRF makes no warranty as to the fitness of the database for the purpose described herein.

You understand and acknowledge that the MMRF is the recipient of the database under a licensing agreement with the MEDSTAT Group that restricts the purposes and duration of such access to the database, and that if for any reason MMRF terminates that relationship with the MEDSTAT Group, your access to the database shall also be terminated, and that you agree to hold the MMRF harmless of any and all losses you may incur as a result of termination. Further, you acknowledge that you are aware that the current license agreement between MMRF and the MEDSTAT Group terminates effective August 31, 2013. If for any reason you terminate your relationship with MMRF, or MMRF terminates its relationship with you, you acknowledge that access to the database for any purpose shall from that point forward, be prohibited.

You acknowledge and agree that the database is the proprietary and confidential property of the MEDSTAT Group, and that title thereto shall remain the exclusive property of MEDSTAT, except that you shall own the final dissertation you develop hereunder, provided, that you include the following copyright attribution to MEDSTAT: "Copyright© 2009 (use copyright year on Data) The MEDSTAT Group, Inc. All Rights Reserved." Further, you agree to maintain the confidential nature of the database and shall not copy, reproduce, or duplicate the database, in whole, or in part.

Mary Bergaas — Yen-Wen Chen

Mary Bergaas

Chief Operating Officer